Acta Scientific NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 7 Issue 8 August 2023

Research Article

Gibberellin of GA-4 from Bitter Gourd (*Momordica charantia*)

Dine Agustine[1], Iman Permana Maksum[1], Ika Wiani[1], Safri Ishmayana[1], Reginawanti Hindersah[2] and Ukun MS Soedjanaatmadja[1]*

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, West Java, Indonesia

[2]Department of Soil Science and Resources, Faculty of Agriculture, Universitas Padjadjaran, West Java, Indonesia

***Corresponding Author:** Ukun MS Soedjanaatmadja, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, West Java, Indonesia.

DOI: 10.31080/ASNH.2023.07.1285

Received: June 27, 2023

Published: July 24, 2023

Abstract

Gibberellin is one of phytohormone which has function to stimulate plant growth, especially for accelerating the growth of shoot and fruiting of plants. Gibberellin (GAs) are very important group of phytohormones involved in seed germination, vegetative growth, flowering and fruit development, being only four of the 136 known have more bio-active activity than other, they are GS-1, GA-3, GA-4 and GA-7. The objective of study is to isolate and identify gibberellin of GA-4 from bitter gourd (*Momordica. Charantia*), from Majalengka of West Java-Indonesia Plantation. Isolation and purification of GA-4 were carried out by thin-layer chromatography, adsorption column chromatography on Silica Gel G-60. while characterization of GA-4 were carried out by Reversed Phase High Performance Liquid Chromatography (RP-HPLC), Fourier Transform Infrared (FT-IR), and Mass Spectroscopy (MS). The results of RP-HPLC and FTIR of gibberelline of GA-4 isolates showed a retention time and wave number which was relatively similar to the GA-4 standard. From the result of MS analysis, the mass spectrum shows the peak of the molecular ion [M+H] + at m/z 355.1501 as the measured mass, and the calculated mass is 355.1521 with the molecular formula of C19H24O5Na, and the degree of saturation or DBE is 7. The measured molecular mass and the number of DBEs correspond to the molecular mass of GA-4. From the RP-HPLC found that GA-4 content in *M.charantia* was 6.06 mg/g dry weight.

Keywords: Bitter Gourd; Gibberellin; *Momordica Charantia*; Phytohormone; Soybean

Introduction

Bitter gourd plant (*M. charantia* L) is a horticultural plant known as bitter gourd, bitter melon and bitter squash which belongs to the Cucurbitaceae family which has a characteristic bitter taste. Behind the bitter taste, bitter melon plants have many properties and benefits both in terms of health and as a potential source of food [1]. Bitter melon can grow well and is widely cultivated in tropical and sub-tropical regions [2]. Bitter gourd is very easy to find and is found in almost all regions in Indonesia and has many local names, for example in Java it is known as paria or pare, in Sumatra it is known as prieu, fori, prepare, kambeh, pare; in Nusa Tenggara they are called paya, truwuk paitap, paliak, pariak, pania and pepule while in Sulawesi they are called poya, pudu, pentum [3].

The various benefits of bitter melon are closely related to the substances or compounds they contain. The Momordica genus is one of the genera rich in the diversity of secondary metabolites such as terpenoids, diterpenoids, flavonoids and other phenolic derivatives. The content of secondary metabolites from this genus has various biological activities [4]. More than 90 terpenoids type cucurbitins have been isolated from fruits, seeds, roots, stems and leaves [5]. Some of these chemical compounds are biologically active and others may become target molecules for future research.

According to Hopkins [6], gibberellin plays an important role in seed germination and mobilizes food reserves contained in the endosperm during the early growth of the embryo. The mobilization is regulated by several hydrolytic enzymes, especially the α-amylase enzyme. Gibberellin can control the germination of seeds of various types of plants in nature and can replace the role of light and temperature in increasing germination. One of the growth regulators that can trigger germination and then growth is gibberellin which plays a role in cell wall development, cell enlargement, root growth, flowering induction and cell division. Gibberellin will play a role in the germination phase where the formation of α-amylase enzymes occurs in the condition of the aleurone layer, affecting the elongation of plant segments, with an increase in the number and size of cells in these segments.

