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α-Mangostin Suppresses TNF-α-Induced Biomarkers of Endothelial Dysfunction

(α-Mangostin Menyekat Penanda Bio Disfungsi Endotel Teraruh TNF-α)

LUQMAN JAYA[1], MIERRAH-NATASHA MUHAMMAD NASIR[2], SITI-MARLIANA JASNI[1], NOR HISAM ZAMAKSHSHARI[2] & ERNA-ZULAIKHA DAYANG[1,*]

[1]Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[2]Faculty of Resources Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

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ABSTRACT

Sepsis is a condition of dysregulated response to infection. Endothelial dysfunction is a key event in the onset and progression of sepsis, marked by a heightened pro-inflammatory response. Tumour necrosis factor-alpha (TNF-α), a major cytokine elevated in sepsis, induces the expression of adhesion molecules and cytokines which leads to the leukocyte recruitment. With limited treatment options to counteract sepsis-induced inflammation, natural compounds offer promising alternatives. α-Mangostin, a xanthone from *Garcinia mangostana*, exhibits anti-inflammatory properties, but its effects on TNF-α-activated endothelial cells remain underexplored. Herein, this study aimed to investigate the potential of α-Mangostin in modulating the inflammatory response of TNF-α-stimulated endothelial cells. Firstly, the structure of isolated α-Mangostin was confirmed via NMR and FTIR and the optimal non-toxic concentration of α-Mangostin in endothelial cells was pre-determined. Subsequently, a range of concentrations of α-Mangostin between 0.1-10 μM was added into TNF-α-stimulated human umbilical vein endothelial cells (HUVECs). After 4 h of exposure to TNF-α, cell lysates and medium supernatants were collected, and the effect of α-Mangostin in attenuating adhesion molecules (E-selectin, VCAM-1, and ICAM-1) and pro-inflammatory cytokine (IL-6) expression was measured using Western blot and ELISA, respectively. Additionally, the effect of the therapeutic concentration of α-Mangostin relative to an NF-κB inhibitor, BAY-11-7082, at a similar concentration, was compared. Cell viability assay, CCK-8, showed the IC_{50} of HUVEC against α-Mangostin to be 10.69 μM. α-Mangostin downregulated E-selectin, VCAM-1, and ICAM-1, with 1 μM and 10 μM showing comparable efficacy. Additionally, α-Mangostin significantly reduced IL-6 secretion from TNF-α-induced endothelial cells from 0.1 μM to 10 μM. Suppression of anti-inflammatory molecules was higher than the positive control, BAY-11-7082, suggesting possible inhibitory action beyond NF-κB activation. These findings highlight the potential of α-Mangostin as a therapeutic candidate to counteract sepsis-related endothelial dysfunction.

Keywords: α-Mangostin; endothelial; *Garcinia mangostana*; inflammation; sepsis

ABSTRAK

Sepsis ialah satu keadaaan tindak balas yang tidak terkawal dalam badan akibat jangkitan. Kegagalan fungsi pada sel endotelial merupakan kunci utama dalam perkembangan sepsis dan ia sering diikuti dengan tindak balas pro-radang yang tidak terkawal. Ketika sepsis berlaku, peningkatan sitokin utama seperti faktor nekrosis tumor (TNF-α) merangsang pembentukan molekul lekatan yang mendorong penarikan leukosit ke arah sel endotelial. Namun, rawatan sepsis sedia ada adalah terhad dan keadaan ini mendorong kepada penerokaan sebatian semula jadi sebagai rawatan alternatif. α-Mangostin ialah xanthone yang boleh dijumpai daripada *Garcinia mangostana.* Kajian sebelum ini telah menilai α-Mangostin sebagai sebatian yang mempunyai ciri anti-radang. Namun, potensi α-Mangostin terhadap endotelial yang telah diaktifkan masih belum diterokai. Oleh itu, kajian ini meneroka potensi α-Mangostin terhadap sel endotelial vena umbilikal manusia (HUVECs) yang dirangsang dan diaktifkan oleh TNF-α. Dalam kajian ini, ekspresi molekul lekat dan sekresi sitokin pro-radang telah dikaji. Selain itu, perbandingan terapeutik α-Mangostin dan perencat NF-κB, BAY-11-7082 juga telah dinilai. Struktur α-Mangostin juga telah dipastikan melalui NMR dan FTIR. Seterusnya, kajian ini telah mengenal pasti kadar kepekatan optimum α-Mangostin yang tidak toksik terhadap HUVECs. Ekspresi molekul lekat telah dilakukan melalui pemblotan Western dan sekresi sitokin telah dinilai melalui ELISA. Melalui analisis sel keviabelan, kajian ini mendapati bahawa nilai IC_{50} α-Mangostin adalah 10.69 μM. α-Mangostin telah mengurangkan ekspresi E-selektin, VCAM-1 dan ICAM-1. Di samping itu, sekresi IL-6 juga telah diturunkan secara signifikan dengan kepekatan 1 μM dan 10 μM menunjukkan kesepadanan. Kajian ini turut menunjukkan bahawa molekul lekat telah diturunkan oleh α-Mangostin lebih banyak berbanding BAY-11-7082, sekali gus membawa kepada kemungkinan α-Mangostin boleh merencat keradangan

melangkaui NF-κB seperti melalui p38 dan MAPK. Penemuan terkumpul ini membuktikan bahawa α-Mangostin merupakan sebatian yang mempunyai potensi terapeutik terhadap sepsis yang berpunca daripada kegagalan fungsi endotelial.

Kata kunci: α-Mangostin; *Garcinia mangostana*; keradangan; sel endotelial; sepsis

INTRODUCTION

Sepsis remains a worldwide health issue that causes 11 million fatalities (World Health Organization 2024). Prolonged exposure of endothelial cells to tumour necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine imperative in sepsis initiation and progression, leads to its impaired homeostasis, which ultimately results in endothelial dysfunction (Maneta et al. 2023; Maruhashi & Higashi 2021; McMullan et al. 2024). The activation of endothelial dysfunction by TNF-α results in a pronounced expression of E-selectin, VCAM-1, and ICAM-1. These are endothelial adhesion molecules important in rolling, adhesion, and transmigration of leukocytes into the surrounding tissues (Maneta et al. 2023). Simultaneously, activated and dysfunctional endothelial cells produce pro-inflammatory cytokines such as interleukin-6 (IL-6), which enhances vascular permeability, therefore, aiding the recruitment of more leukocytes (Li et al. 2018). Despite having a crucial role in adhesion and transmigration of immune cells, excessive and prolonged expression of adhesion molecules and pro-inflammatory cytokines may lead to systemic inflammation, leading to tissue damage and ultimately organ failure (Amalakuhan et al. 2016). However, at present, no drug has been developed to counteract endothelial dysfunction associated with sepsis. This fact opens up a path to the discovery of natural products which can display a promising result in ameliorating sepsis by possibly targeting inflammatory response therefore mitigating endothelial dysfunction.

