TRITERPENOIDS FROM THE FRUITS OF BITTER MELON AND THEIR POTENTIAL ANTI-INFLAMMATORY PROPERTIES

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Abstract: *Bitter melon (Momordica charantia) is a traditional medicinal and functional food highly valued for its diverse pharmacological properties, including anti-inflammatory, anti-diabetic, and antioxidant effects. Phytochemical studies of the fruits of M. charantia led to the isolation of three triterpenoid saponins (1-3). Their structures were elucidated using spectroscopic data, including HR-ESI-MS, 1D and 2D NMR, as well as comparison with reference compounds. In particular, their compounds were identified as 3β-malonyl-7β,23-dimethoxycucurbita-5,24-dien-19-al (1), 3β-malonyl-7β-hydroxy-25-methoxycucurbita-5,23-dien-19-al (2), and 3β-malonyl-7β,25-dihydroxycucurbita-5,23-dien-19-al (3), respectively. To evaluate the anti-inflammatory potential of these substances, the production of pro-inflammatory cytokines—IL-6, IL-12p40, and TNF-α—was tested in LPS-stimulated bone marrow-derived dendritic cells (BMDCs). The data showed that the activity of compound 1 was higher than that of the selected drug adezmapimod, which served as the positive control. The findings suggest the potential in vitro anti-inflammatory effects of secondary metabolites from M. charantia.*
Keywords: *Triterpenoid; Anti-inflammatory effect; Momordica charantia; Bitter melon.*

1. Introduction

Inflammation is a fundamental biological response to infection, injury, or stress, characterized by the activation of immune cells and the release of pro-inflammatory mediators such as cytokines, chemokines, and reactive oxygen species (Van Thanh et al., 2019, Vinh et al., 2017). While acute inflammation plays a protective role in host defense and tissue repair, chronic or uncontrolled inflammation is implicated in the pathogenesis of numerous diseases, including autoimmune disorders, metabolic syndrome, neurodegenerative diseases, and cancer (Duyen et al., 2022, Gao et al., 2020, Giang et al., 2022). Consequently, the discovery of effective anti-inflammatory agents remains a major focus in pharmaceutical and biomedical research.

Natural products derived from edible and medicinal plants have garnered increasing attention in food chemistry and drug discovery due to their structural diversity, biological activity, and relatively low toxicity (Cao et al., 2022, Tuan Anh et al., 2021). Compared to synthetic drugs, natural compounds often exhibit better biocompatibility and fewer side effects, making them suitable candidates for long-term use in chronic conditions. Investigating the pharmacological properties of these bioactive constituents not only enhances our understanding of traditional medicine but also supports the integration of natural product-based interventions into modern healthcare strategies (Vinh et al., 2019a, Vinh et al., 2019b).

Bitter melon (*Momordica charantia*), a member of the Cucurbitaceae family, has been widely recognized as both a traditional medicinal herb and a functional food across Asia, Africa, and South America (Hu et al., 2024). *M. charantia* has been used in traditional medicine to manage conditions such as diabetes, gastrointestinal disorders, and inflammatory diseases (Zhang et al., 2025). Moreover, numerous studies have shown that extracts from *M. charantia* exhibit a range of pharmacological activities, including anti-inflammatory, anti-diabetic, anti-cancer, and antioxidant effects (Oyelere et al., 2022). These biological properties are primarily attributed to its

diverse secondary metabolites, such as triterpenoid saponins, flavonoids, alkaloids, and phenolic compounds (Oyelere et al., 2022). Among them, triterpenoids, polysaccharides and saponins are considered key bioactive constituents with notable anti-inflammatory and immunomodulatory potential (Bora et al., 2023). Previous studies have demonstrated that methanol extracts of *M. charantia* fruits exhibit significant inhibitory effects on the production of pro-inflammatory cytokines and reactive oxygen species *in vitro* (Cao et al., 2021). However, detailed studies on the isolation and characterization of individual compounds, as well as the elucidation of their molecular mechanisms in immune cells, remain limited. Therefore, the present study aims to isolate the major constituents from the methanol extract of bitter melon and evaluate their potential anti-inflammatory activities using *in vitro* immune cell models.