Gibberellin is a phytohormone that regulates various aspects of plant growth and development in the entire plant life cycle, such as cell division and elongation, seed germination, seed elongation, stem and hypocotyl elongation. Gibberellins also regulate plant adaptation to biotic and abiotic stresses. However, Among the more than 130 GAs that have been identified from plants, fungi, and bacteria, only a few types of gibberellins, such as GA1, GA3, GA4, and GA7, have biological activity as plant growth and development regulators [7,8]. According to the research of Castro-Camba., *et al*. [9], there is Interactions of Gibberellins with Phytohormones and Their Role in Stress Responses

Indonesia is one of the countries with abundant biological natural resources which has the potential to produce secondary metabolites and has the potential to be developed as a source of biostimulants. Its application aims to increase the efficiency of absorption of nutrients, abiotic stress tolerance improves crop quality [10]. Currently, biostimulants are widely used to increase plant growth, especially horticultural crops and some food crops [11]. The bio-active GAs commonly found in plants are GA1 and GA4, both of which have a hydroxyl group at C-3β, a carboxyl group attached to C-6, and a γ-lactone between C-4 and C-10 [12].

The gibberellin (GA-3) content in the bitter melon fruit and seeds is known to be 4.185 mg/g wet weight. In a study conducted by Tang., *et al*. [13], isolated endogenous hormones from bitter melon plant pistils. Gibberellins promote germination by promoting the secretion of hydrolytic enzymes and weakening the seed coat tissue, as well as promoting cell division and elongation, and gibberellin also recovers seed germination in rice with impaired brassinosteroid signaling [14].

In this study, isolation, purification and characterization of gibberellin, especially GA-4, from bitter melon (M. charantia) will be carried out.

Materials and Methods

Materials: The fruit of bitter melon (M. charantia) which was obtained from a bitter melon plantation in Cipaku Village, Majalengka District, West Java.-Indonesia.

The research method consisted of five stages, including: (1) preparation of bitter melon extract using maceration and evaporation methods, (2) extraction and purification of bitter melon extract (3) separation of compounds using analytical thin layer chromatography, adsorption column chromatography and preparative thin layer chromatography methods. (4) characterization of the isolate by high performance liquid chromatography (HPLC) method with reversed phase C-18 Nucleosyl ODS column, UV detector with λ = 260 nm, analysis with FTIR and determination of molecular mass with Mass Spectroscopy.

The instruments used in this study were glass tools, seeding box, desiccator, thin layer chromatography, vacuum evaporator (Buchi), HPLC (Alltech 8011/2), glass column, C-18 nucleosil ODS column, biolight fluorescence lamp, analytical balance, pH meter, polybag, FTIR (Shimadzu 8400), mass spectrometer (Waters, Xevo Q-Tof MS) and furnace (Thermolyne).

Preparation of extract: First, remove all the dirt on the bitter melon fruit (M. charantia), then wash thoroughly and dry it. Furthermore, the dried fruit were milled to pieces, and 200 grams of the fruit were macerated with 1200 mL methanol for 4 × 24 hours at room temperature. The methanol extract was collected and concentrated with a vacuum evaporator at a temperature below 40°C until concentrate was obtained. Then the concentrate was dissolved with distilled water to 100 mL to obtain a crude extract.

Extraction and partial purification: As much as 25 mL of the crude extract was alkalized with 5% sodium bicarbonate (W/V) solution to pH 8 and extracted with ethyl acetate (40 mL × 2). The water layer was then acidified with 3 N hydrochloric acid to pH 2.5 and then extracted again with ethyl acetate (20 mL × 2). The ethyl acetate fraction was evaporated to dryness in a vacuum evaporator. Than the residue was dissolved in 5 mL of methanol to obtain methanol extract.

Analytical Thin Layer Chromatography (ATLC): Analytical thin-layer chromatography was performed using plates coated with silica gel GF-254. This plate was cut to the size of 6 cm × 2 cm lower and the upper border was marked 0.5 cm of each end. As many as 3-4 drops of methanol extract were placed with a capillary tube on the lower mark and standard (Gibberellin GA-4) was placed next to it. The plate was eluted with a mixture of ethyl acetate: methylene chloride (6:4) until the eluent move to the upper limit. The result was observed under UV light at a wavelength of 254 nm.