Garcinia mangostana (GM) is a tropical fruit that is commonly known as 'manggis' by the Malaysian locals and can be extensively found in Southeast Asia, including Malaysia, Indonesia and Thailand (Agarwal et al. 2020). Anti-inflammatory activity has been reported from this fruit (Setiawan, Budiman & Prasetyo 2023). Ethnomedicinal uses of various parts of the mangosteen plant, such as the rind, bark, and leaves, have been documented for the treatment of skin infections, gonorrhoea, and inflammation-related condition such as oedema (Astuti et al. 2019; Sowmya et al. 2015; Tatiya-aphiradee, Chatuphonprasert & Jarukamjorn 2019). Among the most prominent group of compounds that has been isolated from GM are xanthones. More than 80 distinct xanthone derivatives have been identified from GM, with α-Mangostin, a prenylated xanthone, being recognized as one of the most abundant and extensively studied compounds due to its potent biological activities. α-Mangostin is primarily found in the pericarp of the plant (Majdalawieh et al. 2024) and was previously reported to display anti-inflammatory effects via modulation of nuclear factor kappa-B (NF-κB) (John et al. 2022) and IL-6 receptor (Majdalawieh, Khatib, & Terro 2025). Furthermore, secretion of IL-6 (Li et al. 2018) and expression of adhesion molecules (Singh et al. 2023) subsequent to exposure to pro-inflammatory mediators such as TNF-α, can lead to endothelial dysfunction. Although a downregulation of pro-inflammatory cytokines was demonstrated by α-Mangostin in LPS-stimulated human follicle dermal papilla cells, confirming its anti-inflammatory properties (Kim et al. 2023), its potential role in attenuating inflammatory response in TNF-α-activated endothelial cells has yet to be fully elucidated. Therefore, in this study, the effect of α-Mangostin on TNF-α-exposed endothelial activation was investigated. Subsequently, the α-Mangostin-suppressing effects on the proinflammatory molecules investigated in this study were subsequently compared to those of a standard NF-κB inhibitor, BAY11-7082.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

The AR-grade chemical solvent such as acetone, chloroform, ethyl acetate, and hexane were utilised in the extraction, isolation, and purification of α-Mangostin from *Garcinia mangostana* peel extract. Silica gel 60 (Merck, Kieselgel 60) Art. No. 9385.1000 of particle size 0.040-0.063 mm and Sephadex LH-20 were utilised to isolate and purify α-Mangostin. The deuterated solvent acetone-d6 was used for NMR analysis.

For *in vitro* analyses, including Western blotting and ELISA, human umbilical vein endothelial cells (HUVECs), Endothelial Cell Growth Medium 2 (EGM-2), Supplement Mix C-39216, Detachment Kit, and phosphate-buffered saline (PBS) were obtained from PromoCell (Heidelberg, Germany). Microscopic images were captured using an inverted light microscope at 100× magnification (Nikon, Tokyo, Japan). The CCK-8 cell viability assay kit and bicinchoninic acid (BCA) protein assay kit was purchased from Abcam (Cambridge, UK). Penicillin–streptomycin (Cat. No. 15140-122) was obtained from Thermo Fisher Scientific (New York, USA). For inflammatory stimulation, cells were treated with tumour necrosis factor-alpha (TNF-α) (Gibco, Thermo Fisher Scientific, New York, USA). The NF-κB inhibitor BAY 11-7082 (Cat. No. HY-13453) was obtained from MedChemExpress (New Jersey, USA). RIPA buffer (1×) for protein extraction, chemiluminescence detection reagents and PageRuler Prestained protein ladder (Lot. No. 01242323), were purchased from Thermo Fisher Scientific (Illinois, USA), while Laemmli buffer was obtained from Merck (Darmstadt, Germany). Nitrocellulose membranes were purchased

from Sartorius Stedim Biotech (Göttingen, Germany). Primary antibodies against ICAM-1 and GAPDH were obtained from Bio-Techne (Minnesota, USA), VCAM-1 from Cell Signaling Technology (Massachusetts, USA), and E-selectin from Invitrogen (Massachusetts, USA). The IL-6 ELISA kit (Cat. No. E-EL-H6156) was purchased from Elabscience (Wuhan, China). Protein bands were visualised using a chemiluminescence detection system (Amersham Imager 680, Illinois, USA), and densitometric analysis was performed using ImageJ software (National Institutes of Health, Maryland, USA). The membrane was observed through the chemiluminescence detection system Amersham Imager 680 (Illinois, USA). Microplate reader used to measure the absorbance values was SpectraMax iD3, Molecular Devices, (California, USA). The densitometric analysis was performed through ImageJ software from the National Institute of Health (NIH) (Maryland, USA). Laboratory consumables such as 6-well plates were purchased from BIOFIL (Guangzhou, China), and microcentrifuge tubes were from Axygen, Corning Life Sciences, (California, USA).

EXTRACTION AND ISOLATION OF α-MANGOSTIN FROM *Garcinia mangostana*

The extraction crude extract and isolation of α-Mangostin were performed in accordance with (Zaine et al. 2025) with slight modifications. Three kilograms of *Garcinia mangostana* fruits were collected from Rantau, Negeri Sembilan, Malaysia (voucher specimen: UNIMASNHZ005). Approximately one kilogram of dried pericarp of *Garcinia mangostana* was ground into fine powder using a grinder. Then, the *Garcinia mangostana* pericarp fine powder was extracted successively with 2 L of 100% chloroform for 48 h. The extract was filtered and then evaporated using a rotary evaporator to yield 110 g of dark-viscous semisolid extract. Column chromatography using a glass column packed with silica gel was used to isolate α-Mangostin. Fifty grams (50 g) of crude extract was then introduced to gravity column chromatography over silica gel with eluting solvents of hexane-ethyl acetate and ethyl acetate-methanol to give 20 fractions. Then, fractions 5 to 8 were pooled together and were subjected to another round of chromatography and eluted with hexane-ethyl acetate (1:1) to give a total of 10 fractions. Fraction 3 undergoes purification using Sigma Lipophilic Sephadex LH-20 and methanol to give a yellow solid. Recrystallisation of the yellow solid in chloroform gave alpha-mangosteen as yellow crystals (127 mg) as shown in Figure 1(c). The purification of alpha mangosteen from *Garcinia mangostana* extract is portrayed in a schematic diagram in Figure S1.

CHARACTERIZATION OF ISOLATED α-MANGOSTIN

The characterization of the α-Mangostin purified structure was confirmed using nuclear magnetic resonance (NMR) (Guo et al. 2016) and Fourier Transform Infrared (FTIR)

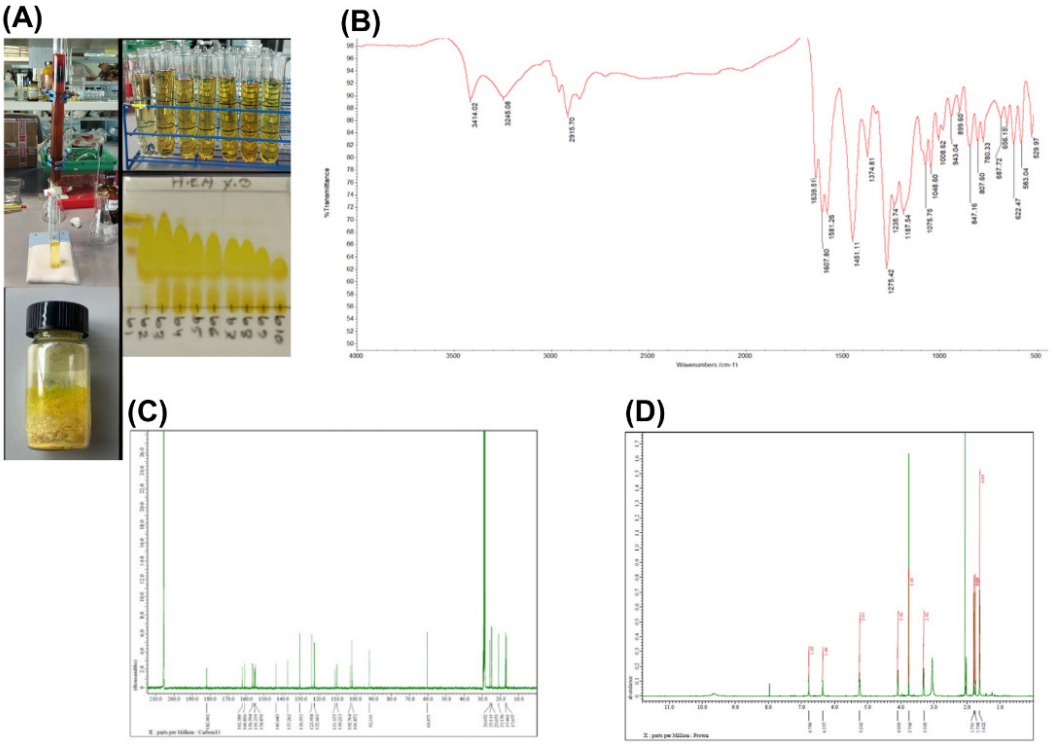

FIGURE 1. (a) The yellow crystal of α-Mangostin, (b) FTIR of spectrum of xanthones, (c) ^{13}C NMR spectrum of isolated α-Mangostin recorded at 125 MHz, and (d) ^{1}H NMR spectrum of isolated α-Mangostin recorded at 500 MHz