2. Research overview

M. charantia, commonly known as bitter melon, has long been utilized in traditional medicine and as a functional food for its wide-ranging health benefits (Oyelere et al., 2022). Numerous studies have documented the pharmacological effects of its extracts and isolated compounds, particularly with regard to anti-diabetic, anti-inflammatory, and antioxidant activities. Among its chemical constituents, triterpenoid saponins, flavonoids, and alkaloids have demonstrated promising biological effects through various mechanisms, such as modulation of glucose metabolism, suppression of pro-inflammatory cytokines, and scavenging of reactive oxygen species (Bora et al., 2023). Despite these encouraging findings, the full therapeutic potential of *M. charantia* remains underexplored. Many secondary metabolites isolated from its fruits and other parts have yet to be thoroughly characterized, and their mechanisms of action in relevant disease models are still poorly understood. Therefore, continued investigation into the bioactive components of *M. charantia* is essential to uncover novel compounds and validate their efficacy and safety for future therapeutic applications.

3. Material and methods

3.1 General experimental procedures

The experimental procedures closely followed those of our prior studies (Hieu et al., 2024, Liu et al., 2023, Minh et al., 2024). NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer. Tetramethylsilane (TMS) was used as the internal standard, and all chemical shifts (δ) are reported in parts per million (ppm), with coupling constants (J) given in hertz (Hz). Structural elucidation was further supported by two-dimensional NMR experiments, including HSQC and HMBC. High-resolution electrospray ionization mass spectrometry (HRESIMS) data were obtained using a Thermo Fisher LC-LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Palo Alto, CA, USA) in positive ion mode. For compound isolation, open column chromatography (CC) was carried out using C18 and silica gel, respectively. Thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60 F254 plates (1.05554.0001), Sephadex LH-20 (GE Healthcare Bio-Sciences AB), and RP-C18 F254S plates (1.15685.0001). Visualization of compounds on TLC plates was achieved under UV light at 254 and 365 nm, followed by spraying with 10% (v/v) aqueous sulfuric acid and subsequent heating to enhance spot detection.

3.2 Sample selection for study

The fruits of *M. charantia* were collected in Me Linh, Hanoi, Vietnam, in May 2023, and taxonomically identified by Dr. Nguyen Cao Cuong (Faculty of Pharmacy and Medicine, University of Dalat, Lam Dong). A voucher specimen (Code: MDR 01) has been deposited in the botanical collection of Faculty of Pharmacy, Thanh Do University, Kim Chung, Hoai Duc, Ha Noi, Viet Nam.

3.3 Purification process

Dried fruits of *M. charantia* (4.5 kg) were extracted three times with ethanol (5 L each) via sonication for 3 hours per extraction. The combined ethanol extracts were concentrated under reduced pressure to yield a crude residue (1200 g). This residue was suspended in distilled water (1.5 L) and sequentially partitioned with *n*-hexane (2 × 1.5 L), dichloromethane CH_2Cl_2 (2 × 1.5 L), and ethyl acetate (EtOAc, 2 × 1.5 L), resulting in three fractions: *n*-hexane (H, 220 g),

CH_2Cl_2 (D, 17 g), EtOAc (E, 31 g), and an aqueous layer.

The CH_2Cl_2-soluble fraction (D, 17 g) was subjected to vacuum liquid chromatography (VLC) on silica gel, using a gradient of MeOH in CH_2Cl_2 (0–100%) to obtain seven subfractions (D1–D7). Fraction D3 (4 g) was further chromatographed on silica gel using CH_2Cl_2–MeOH (25:1, v/v) to yield five subfractions (D3.1–D3.5). Subfraction D3.3 (2.8 g) was subjected to reversed-phase column chromatography on YMC RP-18 and Sephadex LH-20 using acetone–water (4:1, v/v) as the eluent, resulting in the isolation of compounds 1 (2.2 mg), 2 (6.3 mg), and 3 (5.8 mg).