Identification and characterization of GA-4 isolates by Reversed-Phase HPLC, FTIR, and MS: A total of 10 µL fraction containing gibberellin GA-4 which had been purified by preparative TLC, was analyzed by Reversed-Phase (RP) HPLC; using Alltec 8011/2 (C-18 nucleosil ODS column) with UV detector at a wavelength of 254 nm. Analiyzed by FTIR (Shimadzu 8400), and analyzed by Mass Spec. MS (Waters type Xevo Q-Tof MS).

The buffer elution for RP HPLC was performed with an isocratic method using 35% methanol in acetate buffer (pH 3.5) with a flow rate of 0.7 mL/minute. The RP HPLC, FTIR, and MS results were compared to the GA-4 standard.

Results

Analytical Thin Layer Chromatography: Analytical thin layer chromatography was performed to determine the standard Rf. This

Rf value is used to determine the right solvent mixture to purify gibberellin GA-4 compounds. Thin-layer chromatography belongs to solid-liquid chromatography, which in this study silica gel GF-254 was used as the stationary phase and the solvent used was a mixture of ethyl acetate: methylene chloride (6:4) as the mobile phase. Silica gel GF-254 is used because gibberellin has a natural chromophore group so that the use of silica gel GF-254 can provide fluorescence at wavelength 254 [15]. After the calculation, it was determined that the standard Rf value was 0,785, which this value stated that the solvent used was good enough. Compared with an GA-4 standard that was indicates that there is an gibberellin GA-4 compound in the methanol fraction. So it is necessary to further purify it by adsorption column chromatography.

Figure 1: Results of analytical thin layer chromatography on the silica gel GF-254, using a solvent system of chloroform: ethyl acetate: glacial acetic acid (6: 14: 1) from crude extract (1), methanol extract sample (2), and GA-4 standard (3) under UV lamp at λ = 254.

Adsorption column chromatography

Fractions of 2 mL were collected and analyzed for their content using analytical TLC and the result is presented in Figure 1. The fractions that have a spot with Rf value similar to Rf of GA-4 standard were the fractions of 17 to 21.. This indicates that the fraction contains gibberellin (GA-4) compounds. The research was continued with a preparative thin-layer chromatography (TLC) procedure to obtain purer GA-4 compounds.

Preparative Thin Layer Chromatography

Figure 2 shows preparative TLC plate observed under UV light at wavelength 254 nm. Then the parallel stains were scraped off and suspended in methanol. After that, the mixture was centrifuged and decanted to separate the isolates obtained from the KLTP results. The results obtained are concentrated with a vacuum evaporator. The residue was dissolved in 1 mL of methanol for further analysis by reversed-phase high performance liquid chromatography (RP-HPLC) to obtain the concentration of the gibberellin (GA-4) compound.

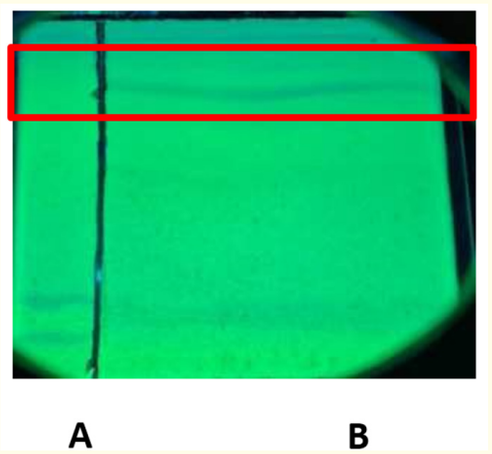

Figure 2: TLC results of Preparative Thin Layer Chromatography on the silica gel GF-254 stationary phase; A: the standard of GA-4, B: the GA-4 isolate from bitter melon. Mobile phase; chloroform-ethyl acetate-acetic acid (6: 14: 1), under ultraviolet light spotting.

Identification of gibberellin GA-4 sample isolates by Reversed-Phase HPLC

The results of GA-4 analysis of the GA-4 standard and GA-4 sample with HPLC are shown in figure 3 and figure 4.