spectroscopy. For NMR the JOEL JNM-ECZ500R at a frequency of 500 MHz was utilized to conduct both proton and carbon NMR analysis. Ten milligrams (10 mg) of isolated α-Mangostin were dissolved in 750 µL of acetone d-6 deuterated solvent. The α-Mangostin sample was run for 3000 scans to obtain the carbon spectrum. Additionally, for FTIR, Attenuated Total Reflectance (AT) spectroscopy with a diamond crystal was carried out utilizing a Perkin Elmer 1605 spectrophotometer to perform an FTIR spectrum analysis (v/cm^{-1}).

HUVECs MAINTENANCE

Human Umbilical Cord Endothelial Cells (HUVECs) were maintained in Endothelial Cell Basal Medium 2 (2% V/V) supplemented with Supplement Mix C-39216 and 1% penicillin-streptomycin. Cells were cultured in T-75 flasks and incubated at the temperature of 37 °C with 5% CO_2. In every 48 to 72 h, the medium in the wells was changed. Then, the cells were trypsinized and passaged upon reaching 90% confluency using DetachKit and sub-passaged to newer T-75 flask. Cells between passages 4 and 7 were used in all experiments to maintain consistency and ensure reliable results.

EFFECT OF α-MANGOSTIN ON CELL VIABILITY

Prior to cell viability assay, HUVECs were seeded with a seeding density of 3×10^4 cells per well in 96-well plate. Then, cell viability was evaluated using the CCK-8 kit after treatment with different concentrations of α-Mangostin. The compound was serially diluted in Endothelial Cell Basal Medium 2 (2% v/v) supplemented with Supplement Mix C-39216, generating final concentrations ranging from (untreated) 0 µM to 50 µM. A volume of 100 µL from each dilution was dispensed into the wells of a 96-well plate. Subsequently, 10 µL of WST-8 solution was added to every well with care. The plate was left for incubation for 4 h at the temperature of 37 °C. Absorbance was then recorded using a microplate reader to determine cell viability at 450 nm.

α-MANGOSTIN AND BAY-11-7082 TREATMENT TOWARDS TNF-A-STIMULATED HUVECs

For experiment procedures, HUVECs were seeded at 3.8×10^4 cells per well in a 6-well plate before they were allowed to adhere to the wells for at least 8 h. Once confluence, cells were treated with α-Mangostin at concentrations between 0.1 and 10 µM, followed by stimulation with TNF-α at 100 ng/mL for 4 h (Li et al. 2015).

For comparative analysis, cells were pre-treated with α-Mangostin (10 µM) or BAY-11-7082 (10 µM) prior to TNF-α stimulation to enable direct comparison at equivalent concentrations. The concentration of 10 µM α-Mangostin was selected for comparison as it represented the highest tested concentration that allowed direct comparison with BAY-11-7082 at an equivalent dose. Furthermore, the selection of 10 µM for BAY-11-7082 was determined due to its non-cytotoxicity after 24 h, with cell lines such as RAW264.7 and HEK293 maintaining high viability at 10-30 µM (Lee et al. 2016). Additionally, HUVECs pre-treated with 10 µM BAY-11-7082 retained normal cobblestone morphology without observable changes (Dayang et al. 2019) supporting its use as positive control in this study.

CELL SUPERNATANT COLLECTION

Following 4 h of TNF-α stimulation, the culture medium was gently removed to prevent disruption of the monolayer and transferred into 1.5 mL microcentrifuge tubes. The samples were subsequently centrifuged at 1,500 × g for 10 min at the temperature of 4 °C. After centrifugation, the supernatants were preserved at −20 °C until analysis was performed.

CELL LYSATE COLLECTION

After collection of cell supernatants, HUVECs were rinsed twice with cold, phosphate-buffered saline (PBS) with a pH of 7.4 to remove residual treatment compounds. Subsequently, 150 µL of RIPA buffer 1X was introduced into each well of the 6-well plate. The plate was kept on ice and gently agitated for 15 min to promote complete cell lysis. The cells were detached using a sterile scraper, and the lysates were transferred with care into 1.5 mL microcentrifuge tubes (c). Centrifugation was carried out at 14,000 × g for 10 min at 4 °C to sediment insoluble debris. The obtained supernatants were collected, transferred into fresh microcentrifuge tubes, and stored at −20 °C until further assays were performed.

EXPRESSION OF ADHESION MOLECULES BY WESTERN BLOT

The bicinchoninic acid (BCA) assay was employed to quantify protein concentrations, in accordance with the supplier's instructions. Then, the western blot analysis was performed with slight modifications (Dayang et al. 2021). A total of 10 µg of protein was mixed with 2.5× Laemmli sample buffer and heated at 95 °C for 10 min. The samples, and the 180 kDa PageRuler Prestained protein ladder were separated by SDS-PAGE on 10% polyacrylamide gels for a total duration of 2 h. Proteins were subsequently transferred onto nitrocellulose membranes (Sartorius Stedim Biotech, Goettingen, Germany) using a wet transfer system at 200 mA for 2 h at 4 °C.

Blocking of the membranes was carried out with 5% BSA prepared in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 h at room temperature. Following blocking, incubation was carried out with rabbit anti-human E-selectin antibody (1:2500) for 1 h on a rocking platform. Three washes with TBST were performed on the membranes before incubation with horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (1:5000) for

1 h under the same conditions. Protein bands were visualized using a chemiluminescence (ECL) reagent and imaged with a chemiluminescence detection.

For additional targets, membranes were stripped with blot stripping buffer for 15 min with gentle rocking to remove bound antibodies. After washing, the membranes were blocked again with 5% BSA in TBST for 1 h at room temperature and then probed sequentially with primary antibodies against VCAM-1 (1:5000) and ICAM-1 (1:3000), each incubated for 1 h with gentle agitation. Prior to densitometric analysis, the bands were normalized to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:5000). Finally, ImageJ was employed in quantifying normalized band intensities from the blotted membrane.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR QUANTIFICATION OF IL-6 LEVELS

Next, ELISA assay was performed following the instructions provided by manufacturers. Briefly, 100 µL of standards, samples, and blanks were dispensed in duplicate into designated wells of a 96-well plate and incubated at the temperature of 37 °C for 90 min. Subsequently, 100 µL of antibody used for biotinylated detection was added, before the wells were incubated for 1 h at the temperature of 37 °C. The following steps include the washing of the wells with wash buffer thrice before 100 µL of HRP conjugate was applied and allowed to react for 30 min. Following five additional washes, 90 µL of substrate reagent was introduced and incubated for 15 min in the dark. Then, 50 µL of stop solution was introduced into each well, and absorbance was read at 450 nm using a microplate reader.

