
Isolation and Identification of Octadecane-3-one Compound From Ethyl Acetate Fraction of *Momordica balsamina* L. Leaves

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Abstract. *Momordica balsamina* L. is a wild plant that generally grows in moist soil and gets a lot of sunlight. The leaves and fruit are reported to have various medicinal and nutritional properties. This study aimed to isolate and identify the compounds contained in the ethyl acetate fraction in *Momordica balsamina* L. The isolation stage was carried out by the maceration method and the separation and purification of the compounds using gravity column chromatography methods to obtain isolate fraction F1.6.2 with a sample weight of 2.4 mg. Identification of compounds using proton nuclear magnetic resonance ($^1\text{H-NMR}$) and carbon nuclear magnetic resonance ($^{13}\text{C-NMR}$) spectroscopy techniques. The proposed identified compound is octadecane-3-one or 3-octadecanone with the molecular formula $\text{C}_{18}\text{H}_{36}\text{O}$.

Keywords: Ethyl acetate fraction, *Momordica balsamina* L, octadecane,

Introduction

Many modern medicines come from plants which are often used in the treatment of various types of diseases. Plants contribute more than 50% of all medicines used throughout the world. Medicinal plants are the most important source of life-saving medicines for the majority of the world's population (Thakur et al., 2009). Indonesia is a country that is rich in natural resources, especially medicinal plants. Various types of diseases can be controlled with traditional medicines derived from plants. Reliance on herbal concoctions made from plants such as pare hutan, the local name of *Momordica balsamina*, has become the impetus for scientific research into the biological effects that *Momordica balsamina* leaves extract may have (Mabasa et al., 2021).

According to research results, the leaves, fruit, seeds, and skin of this plant are known to have various medicinal and nutritional properties. The leaves and fruit extract of *Momordica balsamina* show antiplasmodial activity and are currently used as a malaria drug in Africa. The Leaves and stem extracts also exhibit antimicrobial and hypoglycemic effects. People in Borno state, Nigeria also use forest *Momordica balsamina* leaves as an antiseptic and treatment for stomach aches, in certain areas in South Africa, these leaves are also used as a medicine for sugar diabetes and chronic hypertension (Omokhua-Uyi

and Van Staden, 2020; Thakur et al., 2009; Mabasa et al., 2021; Mshelia et al., 2017; Faujdar et al., 2013; Ludidi et al., 2019).

Reviews based on the activity of *Momordica balsamina* show that this plant has other biological activities such as antimicrobial, antispasmodic, anti-inflammatory, analgesic, anti-HIV, anti-diahorrial, hepatoprotective, anti-malarial, antioxidant, anticancer and wound healing properties (Otimenyin et al., 2008; Serala et al., 2021; Abdulhamid et al., 2023; Agrawal et al., 2018). The main chemical groups of plant origin in *Momordica* species that are recognized as having potential health-promoting effects in diabetes are cucurbitane triterpenoids, saponin glycosides, and anti-HIV proteins (Nagarani et al., 2014). Dependence on herbal ingredients derived from plants, such as *Momordica balsamina*, has recently increased due to the severity and increasing burden of various diseases in humans. The continued use and overharvesting of this herb for medicinal purposes have led to the drive to scientifically validate the biological effects that this extract may exert (Nthulane et al., 2020).

Triterpenes are a large group of structurally diverse natural compounds that are biogenetically derived from isoprene with common structures such as pentacyclic-oleanane, ursane, taraxastane, lupane, and tetracyclic-dammarane and cucurbitane (Sticher, 2020). The medicinal value of this plant is because it contains several chemical compounds which are also known as plant bioactive compounds or secondary metabolite compounds. Secondary metabolites are compounds that have chemical components produced by plants from metabolic processes (Adamu et al., 2015).

A previous study reported that the most abundant compounds in *Momordica balsamina* L. leaves extract are alkaloids, followed by saponins, tannins, reducing compounds, and flavones. The research results also reported the presence of triterpenoid compounds, namely betulinic acid, from the ethyl acetate fraction of *Momordica balsamina* L. leaves taken from the Kano area, Nigeria (Karumi et al., 2004 & Kabir et al., 2021).