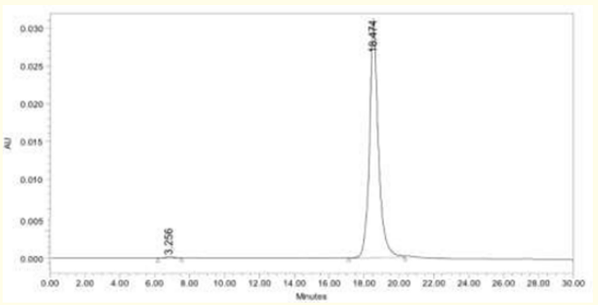

Figure 3: The result of RP-HPLC of the GA-4 standard using a reversed phase ODS Nucleosyl C-18 column, and a UV detector with λ = 254 nm. The mobile phase was 35% methanol in acetate buffer pH 3.5 at a flow rate of 0.7 mL/min.

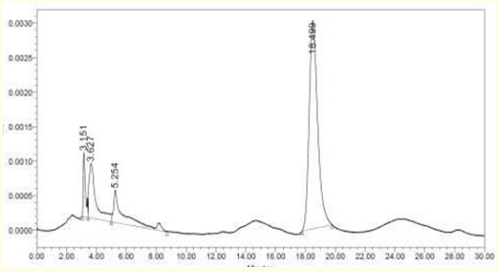

Figure 4: The result of RP-HPLC of the GA-4 isolate sample using a reversed phase ODS Nucleosyl C-18 column, and a UV detector with λ = 254 nm. The mobile phase was 35% methanol in acetate buffer pH 3.5 at a flow rate of 0.7 mL/min.

In the figure 3 shows that at the RP-HPLC retention time of gibberellin GA-4 standar was 18,474 minutes, and the peak indicates that the GA-4 standard used is 100% pure. When compared with the results of the gibberellin (GA-4) sample in the figure 4, there is one dominant peak with a retention time of 18,499 minutes which has a peak and time that is relatively the same as the GA-4 standard. This a little difference in retention time could be caused by the presence of other peaks that caused a shift.

Characterization of gibberellin GA-4 sample isolates by FT-IR

The results of GA-4 analysis of the GA-4 standard and GA-4 sample with FT-IR spectrophotometer are shown in figure 5 and figure 6.

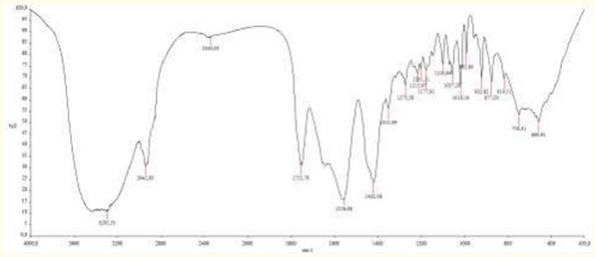

Figure 5: FT-IR spectrum absorption peak of GA-4 standard.

FT-IR spectrum data of the GA-4 standard in Figure 5 show the presence of several absorption peaks. The -OH group gives a strong absorption at wave number 3295.53 cm-1, -RCOOH at wave number 2942.93 cm-1, C = O at wave number 1757.79 cm-1, and C = C at wave number 1556 .08 cm-1.

FT-IR spectrum data of the GA-4 isolate sample in Figure 6 shows a stretching vibration that extends from the OH group at wave number 3408 cm-1. This confirms the presence of the hydroxyl and carboxyl groups of GA-4. The weak stretching vibration of C-H sp3 is shown at wave number 2932 cm-1. While the stretching vibration

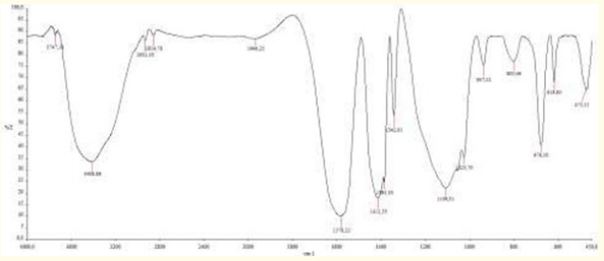

Figure 6: FT-IR spectrum absorption peak of GA-4 isolate sample.