STATISTICAL ANALYSIS

GraphPad Prism version 10 was used as a statistical tool. Data is presented as mean ± standard deviation (S.D.) from three independent experimental replicates. For ELISA outcomes, differences among groups were measured through ANOVA and followed by Dunnet's post hoc test. Densitometric evaluation of Western blot bands was carried out with ImageJ software (NIH, USA), and the resulting intensities were normalized to the housekeeping protein GAPDH. The normalized data were analysed by one-way ANOVA. Any p-values less than 0.05 were considered significant, statistically.

RESULTS AND DISCUSSION

ISOLATION AND STRUCTURAL CHARACTERISATION OF α-MANGOSTIN FROM *Garcinia mangostana* PERICARP

The FTIR spectrum (SI3) gives the typical IR absorptions for xanthones at 3245 cm^{-1}, 1607 cm^{-1}, 1581cm^{-1}, and 1275cm^{-1} as shown in Figure 1(b). Strong absorptions at 3245 cm^{-1} and 1607 cm^{-1} were representative of hydroxyl and conjugate carbonyl stretching, respectively. Meanwhile, the absorption at 1581 m^{-1} was due to the stretching of an aromatic group. The graph of ^{13}C- NMR is shown in Figure 1(c) and ^{1}H- NMR is shown in Figure 1(d). The results are as follows: ^{1}H-NMR (500 MHz, $(CD_3)_2CO$): 1.62(6H, s, H-19 & H-20), 1.74(3H, s, H-14), 1.79(3H, s, H-15), 3.30(3H, s, OCH_3-5), 3.76(2H, d, H-16), 4.09(2H, d, H-11), 5.24(2H, t, H-12 & H-17), 6.37(1H, s, H-4), 6.78(1H, s, H-5). ^{13}C-NMR (125 MHz, $(CD_3)_2CO$): 17.07(C-19), 17.46(C-15), 21.15(C-16), 25.07(C-20), 25.11(C-14), 26.05(C-11), 60.47(OCH_3-7), 92.31(C-4), 101.87(C-9a), 102.76(C-5), 110.21(C-8a), 111.12(C-2), 122.66(C-17), 123.95(C-12), 130.55(C-18 & C13), 137.26(C-8), 143.64(C-7), 154.87(C-10a), 155.37(C-4a), 156.59(C-6), 160.85(C-3), 162.18(C-1) and 182.00(C-9). The ^{1}H and ^{13}C NMR spectra were consistent with literature data reported by Guo et al. (2016) with no significant extraneous signals observed, indicating high purity of the isolated α-Mangostin.

EFFECT OF α-MANGOSTIN ON ENDOTHELIAL CELL VIABILITY

To determine the possible therapeutic range of α-Mangostin for the present study, the effect of the compound on cell viability and subsequently the IC_{50} value were determined. Microscopically, treatment with concentrations of α-Mangostin higher than 12.5 µM resulted in noticeable cell shrinkage, detachment, and reduced confluency, indicating dose-dependent cytotoxic effects. High concentrations of the compound lead to a progressive reduction in cell viability as shown in Figure 2(b). A dose-dependent cytotoxic effect of α-Mangostin was observed on HUVECs, prominently after treatment with concentration of 12.5 µM and above (Figure 2(b)). Dose-response curves were generated from a range of α-Mangostin concentrations and the IC_{50} value was 10.69 µM as shown in Figure 2(c), signifying that α-Mangostin exerts cytotoxic effect by 50% at this concentration. Taken together, as cell viability was significantly reduced at 12.5 µM and IC_{50} was projected at 10.69 µM, the result concluded that the concentration of α-Mangostin that exhibit therapeutic effects should ideally be below 10.69 µM. Nevertheless, a concentration of 10 µM was retained for subsequent assays despite exhibiting approximately slight cytotoxicity, as it remains close to the IC_{50} value and preserves a sufficient population of metabolically active cells for functional evaluation. The inclusion of this concentration was further justified to assess whether higher concentrations could elicit enhanced biological responses and to determine the presence of a dose-dependent effect, which was subsequently observed in the modulation of adhesion molecules and inflammatory markers. Furthermore, the IC_{50} value reflects the potency of a compound, with a lower IC_{50} indicating higher potency in inhibiting a specific biological function or cell viability (Berrouet et al. 2020). The dose-response curve generated from this data demonstrates a typical sigmoidal pattern, with minimal effects at low concentrations and steep decline in viability as the concentration approaches

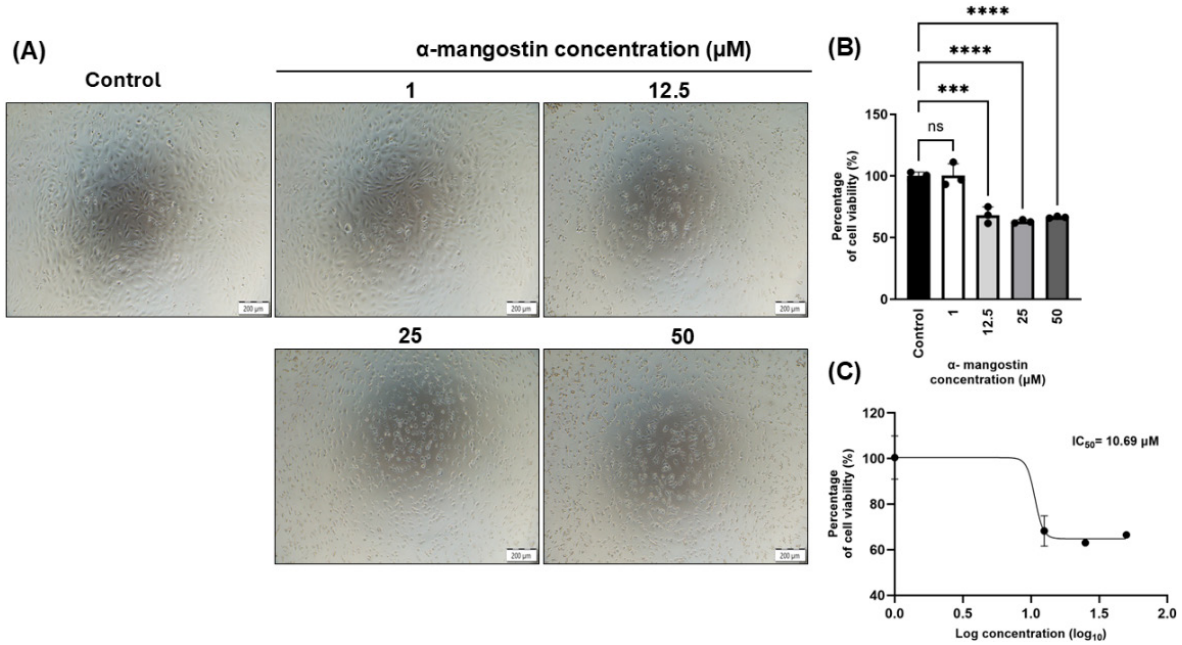

FIGURE 2. (a) The effect of α- Mangostin treatment on HUVECs viability. Cells were exposed to 0, 1, 12.5, 25 and 50 μM for 24 h, (b) Dose-dependent reduction in HUVECs viability following treatment with α-Mangostin with morphological changes seen following addition of increasing concentration of α-Mangostin. The pictures are representative of three independent experiments, (c) Sigmoidal dose-response curve of α-Mangostin in HUVECs generated using GraphPad Prism which the IC_{50} value was calculated based on non-linear regression analysis, indicating the concentration required to reduce cell viability by 50%

and exceeds the IC_{50} value. This dose-dependent action of α-Mangostin suggests that, at lower concentration, it can be potentially beneficial due to its anti-inflammatory properties (Herrera-Aco et al. 2019).