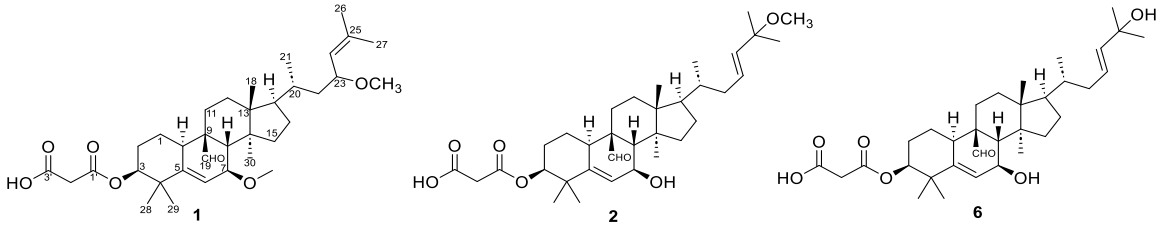
3.4 Cell Culture and Cytokine Assay

The anti-inflammatory effects were carried out similarly to a previous study, with slight modifications (Vinh et al., 2020a, Vinh et al., 2020b). Briefly, BMDCs were seeded into 48-well plates at a density of 1×10^5 cells per 0.5 mL and pretreated with the test compounds at specified concentrations for 1 h. Thereafter, the cells were stimulated with 10 ng/mL lipopolysaccharide (LPS) derived from *Salmonella minnesota* (Alexis, New York, USA). After 18 h of stimulation, supernatants were collected, and the levels of pro-inflammatory cytokines—IL-12p40, IL-6, and TNF-α—were quantified using enzyme-linked immunosorbent assay (ELISA; BD PharMingen, San Diego, CA, USA), following the manufacturer's instructions. All experiments were independently repeated at least three times, and results are expressed as mean ± standard deviation (SD).

4. Results

The dried fruits of *M. charantia* were extracted with EtOH. The crude extract was subsequently partitioned with *n*-hexane, dichloromethane (CH_2Cl_2), and ethyl acetate (EtOAc) against water to yield the corresponding fractions: H (*n*-hexane), D (CH_2Cl_2), and E (EtOAc). Using a combination of chromatographic separation techniques, three triterpenoids (1–3) were successfully isolated from the dichloromethane (D) fraction. Their structures were elucidated through the analysis of 1D and 2D NMR as well as HR-ESI-MS. The isolated compounds were identified as 3*β*-malonyl-7*β*,23-dimethoxycucurbita-5,24-dien-19-al (1), 3*β*-malonyl-7*β*-hydroxy-25-methoxycucurbita-5,23-dien-19-al (2), and 3*β*-malonyl-7*β*,25-dihydroxycucurbita-5,23-dien-19-al (3), respectively. Additionally, detailed NMR data for these triterpenoids are reported here.

Fig.1. Isolated compounds 1–3 isolated from the fruits of *M. charantia*



4.1 3β-malonyl-7β,23-dimethoxycucurbita-5,24-dien-19-al (1)

White amorphous powder, ^{1}H NMR (600 MHz, MeOD): δ_H (ppm): 1.40 (H-1), 1.30 (H-2), 1.93 (H-3), 4.90 (H-4), 5.93 (H-5, br d, J = 4.0 Hz), 3.50 (H-6, d, J = 6.8 Hz), 2.06 (H-7, s), 2.53 (H-8, dd, J = 13.5, 4.0 Hz), 2.40 (H-9, td, J = 14.5, 5.5 Hz), 1.41 (H-10, m), 1.63 (H-11, dd, J = 4.0, 14.0 Hz), 1.32 (H-12, m), 1.90 (H-13, m), 1.50 (H-14, t, J = 10.0 Hz), 0.89 (H-15, s), 9.77 (H-16, s), 1.35 (H-17, m), 0.92 (H-18, d, J = 6.0 Hz), 1.51 (H-19, t, J = 10.0 Hz), 1.34 (H-20, m), 3.92 (H-21, td, J = 4.0, 9.5 Hz), 4.92 (H-22, br s, d), 1.71 (H-23, br d, J = 1.0 Hz), 1.78 (H-24, br d, J = 1.0 Hz), 1.13 (H-25, s), 1.19 (H-26, s), 0.77 (H-27, s), 3.26 (H-28, s), 3.22 (H-29, s), 3.38 (H-30, s). ^{13}C NMR (150 MHz, MeOD): δ_C (ppm) 21.4 (CH_2, C-1), 27.9 (C-2), 80.2 (C-3), 40.5 (C-4), 145.8 (C-5), 121.4 (C-6), 75.5 (C-7), 44.6 (C-8), 49.8 (C-9), 35.8 (C-10), 22.6 (C-11), 29.2 (C-12), 45.8 (C-13), 47.6 (C-14), 34.8 (C-15), 26.2 (C-16), 50.5 (C-17), 14.6 (C-18), 207.5 (C-19), 33.2 (C-20), 19.5 (C-21), 42.3 (C-22), 75.8 (C-23), 126.1 (C-24), 136.8 (C-25), 18.6 (C-26), 25.8 (C-27), 26.6 (C-28), 24.8 (C-29), 18.3 (C-30), 55.6 (CH_3-1), 56.2 (CH_3-2), 166.9 (C-31), 42.3 (C-32), 167.5 (C-33).