Due to the lack of information obtained regarding *Momordica balsamina* L. leaves compared with *Momordica charantia* L. that grow in Indonesia especially North Sulawesi, further research is needed to determine the chemical compounds contained therein (Pakaya et al., 2022; Widiyati et al., 2023). Therefore, this research aims to isolate and identify chemical compounds from the ethyl acetate fraction of pare hutan leaves that grow in the North Sulawesi area. For the isolation stage, extraction, fractionation, and chromatography methods are used, while for identification, proton nuclear magnetic resonance (¹H-NMR) and carbon nuclear magnetic resonance (¹³C-NMR) spectrometry are used.

Methods

The materials used are *Momordica balsamina* L. leaves taken from Rasi village, Ratahan District, Southeast Minahasa Regency, silica gel G60 F254 230-400 mesh (E. Merck 93852), Whatman 42 filter paper, 70% ethanol solvent, n-hexane, ethyl acetate, 86 | JIPI (Jurnal IPA dan Pembelajaran IPA), 8(1), p.85-93, (2024)

methanol. For thin-layer chromatography using aluminum silica gel G60 F254 plates (E. Merck 10554), as a staining agent a 10% H₂SO₄ solution in methanol was used.

The equipment used is a macerator, beaker, measuring cup, pipette, capillary tube, vial, chamber, vacuum, Buchner funnel, separation and purification using gravity column chromatography. For compound analysis, an NMR spectrometer was used. Other equipment that was also used in this research was a scale, fan, blender, aluminum foil, hot plate, tongs, fume cupboard, and a set of rotary evaporators.

Preparation and Extraction

Samples of pare hutan leaves that had been dried using a fan were ground using a blender and then macerated for 3 x 24 hours using 70% ethanol solvent to obtain a filtrate. The filtrate is then evaporated using a rotary evaporator to obtain a thick ethanol extract.

Separation and Purification of Compounds

The ethanol extract (50 g) was added to 50 g of silica gel and crushed, then partitioned repeatedly to separate the compounds based on the level of polarity starting from n-hexane, ethyl acetate, and methanol each 500 mL using a Buchner funnel and vacuum until a filtrate was obtained. then the filtrate is evaporated using an evaporator. The thick extract of the ethyl acetate fraction was then continued to be analyzed using TLC with several solvent volume ratios. The results of each TLC were then sprayed with 10% H₂SO₄ as a stain remover and heated with a hot plate. The solvent ratio that can produce a good separation pattern in TLC will be used as an eluent in gravity column chromatography.

For column chromatography, start with silica gel soaked with the eluent. Then the silica gel slurry is put into the column below which is filled with cotton. Insert slowly until it reaches $\frac{3}{4}$ of the column height, followed by adding 1 g of sample. The eluate from gravity column chromatography was collected in vials at 1 mL/vial and then analyzed using TLC. Each eluate that has the same R_f value is combined. If there are still several stain spots in the fraction being tested, column chromatography must be re-done, this stage is continued until a pure compound is obtained.

Compound Identification

The isolate was then identified using ¹H-NMR and ¹³C-NMR spectroscopy to see the number of protons and carbon contained in the isolate.

Results and Discussion

Sample Preparation and Extraction

The sample preparation results were 1.1 kg of dry powder. This process begins by drying 10 kg of fresh leaves using a fan in a room that is not exposed to direct sunlight to avoid the decomposition of the compounds contained in the sample, then grinding them using a blender to produce pare hutan leaf powder. 1.1 kg of pare hutan leaf powder was

macerated using 70% ethanol solvent for 3x24 hours and produced 12.736 liters of filtrate. The filtrate was then concentrated by evaporation using a rotary evaporator and produced 148 grams of thick ethanol extract of *Momordica balsamina* L. leaves.

Separation and Purification of Compounds

The first stage is to classify compounds based on their level of polarity, namely by carrying out fractionation. This process begins with 50 grams of thick ethanol extract of pare hitam leaves, crushed with 50 grams of G60 F254 silica gel then partitioned successively using the solvents n-hexane, ethyl acetate, and methanol each 500 mL to produce a filtrate from the n fraction. n-hexane 280 mL, ethyl acetate 390 mL, and methanol 490 mL. The filtrate was then evaporated to produce 1.89 g of n-hexane, 13.17 g of ethyl acetate, and 25.11 g of methanol.

