CHEMICAL CONSTITUENT OF METHANOL EXTRACT OF BALINESE MOMORDICA CHARANTIA LEAVES

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ABSTRACT

Momordica charantia (bitter melon) has been used as Balinese traditional medicine for the treatment of many diseases. The methanol extract of M. charantia leaves had shown antioxidant activity. The fractionation of methanol extract of M. charantia leaves was then performed by using silica gel column chromatography and increasing polarity. White crystalline solid was obtained from the extract. Furthermore, the compound was characterized by IR, ^{1}H-NMR, ^{13}C-NMR and identified as Momordicine I. The compound also showed antioxidant activity.

Keywords: *Momordica charantia; Momordicine I; Isolation; natural product; antioxidant*

ABSTRAK

Momordica charantia (pare) merupakan salah satu tanaman yang telah digunakan masyarakat Bali untuk penyembuhan berbagai penyakit. Ekstrak metanol dari daun M. charantia menunjukkan aktivitas antioksidan. Fraksinasi kemudian dilakukan dengan menggunakan silica gel kolom kromatografi dengan peningkatan polaritas. Serbuk putih telah berhasil diisolasi dari ekstrak ini. Selanjutnya senyawa murni tersebut dikarakterisasi dengan IR, ^{1}H-NMR, ^{13}C-NMR dan diidentifikasi sebagai Momordicine I. Senyawa ini juga menunjukkan aktivitas antioksidan.

Kata Kunci: *Momordica charantia; Momordicine I; Isolasi; bahan alam; antioksidan*

INTRODUCTION

Momordica charantia L. is a herbaceous climbing plant in the genus *Momordica* of *Cucurbitaceae* family which is distributed in tropical, subtropical and temperate regions [1-3]. In Bali, *M. charantia* known as “paye” but in Indonesia called “pare”. The fruits, leaves, roots of *M. charantia* have been used in Ayurveda for a number of diseases, as bitter stomachic, laxative, and anthelmintic. Balinese traditional medicines used leaves for herbal drinks called “loloh”.

Loloh are herbal drinks produced from leaves and consumed exclusively in Bali to treat different disease [4]. Balinese people used loloh paye for diabetes [5] and for heartburn [4].

In Asia *M. charantia* is widely used extensively in folk medicine as remedy for diabetes [6-7]. Many phytochemical researches concentrate on the fruit, stem, and seed of *M. charantia* have been reported, which cause isolation cucurbitane types triterpenoid [8-9]. Studies confirmed crude extract of *M. charantia* have activities as antidiabetic [10], antitumor [11] and antioxidant [8]. In this research were to isolate and identify the antioxidant compound from methanol extract of *M. charantia* leaves. The compound was identified by using ^{13}C-NMR and ^{1}H-NMR. Than the antioxidant activity of extract and compound were test by ABTS method.

EXPERIMENTAL SECTION

Materials

M. charantia leaves were obtained from Bedugul, Buleleng-Bali. Leave than dried in air and cut up for extraction process. The organic solvent used such as methanol, ethanol, dimethyl sulfoxide, n-hexane, dichloromethane. Pro-analytic solvent used methanol, *n*-hexane and dichloromethane. Silica gel 60, TLC silica gel GF_{254}, and filter paper whatman 42. Antioxidant assay used 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), potassium persulphate ($K_2S_2O_8$) and gallic acid as positive control.

Instrumentation

Melting point was determined on Fischer John melting apparatus and uncorrected. NMR spectra were recorded on an Agilent 500 MHz with console system DD2 in frequency 500 MHz (^{1}H) and 125 MHz (^{13}C). The antioxidant activity used UV-Vis spectra.

Procedure

Extraction and Isolation

M. charantia leaves were extracted by using 10 L methanol for 3 x 24 hours at room temperature. After extraction, extract were concentrated to dry ness on rotatory evaporator under low pressure to give crude methanol extract. The 50 gram crude extract was separated by using column chromatography vacuum (CCV) and eluted by increasing polarity from dichloromethane 100%, ethyl acetate 100% and methanol 100%. All of fraction will monitor used TLC to give five fractions (F_P-F_T). Fraction F_R was separated again by using CCV by silica gel column with gradient mixture ethyl acetate: *n*-hexane (5:95→40:60) to give five fractions.

Fraction R6 was purified by using CCV with gradient mixture ethyl acetate: *n*-hexane (0:100→70:30) as eluent to give three different fraction. Fraction R6b must be evaporating by using *Buchi Rotatory evaporator* than get 680 mg white solid compound.

The white solid compound was recrystallized with dichloromethane and *n*-hexane to give white crystalline solid (489 mg). The crystal melted at 128-130 °C. The compound was characterized at Institute Technology Bandung by using NMR spectra.