α-MANGOSTIN AMELIORATES EXPRESSION OF ADHESION MOLECULES AND IL-6 IN TNF-α-STIMULATED HUVECs

To evaluate the potential of α-Mangostin in elucidating endothelial dysfunction, the expression of adhesion molecules was measured in TNF-α-stimulated HUVECs. As shown in Figure 3(a), TNF-α exposure resulted in the high expression of adhesion molecules compared to the unstimulated control. However, treatment with α-Mangostin resulted in visibly reduced band intensities for these adhesion molecules when compared to the TNF-α-only group, demonstrating a dose-dependent suppression of adhesion molecule expression.

Correspondingly, densitometric analysis demonstrated a significant upregulation of E-selectin expression in the TNF-α over the unstimulated control group ($p < 0.05$). Similarly, bands corresponding to VCAM-1 and ICAM-1 were shown to upregulated in TNF-α-treated cells compared to unstimulated control. Quantification of band intensities showed a significant increase in the expression of both VCAM-1 ($p < 0.05$) and ICAM-1 ($p < 0.05$) following TNF-α stimulation, indicating endothelial activation in response to the pro-inflammatory cytokine. The analysis further showed the 0.1 μM of α-Mangostin concentration showed no significant difference towards the E-selectin expression ($p > 0.05$), VCAM-1 ($p > 0.05$) and ICAM-1 ($p > 0.05$) in relative to TNF-α treatment alone. The treatment with α-Mangostin at 1.0 μM significantly reduced the expression levels of VCAM-1 ($p < 0.05$) and ICAM-1 ($p < 0.05$) compared to TNF-α-stimulated HUVECs. At higher concentration, 10 μM α-Mangostin significantly reduced the expression of E-selectin (Figure 3(b)-3(d)).

The TNF-α–stimulated HUVECs model is a widely established and extensively validated *in vitro* system for studying endothelial inflammation and is commonly used to assess the anti-inflammatory effects of pharmacological compounds. Furthermore, high expression of adhesion molecules such as E-selectin, VCAM-1 and ICAM-1 and IL-6 is well known to be an indicator of endothelial dysfunction, including clinical observation (Alfaro et al. 2024; Dri et al. 2023; Liu & Chen 2024). In this study, elevated levels of these adhesion molecules and IL-6 were observed in LPS-stimulated HUVECs. This confirms the responsiveness of the endothelial cells to inflammatory stimulation and is consistent with previously reported endothelial activation models. Moreover, the anti-inflammatory effects of α-Mangostin were evaluated relative to the TNF-α–only stimulated group, which served as the disease-mimicking condition. α-Mangostin

demonstrated a clear concentration-dependent suppression of TNF-α–induced adhesion molecules and IL-6 secretion, indicating targeted modulation of inflammatory signalling rather than nonspecific cytotoxic effects. This is further supported by the observation that significant anti-inflammatory effects were also observed at 1 µM, a concentration that maintained relatively high cell viability, thereby reducing the likelihood that the observed effects were solely attributable to cytotoxicity.

The downregulation of adhesion molecules suggests that α-Mangostin may effectively inhibits endothelial activation, which is a critical step in sepsis (McMullan et al. 2024). This effect is likely mediated through suppression of NF-κB signalling, as previous studies show that TNF-α triggers NF-κB-mediated VCAM-1 as well as ICAM-1 expression in endothelial cells (Chu et al. 2017). Parallel with this data, previous study have reported that xanthones are shown to reduce adhesion molecule such as ICAM-1 protein levels in HUVECs models (Madan et al. 2002). Moreover, a previous study reported that α-Mangostin significantly mitigated LPS-induced upregulation of both VCAM-1 and ICAM-1 protein in human dental pulp cells, proving its role in mitigating vascular inflammation (Kim et al. 2023).

The level of IL-6 in the TNF-α-stimulated HUVECs was quantified using ELISA to investigate whether α-Mangostin modulates inflammatory cytokine secretion. As shown in Figure 3(e), TNF-α stimulation significantly increased IL-6 production compared to untreated controls ($p < 0.05$). Treatment with α-Mangostin at concentrations of 0.1, 1, and 10 µM resulted in the reduction of IL-6 secretion. There was no significant difference between 0.1 µM and 1 µM α-Mangostin ($p > 0.05$). However, the highest concentration of α-Mangostin (10 µM) significantly suppressed IL-6 release compared to TNF-α alone ($p < 0.05$). The present study demonstrates that α-Mangostin significantly reduces TNF-α–induced IL-6 secretion in HUVECs, similar with previous reports highlighting its anti-inflammatory properties (Widowati et al. 2016; Yiemwattana & Kaomongkolgit 2015). More importantly, treatment with 10 µM α-Mangostin resulted in a more pronounced suppression when compared to 1 µM α-Mangostin of all three adhesion molecules indicating a clear dose-dependent inhibitory effect. Although 10 µM of α-Mangostin was associated with some degree of cytotoxicity, this concentration was retained for subsequent analyses, as it still preserved a sufficient population of metabolically active cells to mount a measurable biological response. The selection of this concentration was therefore based on a balance between achieving maximal anti-inflammatory efficacy and maintaining adequate cell viability. Nevertheless, the observed cytotoxicity at higher concentrations suggests that future studies should explore strategies to improve the therapeutic

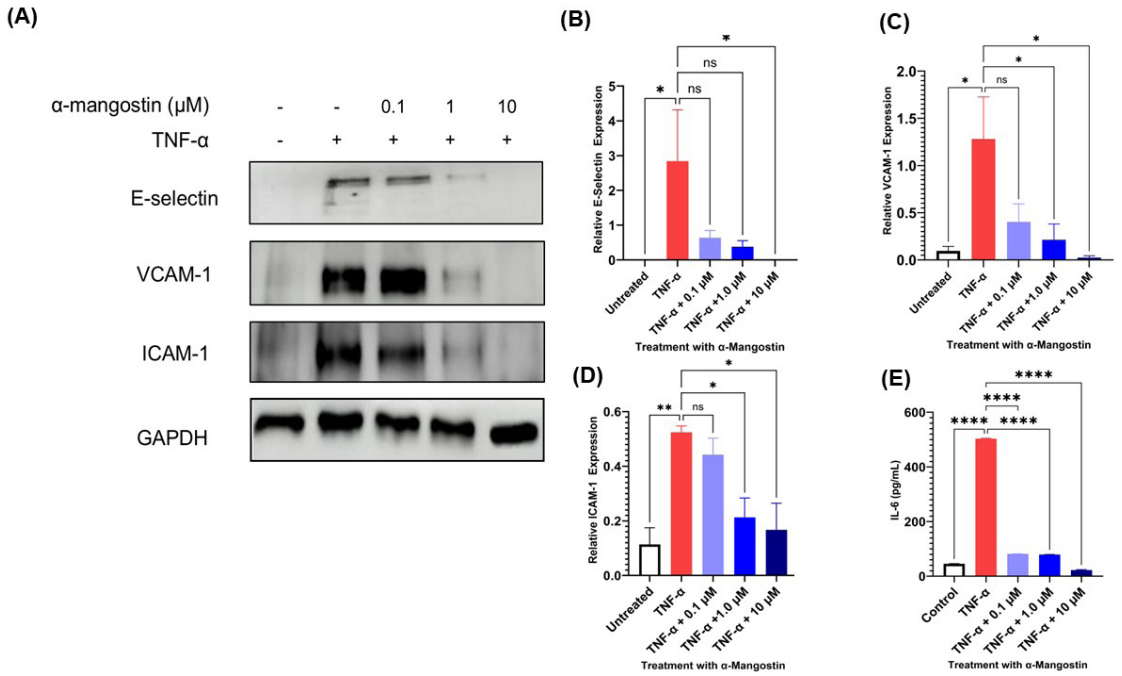

FIGURE 3. (a) Representative western blot depicting E-selectin, VCAM-1, and ICAM-1 protein in HUVECs stimulated with different concentrations of α-Mangostin (0.1, 1, and 10 µM), together with untreated control, TNF-α alone, and GAPDH as the loading control, (b) Densitometric analysis of E-selectin, (c) VCAM-1, and (d) ICAM-1 protein synthesis, normalized to GAPDH. Data are shown as mean ± standard error of the mean (SEM) from three independent replicates (n = 3). Statistical significance was defined at $p < 0.05$ (*) or $p < 0.01$ (**). (e) IL-6 concentrations in HUVECs following treatment, expressed as mean ± SEM (n = 3), with significance indicated at *$p < 0.05$ and ***$p < 0.0001$