C = C is shown at wave number 1578 cm-1. Then the presence of a methylene group (-CH2-) is indicated at wave number 1412 cm-1.

Characterization of gibberellin GA-4 sample isolates by Mass-spec.

The results of GA-4 sample isolate by Mass Spec., is shown in Figure 7.

The presence of GA-4 in bitter melon isolates was also confirmed from the results of mass spectrum measurements. The mass spectrum shows the peak of the molecular ion [M+H]+ at m/z 355.1501 as the measured mass and the calculated mass is 355.1521 with the molecular formula C19H24O5Na, and the degree of saturation or DBE is 7. The measured molecular mass and the number of DBEs correspond to the molecular mass of GA-4.

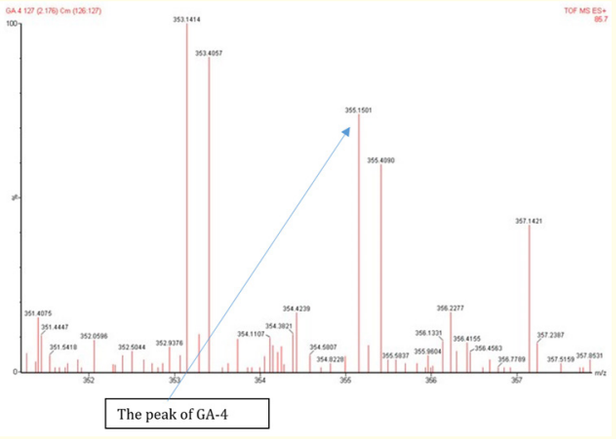

Figure 7: Mass spectrum of GA-4 isolate (GA-4 [M+Na] + = 355.1501).

From the results of analysis using RP-HPLC, FT-IR, and Mass Spec. it can be proven that the bitter gourd (M. charantia) sample isolates contain the gibberellin compound of GA-4.

Discussion

In plants, gibberellins (GAs) play important roles in regulating growth and development. Early studies revealed the large chemodiversity of gibberellins in plants, but only GA-1, GA-3, GA-4, and GA-7 show biological activity that controls plant development [16]. Isolation and characterization of gibberellin GA-4 from the bitter gourd fruit (M. charantia) was an innovative result. Previous research on gibberellin in bitter gourd was limited to the type of gibberellin GA-3, while research on the content of gibberellin GA-4 has not been carried out by previous researchers. In a study conducted by Tang., *et al*. [13], obtained a gibberellin hormone (GA_3) content was 1.06x10-3 mg/g, which was successfully isolated from bitter melon pistils (M. charantia). Research result of Susilo., *et al*. [17] obtained that GA-3 from fruit and seeds of bitter gourd was 4.185 mg/g wet weight, and from the research results of Nindita [18], obtained GA-3 from bitter melon seeds was 2.58 mg/g dry weight

Identification of GA-4 standard using RP-HPLC resulted in a chromatogram showing the dominant peak of standard GA-4 at a retention time of 18.474 minutes. Chromatogram from RP-HPLC of GA-4 isolates from bitter melon had a dominant peak at a retention time of 18.499 minutes. Compared with the results of the GA-4 standard, the peaks were relatively the same. And according to the comparison of the retention time of the GA-4 standard and the GA-4 sample states that the isolated sample contains GA-4 compound with a purity level of 68.46%. This difference in retention time could be due to the presence of impurities or other components detected in the isolate. By comparing the sample peak area and the standard peak area multiplied by the standard concentration used for RP-HPLC, obtained the GA-4 concentration in the bitter gourd (M.charantia) sample was 6.06 mg/g dry weight.