4.2 3β-malonyl-7β-hydroxy-25-methoxycucurbita-5,23-dien-19-al (2)

White amorphous powder, ^{1}H NMR (600 MHz, MeOD): δ_H (ppm): 4.83 (br s, H-3), 5.90 (s), 4.09 (d, J = 4.5 Hz, H-7), 2.02 (s, H-8), 2.56 (d, J = 12.0 Hz), 2.30 (td, J = 14.5, 9.5 Hz, H-), 1.51 (m), 1.64 (m), 1.39 (d, J = 8.5 Hz), 1.89 (s), 1.49 (s), 0.87 (s), 9.72 (s), 1.54 (m), 0.90 (d, J = 6.5 Hz), 2.18 (d, J = 10.5 Hz), 1.77 (m), 5.49 (m), 5.38 (d, J = 15.5 Hz), 1.24 (s), 1.24 (s), 1.09 (s), 1.17 (s), 0.73 (s), 3.13 (s), 3.33 (s). ^{13}C NMR (150 MHz, MeOD): δ_C (ppm) 21.6 (C-1, CH_2), 25.5 (C-2, CH_2), 79.5 (C-3, CH), 40.4 (C-4, C), 145.3 (C-5, C), 122.9 (C-6, CH), 66.3 (C-7, CH), 49.2 (C-8, CH), 49.6 (C-9, C), 35.5 (C-10, CH), 22.6 (C-11, CH_2), 28.3 (C-12, CH_2), 45.4 (C-13, C), 47.7 (C-14, C), 34.7 (C-15, CH_2), 27.5 (C 16, CH_2), 49.9 (C-17, CH), 14.9 (C-18, CH_3), 209.4 (C-19, C), 36.0 (C-20, CH), 18.5 (C-21, CH_3), 39.6 (C-22, CH_2), 128.5 (C-23, CH), 136.9 (C-24, CH), 75.1 (C-25, C), 26.2 (C-26, CH_3), 25.9 (C-27, CH_3), 26.7 (C-28, CH_3), 25.1 (C-29, CH_3), 18.0 (C-30, CH_3), 50.3 (C-31, CH_3), 166.2 (C-32, C), 42.1 (C-33, CH_2), 169.3 (C-34, C).

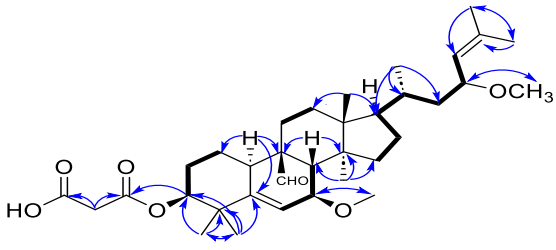
4.3 3β-malonyl-7β,25-dihydroxycucurbita-5,23-dien-19-al (3)

