



FULL ARTICLE

The role of myricetin from *Rhodomyrtus tomentosa* (Aiton) Hassk fruits on downregulation of FcεRI-mediated mast cell activation

Thanh Sang Vo¹ | Tin Thanh Le² | So-Yeon Kim³ | Dai-Hung Ngo⁴¹NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam²Faculty of Chemistry, Ho Chi Minh City University of Education, Ho Chi Minh City, Vietnam³Food Safety and Processing Research Division, National Institute of Fisheries Science, Busan, South Korea⁴Faculty of Natural Sciences, Thu Dau Mot University, Thu Dau Mot City, Vietnam**Correspondence**Thanh Sang Vo, NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.
Email: vtsang@ntt.edu.vnDai-Hung Ngo, Faculty of Natural Sciences, Thu Dau Mot University, Thu Dau Mot City, Binh Duong Province, Vietnam.
Email: hungnd@tdmu.edu.vn**Funding information**

National Foundation for Science and Technology Development, Grant/Award Number: 106-NN.02-2016.68

Abstract

Rhodomyrtus tomentosa was reported to contain various bioactive metabolites, especially phenolic compounds. In the present study, the suppressive activity of phenolic compound from *R. tomentosa* fruits on mast cell activation was investigated in vitro. The result showed that myricetin was isolated from *R. tomentosa* fruits and its characterization was identified by nuclear magnetic resonance spectroscopy. Notably, myricetin was found to be effective in inhibition of mast cell degranulation by attenuating the release of β -hexosaminidase and the elevation of intracellular calcium. Moreover, myricetin exhibited inhibitory effect on the production of IL-4 and Tumor necrosis factor alpha (TNF- α) in a concentration-dependent manner. Furthermore, high antioxidant activity of myricetin due to scavenging 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and ABTS⁺ radicals was also evidenced. Notably, the activation of FcεRI-mediated signaling molecules including Syk, PLC γ , and NF- κ B was also suppressed by myricetin treatment. Accordingly, myricetin from *R. tomentosa* fruits could be suggested as a functional food for the amelioration of allergic diseases.

Practical applications

Polyphenol have been shown to exert various biological activities and health beneficial effects. Results from the present study revealed that myricetin from *R. tomentosa* fruits possesses the inhibitory effect on allergic response in mast cells. Therefore, myricetin from *R. tomentosa* fruits could be developed as a functional ingredient for the amelioration of allergic diseases.

KEYWORDSallergy, degranulation, mast cells, myricetin, *Rhodomyrtus tomentosa*

1 | INTRODUCTION

Mast cells exert an important function in allergic inflammatory reaction due to the pathogenesis of various disorders such as allergic asthma, food allergies, anaphylaxis, and atopic dermatitis (Kumar & Sharma, 2010). The activation of mast cells is triggered through cross-linking of immunoglobulin E by allergens on cell surface FcεRI

receptor. It associates to activation of tyrosine kinase, production of reactive oxygen species (ROS), phosphorylation of mitogen-activated protein kinases (MAPKs), translocation of nuclear factor NF- κ B, and induction of degranulation (Gilfillan & Tkaczyk, 2006; Kalesnikoff & Galli, 2008). Notably, the degranulation of mast cell leads to the release of different inflammatory mediators, especially histamine and cytokines (Turner & Kinet, 1999). Numerous acute

and chronic allergic responses such as airway constriction, mucous production, recruitment of major effector cells into tissue sites, and enhancement of pro-inflammatory cytokine production are induced by these mediators (Galli, Tsai, & Piliponsky, 2008;utel, Blaser, & Akdis, 2005). Hence, mast cell degranulation is considered as a therapeutic target for the development of various anti-allergic drugs so far (Siebenhaar, Redegeld, Bischoff, Gibbs, & Maurer, 2018).