Conclusion

Isolation and characterization of gibberellin (GA-4) from the bitter gourd (M. Charantia) were carried out by thin-layer chromatography, adsorption column chromatography, RP-HPLC), FT-IR and MS, were found the good result. The results of HPLC and FTIR of gibberelline of GA-4 sample isolates showed a retention time and wave number which was relatively similar to the GA-4 standard. The mass spectrum shows the peak of the molecular ion [M+H]+ at m/z 355.1501 as the measured mass, and the calculated mass is 355.1521 with the molecular formula C19H24O5Na, and the degree of saturation or DBE is 7. The measured molecular mass and the number of DBEs correspond to the molecular mass of GA-4. From the RP-HPLC found that GA-4 content in *M.charantia* was 6.06 mg/g dry weight.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors are grateful for the Laboratory facilities in Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Padjadjaran and to the Executive Board of Universitas Padjadjaran, Dean of the Faculty of Mathematics and Natural Sciences, Head of the Department of Chemistry, Head and the entire staff of Biochemistry Laboratory. We would like also to thank to all those who have given advice, motivation and materially support.

Bibliography

1. Subahar and Tati SS. "Efficacy and Benefits of Disease Exterminator Bitter Gourd: Healthy with Traditional Herb" First ed. PT Agromedia Pustaka, Jakarta (2004).

2. QH Ipaulle and Dody Kastono. "The effects of Main Stem and Primary Branches Pruning on The Yield and Quality of Bitter Gourd (*Momordica charantia* L.)" Vegetalika 9.3 (2020): 474-487.

3. Susilawati and Hermansyah. "Antiplasmodium Potential Test of Bitter Gourd Extract (*Momordica charantia* L) against Plasmodium falcifarum". Molekul 9.1 (2014): 13-17.

4. Suleman Duengo and Weny JA Musa. "Phytochemical Screening of Methanol Extract in Bitter Gourd (*Momordica charantia* L)". *Enthropi Journal* 11 (2016): 223-225.

5. Chiy-Rong Chen., *et al*. "Cucurbitane-Type Triterpenoids from *Momordica charantia*". *Natural Product Communications* 12. 6 (2017): 889-892

6. Hopkin WG." Introduction to Plant Physiology". John Wiley and Sons, Inc. Singapore (1985).

7. Gao X., *et al*. "Hormon Metabolism and Signaling in Plants: Gibberellins". Shanghai: Elsevier Ltd. (2017).

8. Binenbaum J., *et al*. "Giberelin localization and transport in plants". *Trends in Plant Science* 23.5 (2018): 410-421.

9. Castro-Camba R., *et al*. "Interactions of Gibberellins with Phytohormones and Their Role in Stress Responses". *Horticulturae* 8 (2022): 241.

10. Du Jardin P. "Plant Biostimulants: Definition, Concept, Main Categories, and Regulation". *Scientia Horticulturae* 196 (2015): 3-4.

11. Calco P., *et al*. "Agricultural Uses of Plant Biostimulants. Plant Soil". Canada: Departement of Biology, University of British Columbia (2014).

12. Buchanan BB., *et al*. "Biochemistry and Molecular Biology of Plants". Second Edition. John Wiley and Sons, Ltd. Chichester (2015).

13. Tang Y., *et al*. "The use of HPLC in determination of endogenous hormones in anthers of bitter melon". *Journal of Life Sciences* 5 (2011): 139-142.

14. Li QF., *et al*. "Gibberellin recovers seed germination in rice with impaired brassinosteroid signalling". *Plant Science* 293 (2020): 110435.

15. Lu Q., *et al*. "Extraction and Analysis of Auxin in Plants Using Dispersive Liquid-Liquid Microextraction Followed by High-Performance Liquid Chromatography with Fluorescence Detection". *Journal of Agricultural and Food Chemistry* 59 (2010): 2763-2770.

16. Juan He., *et al*. "Gibberellin Metabolism in Flowering Plants: An Update and Perspectives". *Frontiers in Plant Science* 11 (2020): 1-6.

17. Susilo WA., *et al*. "Activity of Gibberellin from *Momordica charantia*". Proceeding of The International Seminar on Chemistry. Jatinangor (2009): 697.

18. Nindita A. "Isolation of the Phytohormone Gibberellins from Bitter Gourd Seeds (*Momordica charantia*) as a Biostimulant on Rice Plant Growth (Oryza sativa). Thesis". Padjadjaran University. Sumedang (2014).