window of α-Mangostin. In particular, lipid-based drug delivery systems, such as liposomal encapsulation, may enhance cellular uptake and bioavailability while reducing off-target cytotoxic effects, thereby enabling effective modulation of endothelial inflammation at lower apparent toxicity. As TNF-α is widely recognised for its role in stimulating endothelial cells, which subsequently triggers the secretion of pro-inflammatory cytokines like IL-6, the ability of α-Mangostin to attenuate this response suggests its potential in mitigating vascular inflammation including the modulation of cytokine signalling pathways. Furthermore, the observed reduction in IL-6 secretion may be attributed to the suppression of NF-κB activation, a key transcription factor regulating IL-6 gene expression in endothelial cells (Liu et al. 2017). Previous studies have shown that α-Mangostin inhibits NF-κB signalling in various inflammatory models (Mohan et al. 2018; Zou et al. 2019), supporting the idea that a similar mechanism may be responsible in HUVECs. Moreover, the reduction in IL-6 complements the inhibitory effects of α-Mangostin observed on adhesion molecule expression in this study, indicating a broader anti-inflammatory role targeting both cytokine and adhesion pathways.

α-MANGOSTIN MORE EFFECTIVELY SUPPRESSED ADHESION MOLECULES EXPRESSION THAN BAY-11-7082 in TNF-α STIMULATED HUVECs AT EQUIVALENT DOSES

Next, the evaluation of adhesion molecules' expression in HUVECs under different treatment conditions: untreated control, TNF-α alone, TNF-α with BAY-11-7082 (10 μM), and TNF-α with α-Mangostin (10 μM) was performed. The upregulation of adhesion molecule expression following TNF-α stimulation as shown in the representative western blot as shown in Figure 4(a). In the blot, the adhesion molecules appeared noticeably more prominent than those in the untreated control. Through densitometric analysis, TNF-α stimulation significantly upregulated the expression of E-selectin, VCAM-1 and ICAM-1 which confirms its role in endothelial activation (Figure 4(b)-4(d)). In addition, the expression levels of the adhesion molecules were reduced significantly after the addition of NF-κB inhibitor, BAY-11-7082. Notably, α-Mangostin treatment were comparable with BAY-11-7082 in reducing E-selectin ($p > 0.05$) and VCAM-1 ($p > 0.05$) but managed to significantly reduce the expression of ICAM-1 ($p < 0.05$) when compared to BAY-11-7082. In this study, western blot and densitometric analysis has shown that α-Mangostin effectively reduces TNF-α-induced upregulation of adhesion molecules in HUVECs with effect comparable to that of an established anti-inflammatory drug as BAY-11-7082. It is well known that the expression of adhesion molecules mediates leukocyte adhesion and is responsible for endothelial activation and vascular inflammation is stimulated by many factors including TNF-α (Singh et al. 2023; Zhang et al. 2020). Interestingly, the reduction of these adhesion molecules by BAY-11-7082, aligns with previous studies indicating the involvement of the NF-κB pathway in TNF-α-mediated endothelial activation (Kiatsoonthon et al. 2024; Yan et al. 2015). BAY-11-7082 which is a potent inhibitor of the NF-κB pathway, selectively and irreversibly prevents TNF-α-induced phosphorylation of IκBα, thereby blocking NF-κB activation and its nuclear translocation (Wang et al. 2016). In previous studies that involved endothelial cells treated with TNF-α, BAY-11-7082 significantly reduces the surface expression of key adhesion molecules such as ICAM-1, VCAM-1, and E-selectin (Catalán et al. 2012).

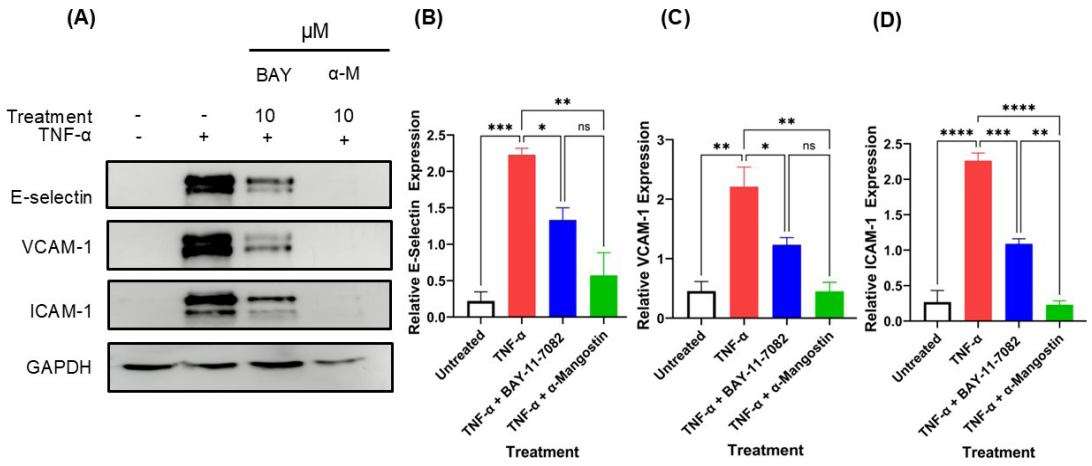

FIGURE 4. (a) Western blot analysis of HUVECs subjected to different treatments: untreated control, TNF-α stimulation, TNF-α with BAY-11-7082 (10 μM), and TNF-α with α-Mangostin (10 μM). Protein bands detected correspond to E-selectin, VCAM-1, and ICAM-1, with GAPDH included as the loading control. (b–d) Quantification of E-selectin, VCAM-1, and ICAM-1 expression after normalization to GAPDH. Results are presented as mean ± SEM from three independent biological replicates (n = 3). Statistical differences were considered significant at $p < 0.05$ (), $p < 0.001$ (**), and $p < 0.0001$ (****)

However, adhesion molecule expressions have been suppressed greatly by α-Mangostin at the concentration of 10 μM. Interestingly, this may be due to its protective role that not only inhibiting NF-κB, but also potentially suppresses additional inflammatory mechanisms such as MAPK or through antioxidant activities. Furthermore, it is observed that α-Mangostin has managed to reduce adhesion molecules better BAY-11-7082, a known standard for NF-κB inhibitor, suggesting its capabilities beyond NF-κB inhibition. It is important to note that other pathway such as p38 MAPK has been observed to stimulate the expression of adhesion molecules, which leads to the possibility of multi-pathway modulation in inflammatory activity in endothelial cells (Wang et al. 2016; Zhong et al. 2012).

CONCLUSIONS

In conclusion, this study demonstrates that α-Mangostin effectively attenuates TNF-α-induced endothelial activation by significantly reducing the synthesis of adhesion molecules and pro-inflammatory cytokine IL-6 in HUVECs. Notably, α-Mangostin exhibited a stronger inhibitory effect on adhesion molecule expression than the standard NF-κB inhibitor BAY-11-7082, suggesting that its anti-inflammatory actions may involve both NF-κB-dependent and independent pathways. These findings point to α-Mangostin as a potentially effective natural compound for reducing vascular inflammation, with its full range of molecular effects still needing to be explored.