Rhodomirtus tomentosa belongs to Myrtaceae family, distributing from Southern to Southeastern Asia. It has long been used as herbal medicine for treatment of diarrhea, dysentery, gynaecopathy, and stomachache in Vietnam, China, Thailand, and Malaysia (Vo, Ngo, Ngo, & Kim, 2019). Recently, *R. tomentosa* has got much attention by researchers due to its nutritional values, phytochemical compositions, and biological activities. A large source of bioactive compounds such as triterpenes, steroids, and phenolics from *R. tomentosa* has been identified in numerous studies (Hamid, Mutazah, & Yusoff, 2017). Moreover, various pharmaceutical values of *R. tomentosa* including anti-inflammatory, antioxidant, antimicrobial, anticancer, anti-diabetic, anti-ulcerogenic, antidepressant, and anti-atherosclerotic activities have been evidenced in different experimental models (Vo & Ngo, 2019). In our previous report, *R. tomentosa* fruit extract (RFE) has been shown to suppress allergic responses in the activated mast cells (Vo, Kim, Ngo, & Ngo, 2019). This activity of RFE was suggested to be due to its biologically active components, especially phenolic compounds. Therefore, the present study was proposed to isolate bioactive polyphenol as well as evaluate its inhibitory activity on mast cell activation.

2 | MATERIALS AND METHODS

2.1 | Reagents

IL-4 and TNF- α kits were purchased from R&D Systems (Minneapolis, MN, USA). Antibodies including p-IkB- α , p50, p65, and β -actin were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA) and p-Syk and p-PLC- γ were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), DNP-BSA, Fura-3/AM, DPPH, and ABTS were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 | Extraction and isolation

The *R. tomentosa* fruit (1 kg) was air-dried under shade and powdered using a grinder before extracted with ethyl acetate (EtOAc) at 40°C for 24 hr. Crude extract was evaporated under vacuum to achieved 11.2 g EtOAc extract. This extract (1 g) was suspended in ethyl acetate and separated by silica gel column. It was respectively eluted with hexan-chloroform (10:0 to 2:8), chloroform-ethyl acetate (10:0 to 2:8), ethyl acetate-ethanol (10:0 to 2:8), and ethanol to achieved 16 fractions. The fraction of ethyl acetate-ethanol

(8:2) was subjected to Supelco column (250 $\times$ 21.2 mm, 10 μ m) of HPLC (Shimadzu LC 8A, Japan), eluted with H₂O-acetonitril (90:10 to 5:95), and detected at 360 nm to obtain compound A (15.1 mg). The compound A was characterized by high-resolution MicroTOF QII Mass Spectrometer (Bruker Daltonics, Bremen, Germany) and NMR (Bruker, 20251 Hamburg, Germany).

2.3 | Cell culture and cell viability

RBL-2H3 cells (Korean Cell Line Bank, Seoul, Korea) were cultured in a humidified atmosphere containing 5% of CO₂ at 37°C using Dulbecco's modified eagle medium (DMEM) supplemented with 5% of heat-inactivated fetal bovine serum (FBS), 10 mM of HEPES buffer, 100 U/ml of penicillin G, and 100 mg/ml of streptomycin.

MTT assay was applied to examine the cell viability. Briefly, RBL-2H3 cells (2×10^4 cells/ml) were exposed to myricetin (10, 20, and 40 μ M) for 24 hr. The cell culture DMEM was then replaced by MTT solution (1 mg/ml). After 4 hr of incubation, dimethyl sulfoxide (100 μ l) was supplemented and the cell viability was measured at 540 nm under a microplate reader (GENios® Tecan Austria GmbH, Austria).

2.4 | Degranulation assay

RBL-2H3 cells (1×10^5 cells/ml) were exposed to myricetin (10, 20, and 40 μ M) for 24 hr. The cells were then sensitized with DNP-specific IgE antibody (1 μ g/ml) and incubated with DNP-BSA (1 μ g/ml) for 60 min. The release level of β -hexosaminidase was measured and calculated as previously described (Vo, Kong, & Kim, 2011). The inhibition of β -hexosaminidase release were expressed as a percentage compared to control: inhibition (%) = $[1 - (\text{absorbance of tested sample} - \text{absorbance of blank}) / (\text{absorbance of control} - \text{absorbance of blank})] \times 100$.

2.5 | The intracellular Ca²⁺ elevation assay

RBL-2H3 cells (1×10^3 cells/ml) were exposed to myricetin (40 μ M) for 24 hr prior to incubating DNP-specific IgE antibody. Fura-3/AM (2 μ M) was then added for 60 min at 37°C before exposed to DNP-BSA for 10 min. The cells were finally fixed by paraformaldehyde 3% and visualized under a fluorescence microscope (CTR 6000, Leica, Wetzlar, Germany).