***Antioxidant activity (*ABTS) free radical scavenging**

Radical scavenging activity was validated by the UV absorbing method. Various concentration of sample was prepared by dissolving sample (10 mg) in DMSO (1 mL). ABTS was dissolved in DMSO and react with 88 µl $K_2S_2O_8$. The sample solution (10 µL) was added by ABTS solution (1 mL). Incubation for 4 minutes at 30°C, the absorbance at 734 nm was measured. Measurement was conducted three times with methanol used as blank.

IC50 Analysis

IC_{50} Calculation based on the percentage inhibition in ABTS method. The IC_{50} value can be determine by line equation/ regression to calculate IC_{50} from the curve.

RESULTS AND DISCUSSION

Compound R6 was white crystalline solid (mp 128), isolated from methanol extract in ethyl acetate fraction. The IR spectrum showed characteristic brought bands of hydroxyl groups V_{max} 3412 cm^{-1}, an aldehyde group function (Silverstein, 1991) V_{max} 1707 cm^{-1}, C=C sp^2 V_{max} 1641 cm^{-1}, aliphatic C-H sp^3 V_{max} 2877 and 2949 cm^{-1}. The spectrum of ^{1}H-NMR and ^{13}C-NMR show the presence of an aldehyde group at δ_H 9,88 (1H, s) and δ_C 209,7. Three hydroxyl group at δ_H 3.55 (1H,brs), 4.00 (1H, d) and 4.42 (1H, dt). Another spectrum show two substituted double bond are δ_H 5.91 (1H, d, *J*= 5 Hz) and 5.16 (1H, d, *J*= 10 Hz). ^{13}C-NMR also show the presence of hydroxyl group with chemical shift at δ_C 66,5; 66,8 and 77,0. All of H-NMR and C-NMR data was shown in Table 1. Compound R6b was identified as momordicine I (3,7,23-trihydroxycucurbita-5,24-dien-19-al) on the basis comparison with the literature data [1,13-14]. Some of literatures isolate momordicine I from extract methanol *M. charantia* leave by antibacterial activity and antifeedant activity.

The antioxidant capacity from methanol extract *M. charantia* can be determine by using combination some of method to know the antioxidant activities as in-vitro assay (Frankel). Most of vegetable analyzed show much lower antioxidant capacities assay relative to ABTS assay.

Extract of *M. charantia* leaves and momordicine I were subjected to examination for potential free radical scavenging on ABTS. The methanol extracts of leaves *M. charantia* shown IC_{50} value of 27.58µg/mL. The methanol extract included many secondary metabolic compounds in an important role in antioxidant activity such as flavonoid, terpenoid, saponin, tannin and steroid [12]. Momordicine I show inhibition 36.76%. From the result Momordicine I is less active as an antioxidant agent but the extract is contain active compound. Momordicine I structure can be seen in figure 1.

CONCLUSION

In the present study, 3,7,23-trihydroxycucurbitan-5-24-dien-19-al or Momordicine I was isolated from ethyl acetate fraction extract methanol of *M. charantia* leaves. Methanol extract show antioxidant activity IC_{50} 27.58 µg/mL compare with gallic acid as positive control 1.78 µg/mL. Momordicine I show weak ABTS radical cation scavenging activities 36.76%.

ACKNOWLEDGEMENTS

Grateful Institute Technology Sepuluh November for IR measurement and conduct research in Natural Product and Synthesis Laboratory. Lipi Purwodadi Botanic Garden for identification of *M. charantia L*. Institute Technology Bandung for the measurement of C-NMR and H-NMR.

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Table 1 Data of Momordicine I

Position	^{13}C-NMR	^{1}H-NMR
1	22.2	
2	30.2	
3	77.0	3.55 (1H, brs)
4	42.2	
5	147.3	
6	123.9	5.91 (1H, d, J=5 Hz)
7	66.8	4.00 (1H, d, J=5 Hz)
8	50.8	2.40 (1H, m)
9	52.0	
10	37.7	2.58 (1H, d, J= 10 Hz)
11	23.3	
12	29.8	
13	46.7	
14	49.0	
15	35.6	
16	28.6	
17	51.2	
18	15.3	
19	209.7	9.88 (1H, s)
20	33.7	1.96 (1H, m)
21	19.2	1.00 (3H, d, J=5 Hz)
22	45.5	
23	66.5	4.42(1H, dt, J=10 Hz)
24	133.4	5.16 (1H, d, J=10 Hz)
25	130.4	
26	18.7	1.70 (s)
27	26.0	1.67(s)
28	25.9	
29	27.7	
30	18.1	

Figure 1 Structure of Momordicine I