White amorphous powder, ^{1}H NMR (500 MHz, MeOD): δ_H (ppm): 1.73 (m), 1.40 (m), 1.90 (m), 1.73 (m), 4.84 (br s), 5.91 (d, J = 4.5 Hz), 4.11 (d, J = 5.2 Hz), 2.01 (s), 2.56 (dd, J = 12.8, 4.1 Hz), 2.32 (td, J = 14.6, 5.6 Hz), 1.47 (m), 1.63 (m), 1.36 (m), 1.90 (m), 1.47 (m), 0.88 (s), 9.72 (s), 1.53 (m), 0.89 (d, J = 6.4 Hz), 2.16 (dd, J = 10.7, 6.6 Hz), 1.74 (m), 5.59 (m), 5.59 (m), 1.30 (s), 1.30 (s), 1.11 (s), 1.19 (s), 0.75 (s), 3.32 (d, J = 1.8 Hz). ^{13}C NMR (125MHz, MeOD): δ_C (ppm) 21.6 (C-1, CH_2), 25.7 (C-2, CH_2), 79.5 (C-3, CH), 40.4 (C-4, C), 145.4 (C-5, C), 122.9 (C-6, CH), 66.4 (C-7, CH), 49.5 (C-8, CH), 50.0 (C-9, C), 36.1 (C-10, CH), 22.6 (C-11, CH_2), 29.0 (C-12, CH_2), 45.6 (C-13, C), 47.8 (C-14, C), 34.7 (C-15, CH_2), 27.6 (C-16, CH_2), 49.9 (C-17, CH), 15.0 (C-18, CH_3), 209.8 (C-19, C), 36.4 (C-20, CH), 18.8 (C-21, CH_3), 39.2 (C-22, CH_2), 125.4 (C-23, CH), 139.8 (C-24, CH), 71.3 (C-25, C), 30.1 (C-26, CH_3), 30.0 (C-27, CH_3), 25.8 (C-28, CH_3), 25.1 (C-29, CH_3), 18.0 (C-30, CH_3), 166.1 (C-31, C), 42.1 (C-32, CH_2), 169.0 (C-33, C).

5. Discussions

Compound 1 identified as an amorphous whitish powder. The molecular formular 1 was deducted as $C_{35}H_{54}O_7$ based on the sodium ion peak at 609.3759 $[M+Na]^+$ (calcd. for $C_{35}H_{54}O_7Na^+$, 609.3762) based on HR-ESI-MS spectrum. The NMR spectra of **1** exhibited seven methyl groups, including [δ_H 0.89 (3H, s, H-18), 0.92 (3H, d, J = 6.0 Hz, H-21), 1.71 (3H, d, J = 1.0 Hz, H-26), 1.78 (3H, d, J = 1.0 Hz, H-27), 1.13 (3H, s, H-28), 1.19 (3H, s, H-29), and 0.77 (3H, s, H-30)]; two methoxy signals [δ_H 3.22 and 3.26 (each 3H, s)]; and one aldehyde signal [δ_H 9.77 (1H, s)]. The ^{13}C NMR data combined with HSQC showed a total of 35 signals. It was analyzed as consisting of 7 methyl groups, 8 methylene groups, 7 methine groups, and 5 quaternary carbons. Moreover, two olefinic signals [(δ_H 5.94 (1H, br d, J = 4.0 Hz, H-6), and δ_H 4.92 (1H, d, J = 9.5 Hz, H-24), were also observed. The NMR data of compound **1** revealed that it is a cucurbitane triterpenoid, a major component of *M. charantia*. The planar structure of compound 1 was identified based on COSY and HMBC correlations. In particular, HMBC correlations from H-28 (δ_H 1.13) and H-29 (δ_H 1.19) to C-3 (δ_C 80.2) were observed. Additionally, the detailed HMBC and COSY correlations of compound 1 are presented in Figure 2. Comparing the NMR data of compound 1 with momordicine I, a compound identified from *M. charantia*, allowed for the deduction of the relative configuration of compound 1 (Mekuria et al., 2005). Consequently, the structure of 1 was elucidated as 3β-malonyl- 7β,23-dimethoxycucurbita-5,24-dien-19-al.

Fig.2. Important COSY (bold black strokes) and HMBC (blue arrow) cross-peaks of 1



Using the same structural elucidation method applied to compound 1, compounds 2 and 3 were identified as 3β-malonyl-7β-hydroxy-25-methoxycucurbita-5,23-dien-19-al (2), and 3β-malonyl-7β,25-dihydroxycucurbita-5,23-dien-19-al (3), respectively.

Indeed, compound 2 was also isolated as an

amorphous whitish powder. The molecular formula of 2 was assigned as $C_{34}H_{52}O_7$ based on the sodium molecular ion peak at *m/z* 595.3652 $[M + Na]^+$, calculated as 595.3605. The NMR data for 2 are very similar to those for 1, with minor differences at the C-7, C-23, and C-25 positions. This was verified by COSY and HMBC experiments. In particular, the COSY correlations from H-6 (δ_H 5.90) to H-7 (δ_H 4.09) and the HMBC correlations from the methyl groups H-26 and H-27 to C-25 (δ_C 75.1) confirmed the presence of a methine hydroxyl group at C-7 and a quaternary carbon at C-25. Therefore, compound 2 was identified as 3*β*-malonyl-7β-hydroxy-25-methoxycucurbita-5,23-dien-19-al.