2.6 | Measurement of cytokine production

RBL-2H3 cells (2×10^5 cells/ml) were exposed to myricetin (10, 20, and 40 μ M) for 24 hr. The cells were then sensitized with DNP-specific IgE antibody (1 μ g/ml) and incubated with DNP-BSA

(1 µg/ml) for 24 hr. The amount of TNF-α and IL-4 production in culture supernatant was measured regarding the protocol of R&D systems.

2.7 | DPPH scavenging assay

The assay was examined as previously described (Vo, Le, & Ngo, 2018). Briefly, 100 µl of myricetin (2–16 µM) was mixed with 100 µl of DPPH solution. After 30 min of incubation, the absorbance of the mixture was measured at 490 nm by using Genova Nano (Jenway, UK). The scavenging activity was identified as shown below:

$$\text{DPPH scavenging activity} = \left[(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}) / \text{OD}_{\text{control}} \right] \times 100\%$$

2.8 | 2,2-azinobis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS) scavenging assay

The assay was examined as described by Vo et al. (2018). Briefly, 900 µl of ABTS⁺ solution was mixed with 100 µl of myricetin (2–16 µM) for 45 s. Measurement was taken immediately at 734 nm after 15 min of incubation. The scavenging activity was identified as shown below:

$$\text{ABTS}^+ \text{ scavenging activity} = \left[(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}) / \text{OD}_{\text{control}} \right] \times 100\%$$

2.9 | Western blot analysis

RBL-2H3 cells were exposed to myricetin (10, 20, and 40 µM) for 24 hr. The cells were then sensitized with DNP-specific IgE antibody (1 µg/ml) and incubated with DNP-BSA (1 µg/ml) for 10 min. RIPA lysis buffer was applied for cell lysis, and the target protein was detected as described by Vo and colleagues (2011).

2.10 | Statistical analysis

Data were analyzed using Pair sample *T* test of statistical package for the social sciences (SPSS). The statistical differences among groups were considered significant at $p < .05$.

3 | RESULTS AND DISCUSSION

3.1 | Structure evaluation of myricetin from *R. tomentosa*

Compound A was obtained as yellow needles. The ¹H-NMR and ¹³C-NMR spectrum were showed in Figure 1. The ¹H-NMR spectrum showed a down field signal at δ 12.15 of a chelated hydroxyl group (1H, s, 5-OH). It also exhibited a characteristic *meta*-coupling proton signal at δ 6.26 (1H, d, *J* = 2.0 Hz, H-6) and 6.50 (1H, d, *J* = 2.0 Hz, H-8) for the A-ring of flavonoid. The AX coupling system at δ 7.42 (2H, s) was assigned to H-2' and H-6' of B ring. The ¹³C-NMR spectrum showed 13 signals for 15 carbons of a flavone skeleton including a conjugated ketone group at δ_c 176.6 (C-4) (Table 1). By comparing the NMR spectral data with those reported in literature, the structure of compound A was determined as myricetin (Figure 2) (Mohammed, 2015).

3.2 | The inhibitory effect of myricetin on mast cell degranulation

Mast cells were found to express high level of FcεRI receptor and play a key function in allergic reactions (Gurish & Austen, 2001). The activation of mast cells causes the elevation of intracellular calcium, followed by the degranulation and the release of various pro-inflammatory