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REFERENCES

Agarwal, G., Carcache, P.J.B., Addo, E.M. & Kinghorn, A.D. 2020. Current status and contemporary approaches to the discovery of antitumor agents from higher plants. *Biotechnology Advances* 38: 107337.

Alfaro, E., Díaz-García, E., García-Tovar, S., Galera, R., Casitas, R., Torres-Vargas, M., López-Fernández, C., Añón, J.M., García-Río, F. & Cubillos-Zapata, C. 2024. Endothelial dysfunction and persistent inflammation in severe post-COVID-19 patients: Implications for gas exchange. *BMC Medicine* 22: 242.

Amalakuhan, B., Habib, S.A., Mangat, M., Reyes, L.F., Rodriguez, A.H., Hinojosa, C.A., Soni, N.J., Gilley, R.P., Bustamante, C.A., Anzueto, A., Levine, S.M., Peters, J.I., Aliberti, S., Sibila, O., Chalmers, J.D., Torres, A., Waterer, G.W., Martin-Loeches, I., Bordon, J., Blanquer, J., Sanz, F., Marcos, P.J., Rello, J., Ramirez, J., Solé-Violán, J., Luna, C.M., Feldman, C., Witzenrath, M., Wunderink, R.G., Stolz, D., Wiemken, T.L., Shindo, Y., Dela Cruz, C.S., Orihuela, C.J. & Restrepo, M.I. 2016. Endothelial adhesion molecules and multiple organ failure in patients with severe sepsis. *Cytokine* 88: 267-273.

Astuti, K.W., Wijayanti, N.P.A.D., Yustiantara, P.S., Laksana, K.P. & Putra, P.S.A. 2019. Anti-inflammatory activity of mangosteen (*Garcinia mangostana* Linn.) rind extract nanoemulgel and gel dosage forms. *Biomedical and Pharmacology Journal* 12(4): 1767-1774.

Berrouet, C., Dorilas, N., Rejniak, K.A. & Tuncer, N. 2020. Comparison of drug inhibitory effects IC_{50} in monolayer and spheroid cultures. *Bulletin of Mathematical Biology* 82(6): 68.

Catalán, Ú., Fernández-Castillejo, S., Pons, L., Heras, M., Aragonés, G., Anglès, N., Morelló, J-R. & Solà, R. 2012. Alpha-tocopherol and BAY 11-7082 reduce vascular cell adhesion molecule in human aortic endothelial cells. *Journal of Vascular Research* 49(4): 319-328.

Chu, L-Y., Hsueh, Y-C., Cheng, H-L. & Wu, K.K. 2017. Cytokine-induced autophagy promotes long-term VCAM-1 but not ICAM-1 expression by degrading late-phase IκBα. *Scientific Reports* 7(1): 12472.

Dayang, E-Z., Luxen, M., Kuiper, T., Yan, R., Rangarajan, S., van Meurs, M., Moser, J. & Molema, G. 2021. Pharmacological inhibition of focal adhesion kinase 1 (FAK1) and anaplastic lymphoma kinase (ALK) identified via kinome profile analysis attenuates lipopolysaccharide-induced endothelial inflammatory activation. *Biomedicine & Pharmacotherapy* 133: 111073.

Dayang, E-Z., Plantinga, J., ter Ellen, B., van Meurs, M., Molema, G. & Moser, J. 2019. Identification of LPS-activated endothelial subpopulations with distinct inflammatory phenotypes and regulatory signaling mechanisms. *Front. Immunol.* 10: 1169. doi: 10.3389/fimmu.2019.01169

Dri, E., Lampas, E., Lazaros, G., Lazarou, E., Theofilis, P., Tsioufis, C. & Tousoulis, D. 2023. Inflammatory mediators of endothelial dysfunction. *Life* doi:10.3390/life13061420

Guo, M., Wang, X., Lu, X., Wang, H. & Brodelius, P.E. 2016. α-mangostin extraction from the native mangosteen (*Garcinia mangostana* L.) and the binding mechanisms of α-mangostin to HSA or TRF. *PLoS ONE* 11(9): e0161566.

Herrera-Aco, D.R., Medina-Campos, O.N., Pedraza-Chaverri, J., Sciutto-Conde, E., Rosas-Salgado, G. & Fragoso-González, G. 2019. Alpha-mangostin: Anti-inflammatory and antioxidant effects on established collagen-induced arthritis in DBA/1J mice. *Food and Chemical Toxicology* 124: 300-315.

John, O., Mushunje, A., Surugau, N. & Guad, R. 2022. The metabolic and molecular mechanisms of α-Mangostin in cardiometabolic disorders (review). *International Journal of Molecular Medicine* 50(3): 120-157.

Kiatsoonthon, K., Phimthong, N., Potikanond, S., Wikan, N. & Nimlamool, W. 2024. Panduratin A inhibits TNF alpha-stimulated endothelial cell activation through suppressing the NF-κB pathway. *Biomolecules* 15(1): 34-49.

Kim, Y-S., Jang, J-H., Koh, J-T., Hwang, Y-C., Oh, W-M. & Lee, B-N. 2023. Alpha-mangostin suppresses LPS-induced inflammation in human dental pulp cells. *Applied Sciences* 13(2): 681-690.

Lee, H.N., Jang, H.Y., Kim, H.J., Shin, S.A., Choo, G.S., Park, Y.S., Kim, S.K. & Jung, J.Y. 2016. Antitumor and apoptosis-inducing effects of α-mangostin extracted from the pericarp of the mangosteen fruit (*Garcinia mangostana* L.) in YD-15 tongue mucoepidermoid carcinoma cells. *International Journal of Molecular Medicine* 37(4): 939-948.

Li, M., van Esch, B.C.A.M., Henricks, P.A.J., Garssen, J. & Folkerts, G. 2018. Time and concentration dependent effects of short chain fatty acids on lipopolysaccharide- or tumor necrosis factor α-induced endothelial activation. *Frontiers in Pharmacology* 9: 0023.

Li, S., Xu, J., Yao, W., Li, H., Liu, Q., Xiao, F., Irwin, M.G., Xia, Z. & Ruan, W. 2015. Sevoflurane pretreatment attenuates TNF-α-induced human endothelial cell dysfunction through activating eNOS/NO pathway. *Biochemical and Biophysical Research Communications* 460(3): 879-886.

Liu, Y. & Chen, L. 2024. Impact of interleukin 6 levels on acute lung injury risk and disease severity in critically ill sepsis patients. *World Journal of Clinical Cases* 12(23): 5374-5381.

Liu, T., Zhang, L., Joo, D. & Sun, S-C. 2017. NF-κB signaling in inflammation. *Signal Transduction and Targeted Therapy* 2(1): 17023.

Madan, B., Singh, I., Kumar, A., Prasad, A.K., Raj, H.G., Parmar, V.S. & Ghosh, B. 2002. Xanthones as inhibitors of microsomal lipid peroxidation and TNF-α induced ICAM-1 expression on human umbilical vein endothelial cells (HUVECs). *Bioorganic & Medicinal Chemistry* 10(11): 3431-3436.

Majdalawieh, A.F., Khatib, B.K. & Terro, T.M. 2025. α-mangostin is a xanthone derivative from mangosteen with potent immunomodulatory and anti-inflammatory properties. *Biomolecules* 15(5): 681.

Majdalawieh, A.F., Terro, T.M., Ahari, S.H. & Abu-Yousef, I.A. 2024. α-mangostin: A xanthone derivative in mangosteen with potent anti-cancer properties. *Biomolecules* 14(11): 1382.