Compound 3 was also obtained as a white amorphous powder. The NMR data for compound 3 were very similar to those of compound 2, with minor differences observed at the C-25 position. In particular, the comparison of the chemical shift at C-25 (from δ_C 71.3 in compound 2 to δ_C 75.1 in compound 3) indicated the presence of an alcohol adjacent to a quaternary carbon at C-25. Thus, compound 3 was identified as 3*β*-malonyl-7β-hydroxy-25-methoxycucurbita-5,23-dien-19-al.

Regarding the evaluation of potential anti-inflammatory effects of isolated compounds from *M. charantia*, compound 1 significantly inhibited the production of TNF-α, IL-6, and IL-12p40 in lipopolysaccharide-stimulated bone marrow-derived dendritic cells, with IC_{50} values of 0.553 ± 0.01, 0.857 ± 0.02, and 2.143 ± 0.02 μM, respectively. Adezmapimod, an inhibitor of cytokine suppressive binding protein/p38 kinase, was selected as a positive control. In this study, adezmapimod demonstrated IC_{50} values for TNF-α, IL-6, and IL-12 production of 5.0 ± 0.01, 3.5 ± 0.02, and 7.2 ± 0.02 μM, respectively. Thus, compound 1 significantly inhibited the production of multiple pro-inflammatory cytokines. Further *in vivo* studies need to be carried out to investigate the underlying inflammatory properties of compound 1.

6. Conclusion

In summary, phytochemical investigation of the ethanol extract from the fruits of *M. charantia* led to the isolation of three triterpenoids (**1-3**). Their structures were elucidated based on detailed spectroscopic analyses, including NMR and mass spectrometry. The anti-inflammatory potential of the isolated compounds was evaluated using an *in vitro* LPS-stimulated BMDC model. Compound **1** demonstrated significant inhibitory effects on the production of pro-inflammatory cytokines, including IL-6, IL-12p40, and TNF-α. These findings highlight the promising *in vitro* anti-inflammatory activity of secondary metabolites from M. charantia and support their potential as therapeutic agents. However, further *in vivo* and in silico studies are warranted to validate their efficacy and safety in clinical settings.

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CÁC HỢP CHẤT TRITERPENOID TỪ QUẢ MƯỚP ĐẮNG VÀ TIỀM NĂNG CHỐNG VIÊM CỦA CHÚNG

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Tóm tắt: *Mướp đắng (Momordica charantia) là một loại thực phẩm chức năng và dược liệu truyền thống, được đánh giá cao nhờ các hoạt tính dược lý đa dạng như chống viêm, chống đái tháo đường và chống oxy hóa. Các nghiên cứu hóa thực vật trên quả của M. charantia đã dẫn đến việc phân lập được ba saponin triterpenoid (1–3). Cấu trúc của các hợp chất này được xác định thông qua dữ liệu phổ, bao gồm HR-ESI-MS, NMR 1D và 2D, cũng như so sánh với các hợp chất tham chiếu. Cụ thể, các hợp chất này được xác định tương ứng là 3β-malonyl-7β,23-dimethoxycucurbita-5,24-dien-19-al (1), 3β-malonyl-7β-hydroxy-25-methoxycucurbita-5,23-dien-19-al (2) và 3β-malonyl-7β,25-dihydroxycucurbita-5,23-dien-19-al (3). Để đánh giá tiềm năng chống viêm của các chất này, khả năng ức chế sản sinh các cytokine tiền viêm—IL-6, IL-12p40 và TNF-α—đã được thử nghiệm trên tế bào đuôi gai có nguồn gốc từ tủy xương (BMDCs) được kích thích bởi LPS. Dữ liệu cho thấy hợp chất 1 có hoạt tính cao hơn cả thuốc adezmapimod, được sử dụng làm đối chứng dương. Các kết quả này cho thấy tiềm năng chống viêm in vitro của các hợp chất thứ cấp phân lập từ M. charantia.*

Từ khóa: *Các hợp chất triterpenoid; Hiệu ứng chống viêm; Momordica charantia; Mướp đắng.*