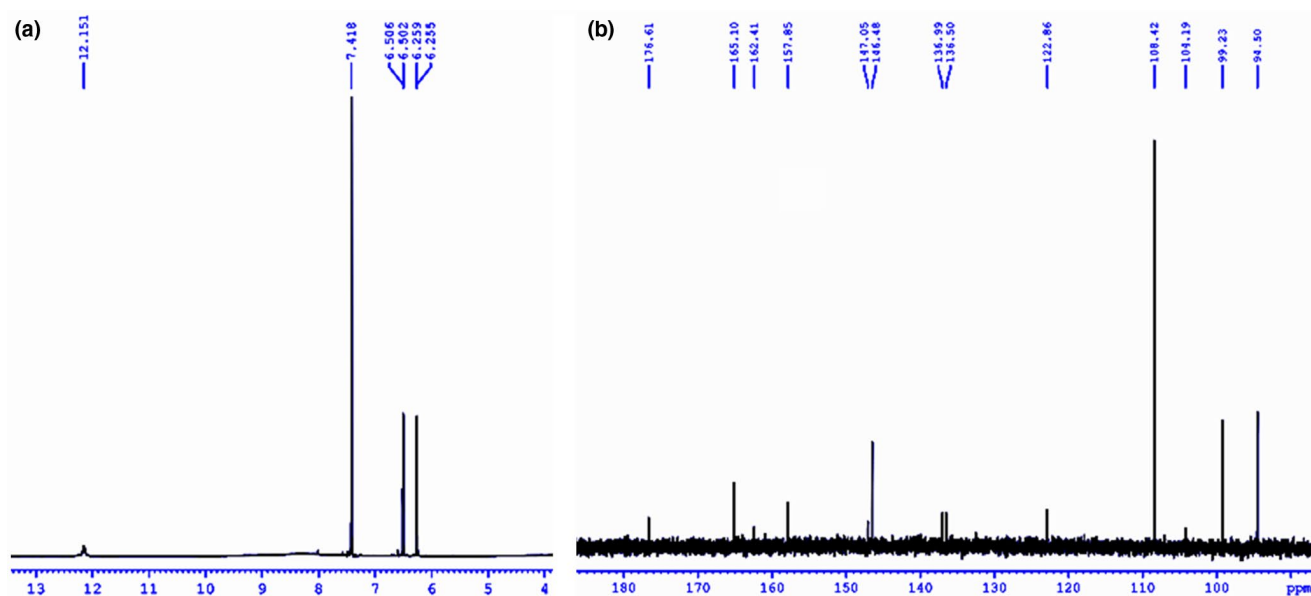


FIGURE 1 Spectrum ¹H-NMR (a) and ¹³C-NMR (b) of myricetin from *R. tomentosa* fruits

TABLE 1 ^1H (500 MHz) and ^{13}C (125 MHz) NMR data for compound A in acetone- d_6

C	Compound A		Myricetin ^a	
	δ_{H} (mult., $J = \text{Hz}$)	δ_{C}	δ_{H} (mult., $J = \text{Hz}$)	δ_{C}
2	—	147.1	—	147.1
3	—	136.5	—	136.1
4	—	176.6	—	176.0
5	—	162.4	—	160.9
6	6.26 (1H, d, 2.0)	99.2	6.13 (brs)	98.6
7	—	165.1	—	164.4
8	6.50 (1H, d, 2.0)	94.5	6.41 (brs)	93.7
9	—	157.9	—	156.5
10	—	104.2	—	103.2
1'	—	122.9	—	121.1
2', 6'	7.42 (2H, s)	108.4	7.17 (s)	107.5
3', 5'	—	146.5	—	146.0
4'	—	137.0	—	136.2
5-OH	12.15 (1H, s)	—	—	—

^a δ_{H} and δ_{C} of myricetin recorded in DMSO- d_6 .

mediators (Galli & Tsai, 2012). Especially, β -hexosaminidase is considered as a marker of mast cell degranulation and often used to screen anti-allergic agents from natural products (Fukuishi et al., 2014). In this study, the suppressive effect of myricetin on mast degranulation was examined via measuring β -hexosaminidase release from RBL-2H3 cells. The result showed that myricetin was effective in suppression of mast cell degranulation in a concentration-dependent manner. The β -hexosaminidase release was inhibited up to 21%, 43%, and 68% in the presence of myricetin (10, 20, and 40 μM , respectively) (Figure 3a). According to Ko and colleagues, piceatannol (50 μM) inhibited 60% of histamine and β -hexosaminidase release from RBL-2H3 cells (Ko et al., 2013). Likewise, Kim and colleagues have determined that hispidulin (30 μM) inhibited 60% of β -hexosaminidase release from RBL-2H3 cells (Kim, Min, Kim, Kim, & Kwon, 2019). Meanwhile, Weng, Patel, Panagiotidou, and Theoharides (2015) showed that luteolin (100 μM) inhibited about 70% of β -hexosaminidase release from LAD2 cells. On the contrary, tea polyphenols such as epigallocatechin and epicatechin gallate were showed to inhibit 45% and 59% of histamine release at the concentration of 100 μM , respectively (Matsuo, Yamada, Shoji, Mori, & Sugano, 1997). Especially, 6,6'-bieckol (25 μM) and phlorofucofuroeckol B (25 μM) from *Eisenia Arborea* were reported to inhibit 71.1% and 90.4% of β -hexosaminidase release from RBL-2H3 cells (Sugiura et al., 2007). As the result, the inhibition of myricetin on mast cell degranulation was stronger than that of luteolin and tea polyphenols, similar with that of piceatannol and hispidulin, and slightly weaker than that of phlorotannins such as 6,6'-bieckol and phlorofucofuroeckol B. On the contrary, the parallel experiment showed that myricetin significantly decreased intracellular Ca^{2+} elevation in the activated cells