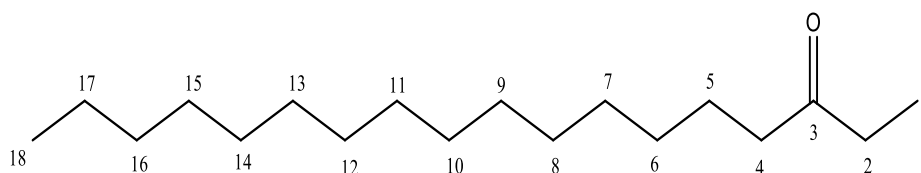
The second stage is gravity column chromatography (CC). CC was carried out using 1 g sample of ethyl acetate fraction extract, 27 g silica gel stationary phase, and ethyl acetate mobile phase (eluent): n-hexane (3:7) which had been analyzed using thin layer chromatography (TLC) which was able to provide a good separation pattern. The eluate produced in the KKG process was 706 vials which were then analyzed again using TLC. Based on the TLC results, eluates with the same *r_f* value were combined, evaporated then weighed and 3 fractions were obtained and analyzed again by TLC. The TLC results showed that F1, F2, and F3 still had some stains, which means there were no relatively pure compounds, so column re-chromatography was carried out on 160 mg of F1 using the eluent ethyl acetate: n-hexane (2:8) which had been analyzed by TLC. The eluate produced by F1 column re-chromatography was 700 vials. The eluates were then analyzed again using TLC and produced 9 fractions. The F1.6 fraction of 71.2 mg was again subjected to column chromatography using the eluent ethyl acetate: n-hexane (3:7) which had been analyzed and was able to provide a good separation pattern on TLC. The eluate obtained from F1.6 column re-chromatography was 770 vials. The eluates were then analyzed again using TLC and produced 18 fractions. The TLC results showed that the F1.6.2 fraction had one stain and it could be stated that the compound isolated from F1.6.2 was relatively pure. The relatively pure F1.6.2 fraction compound was then analyzed using TLC with several eluent comparisons to test its purity.

Nuclear Magnetic Resonance (NMR) Analysis

The Isolate F1.6.2 (2.4 mg) was then analyzed using ¹H-NMR and ¹³C-NMR with Chloroform-D (CDCl₃) solvent at a frequency of 500 MHz for protons and 125 MHz for carbon. The results of ¹H-NMR and ¹³C-NMR spectroscopy data are tabulated in the Table 1. Based on ¹H-NMR and ¹³C-NMR spectrum data, it shows the following signals: Signal at chemical shift (δC) 177.76 ppm indicates the presence of a C=O group, signal at (δC) 37.19 - (δC) 22, 79 ppm indicates an identical CH₂ group and is supported by a chemical shift (δH) 1.62 - (δH) 1.24 ppm, signals at (δC) 19.82 and (δC) 14.24 ppm indicate the presence of an identical CH₃ group and supported by chemical shifts (δH) 0.88 and (δH) 0.82 ppm. These signals indicate the presence of open-chain ketone compounds in isolate F1.6.2 (Rahelivao et al., 2017).

Table 1. ¹H-NMR and ¹³C-NMR Spectroscopic Data

Position	Fraction 1.6.2	
	δ H	δ C
1	0.82 (<i>d</i> , J = 12.5 Hz)	19.82
2	2.33 (<i>t</i> , J = 7.5 Hz)	33.71
3	-	177.76
4	1.24 (<i>s</i>)	37.19
5	1.62 (<i>d</i> , J = 7.5 Hz)	24.79
6	1.24 (<i>s</i>)	27.18
7	1.24 (<i>s</i>)	29.15
8	1.24 (<i>s</i>)	29.34
9	1.24 (<i>s</i>)	29.47
10	1.24 (<i>s</i>)	29.54
11	1.24 (<i>s</i>)	29.69
12	1.24 (<i>s</i>)	29.79
13	1.27 (<i>s</i>)	30.13
14	1.32 (<i>s</i>)	30.26
15	1.24 (<i>s</i>)	32.02
16	1.24 (<i>s</i>)	32.84
17	0.86 (<i>t</i> , J = 7 Hz)	22.79
18	0.88 (<i>s</i>)	14.24