Maneta, E., Aivalioti, E., Tual-Chalot, S., Emini Veseli, B., Gatsiou, A., Stamatelopoulos, K. & Stellos, K. 2023. Endothelial dysfunction and immunothrombosis in sepsis. *Frontiers in Immunology* 14: 1144229.

Maruhashi, T. & Higashi, Y. 2021. Pathophysiological association between diabetes mellitus and endothelial dysfunction. *Antioxidants* 10(8): 1306.

McMullan, R.R., McAuley, D.F., O'Kane, C.M. & Silversides, J.A. 2024. Vascular leak in sepsis: Physiological basis and potential therapeutic advances. *Critical Care* 28(1): 97.

Mohan, S., Syam, S., Abdelwahab, S.I. & Thangavel, N. 2018. An anti-inflammatory molecular mechanism of action of α-mangostin, the major xanthone from the pericarp of *Garcinia mangostana*: an *in silico, in vitro* and *in vivo* approach. *Food & Function* 9(7): 3860-3871.

Setiawan, A.A., Budiman, J. & Prasetyo, A. 2023. Anti-inflammatory potency of mangosteen (*Garcinia mangostana* L.): A systematic review. *Open Access Macedonian Journal of Medical Sciences* 11(Feb): 58-66.

Singh, V., Kaur, R., Kumari, P., Pasricha, C. & Singh, R. 2023. ICAM-1 and VCAM-1: Gatekeepers in various inflammatory and cardiovascular disorders. *Clinica Chimica Acta* 548: 117487.

Sowmya, V., Kalekhan, F., Kamath, K. & Baliga, M.S. 2015. Fruits in the prevention of cataractogenesis by targeting the aldose reductase: Promise from preclinical observations. In *Foods and Dietary Supplements in the Prevention and Treatment of Disease in Older Adults,* edited by Watson, R.R. Academic Press. pp. 105-109.

Tatiya-aphiradee, N., Chatuphonprasert, W. & Jarukamjorn, K. 2019. Anti-inflammatory effect of *Garcinia mangostana* Linn. pericarp extract in methicillin-resistant *Staphylococcus aureus*-induced superficial skin infection in mice. *Biomedicine & Pharmacotherapy* 111: 705-713.

Wang, Y., Cao, J., Fan, Y., Xie, Y., Xu, Z., Yin, Z., Gao, L. & Wang, C. 2016. Artemisinin inhibits monocyte adhesion to HUVECs through the NF-κB and MAPK pathways *in vitro*. *International Journal of Molecular Medicine* 37(6): 1567-1575.

World Health Organization. 2024. *Sepsis.* https://www.who.int/news-room/fact-sheets/detail/sepsis

Widowati, W., Darsono, L., Suherman, J., Fauziah, N., Maesaroh, M. & Erawijantari, P.P. 2016. Anti-inflammatory effect of mangosteen (*Garcinia mangostana* L.) peel extract and its compounds in LPS-induced RAW264.7 cells. *Natural Product Sciences* 22(3): 147.

Yan, S., Zhang, X., Zheng, H., Hu, D., Zhang, Y., Guan, Q., Liu, L., Ding, Q. & Li, Y. 2015. Clematichinenoside inhibits VCAM-1 and ICAM-1 expression in TNF-α-treated endothelial cells via NADPH oxidase-dependent IκB kinase/NF-κB pathway. *Free Radical Biology and Medicine* 78: 190-201.

Yiemwattana, I. & Kaomongkolgit, R. 2015. Alpha-mangostin suppresses IL-6 and IL-8 expression in *P. gingivalis* LPS-stimulated human gingival fibroblasts. *Odontology* 103(3): 348-355.

Zaine, N.F.Z., Zamakshshari, N.H., Abd Halim, A.N., Yi Mian, V.J. & Ngui Sing, N. 2025. Isolation, derivatization, and anti-microbial evaluation of secondary metabolites from *Garcinia dryobalanoides*. *Natural Product Research* 39(22): 6383-6389.

Zhang, Y., Liu, H., Tang, W., Qiu, Q. & Peng, J. 2020. Resveratrol prevents TNF-α-induced VCAM-1 and ICAM-1 upregulation in endothelial progenitor cells via reduction of NF-κB activation. *Journal of International Medical Research* 48(9): 0300060520945131.

Zhong, X., Li, X., Liu, F., Tan, H. & Shang, D. 2012. Omentin inhibits TNF-α-induced expression of adhesion molecules in endothelial cells via ERK/NF-κB pathway. *Biochemical and Biophysical Research Communications* 425(2): 401-406.

Zou, W., Yin, P., Shi, Y., Jin, N., Gao, Q., Li, J. & Liu, F. 2019. A novel biological role of α-mangostin via TAK1–NF-κB pathway against inflammatory. *Inflammation* 42(1): 103-112.

*Corresponding author; email: ahdezulaikha@unimas.my

Supplementary Data

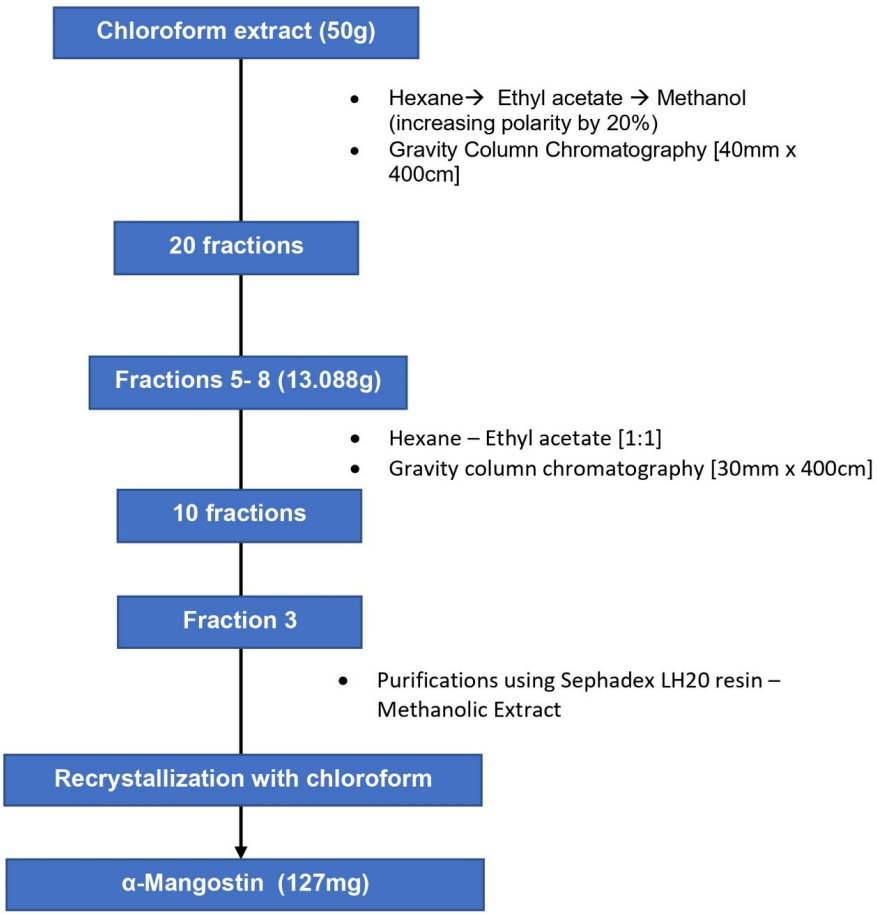

FIGURE S1. Schematic Diagram of Purification Process of α- Mangostin from *Garcinia mangostana* Extract