**Figure 1.** Proposed structure of Isolate F1.6.2; Octadecan-3-one or 3-Octadecanone.

The chemical shift data in the comparative literature, as seen in Table 2 and Figure 2, has the same main chain as the isolate, but in the structure of the comparative literature there are methyl groups at C atom numbers 6, 10 and 17, whereas in the isolate F1.6.2 is missing. The compound (2*S*,4*R*,5*S*)-4-bromo-2-(*tert*-butyldimethylsilyloxy)-5-hydroxy-3-octadecanone shows a similar shift at C1, C-6 to C-18 with a range of 0.92 – 1.38 ppm at ¹H -NMR and range 18 – 31 ppm. Meanwhile, C-2 to C-5 have differences due to the functional group attached to the carbon atom. The compound (3*R*,5*S*)-3,5-dihydroxy-3,5-*O*-isopropylidene-2-octadecanone is also reported to have a similar range to (2*S*,4*R*,5*S*)-4-bromo-2-(*tert*-butyldimethylsilyloxy)-5-hydroxy-3-octadecanone (Esteve et al., 2011).

Table 2. The Comparison literature $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ Spectroscopic Data

Posisi	6,10,17-trimethyl-2-octadecanone (Sobeh, et al., 2016)	
	δH	δC
1	2.06, s	29.99
2	-	209.5
3	2.33, t, (J = 7.8)	44.3
4	1.48, m	21.59
5	-	36.64
6	1.31, m	32.94
7	-	37.38
8	1.12, m	24.57
9	-	37.43
10	1.19, m	32.82
11	-	37.55
12	-	29.85
13	-	29.85
14	-	29.85
15	1.22, m	24.94
16	1.07, m	39.51
17	1.45, m	28.13
18	1.00, d, (J = 6.6)	22.77
19	1.00, d, (J = 6.6)	22.87
20	0.79, d, (J = 6.6)	19.73
21	0.77, d, (J = 6.6)	19.89

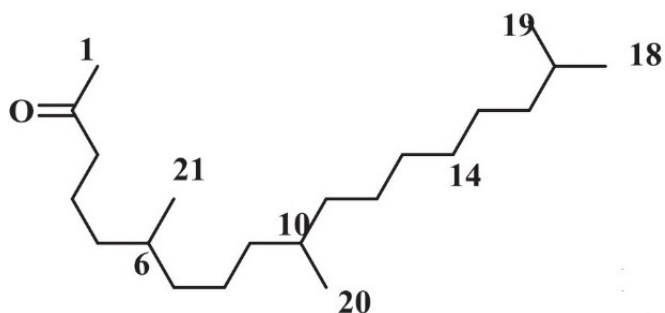


Figure 2. Structure of 6,10,17-trimethyl-2-octadecanone

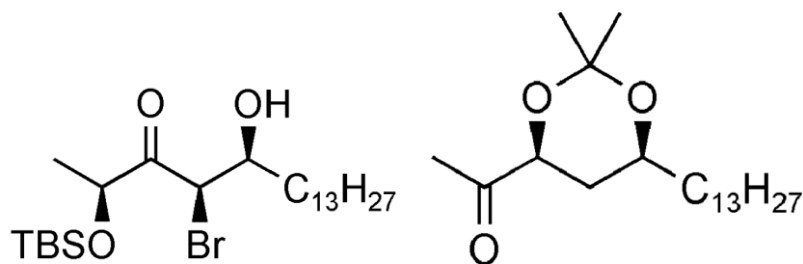


Figure 3. (2S,4R,5S)-4-bromo-2-(tert-butyldimethylsilyloxy)-5-hydroxy-3-octadecanone and (3R,5S)-3,5-dihydroxy-3,5-O-isopropylidene-2-octadecanone.

Conclusion

Isolate F1.6.2 of the ethyl acetate fraction of *Momordica balsamina* L. leaves shows the suspected presence of a ketone compound with the molecular formula $C_{18}H_{36}O$ and the proposed name octadecan-3-one or 3-octadecanone.

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