at the concentration of 40 μM (Figure 3b). Indeed, the fluorescence intensity was lowered by myricetin treatment as compared to the control group (absence of myricetin). It was evidenced that intracellular Ca^{2+} elevation is necessary for the degranulation in mast cells (Aketani, Teshima, Umezawa, & Sawada, 2001). Therefore, the inhibition of myricetin on mast cell degranulation may associate to suppression of Ca^{2+} elevation in the IgE-activated mast cells. In agreement with this suggestion, several studies have also reported that the inhibitory activity of a certain agent on mast cell degranulation has been attributed to the suppression of intracellular Ca^{2+} elevation (Fischer, Paulussen, Nico, & Janssen, 1998; Kim, Kim, Kim, Ro, & Jeoung, 2008; Vo, Ngo, & Kim, 2012). So far, various phytochemical components of plants, especially phenolic compounds, were found to be able to stabilize mast cell membrane (Taur & Patil, 2011). Accordingly, myricetin was suggested to stabilize mast cell membrane, thus leading to the prevention of Ca^{2+} influx and intracellular Ca^{2+} elevation, and subsequent inhibition of mast cell degranulation. In order to exclude the inhibition of myricetin on mast cell degranulation due to cytotoxic effect, MTT test was further performed on RBL-2H3 cells. The result showed that myricetin did not cause any cytotoxic effect on RBL-2H3 cells at the concentration of 10, 20, and 40 μM (Figure 3c). It was also reported that myricetin exhibited no cytotoxicity on RAW 264.7 macrophage cells (Tan et al., 2018), human prostate cancer cells (PC-3) (Xu et al., 2013), and human colorectal carcinoma cells (COLO 205) (Ko, Shen, Lee, & Chen, 2005) in a range concentration of 10–50 μM during 24 hr treatment. It indicates that the suppressive activity of myricetin on degranulation in mast cells was not based on its cytotoxic effect.

3.3 | The inhibitory effect of myricetin on cytokine productions

The activation of mast cells triggers the release of both the stored mediators and newly synthesized mediators such IL-4 and TNF- α (Galli et al., 2008). Notably, IL-4 was reported as a key factor in the pathogenesis of allergic diseases due to the increase in IgE synthesis and the promotion of mast cell development (Nabeshima et al., 2005). Meanwhile, TNF- α play important role in immunologic and inflammatory reactions due to induction of other cytokines production and adhesion molecules expression on endothelial cells (Olejnik & Brzezińska-Błaszczak, 1998). Therefore, downregulation of IL-4 and TNF- α may contribute to the amelioration of allergic responses. In this sense, the role of myricetin on IL-4 and TNF- α production from RBL-2H3 cells was investigated. The result showed that the activation of RBL-2H3 cells caused the release of IL-4 and TNF- α up to (16 ± 1.2) and (193 ± 14) pg/ml, respectively. Meanwhile, myricetin treatment significantly reduced IL-4 and TNF- α production up to (6 ± 0.3) and (71 ± 6) pg/ml at the concentration of 40 μM , respectively (Figure 4). Indeed, the cytokine release is caused by NF- κB activation and translocation, leading to the transcription of various cytokine-related genes (Galli & Tsai, 2012). Recently, numerous natural compounds have been evidenced to decrease cytokine generation via suppression of NF- κB activation in Fc ϵ RI-mediated mast cells. Evidently, nothofagin, a dihydrochalcone of unprocessed rooibos tea,

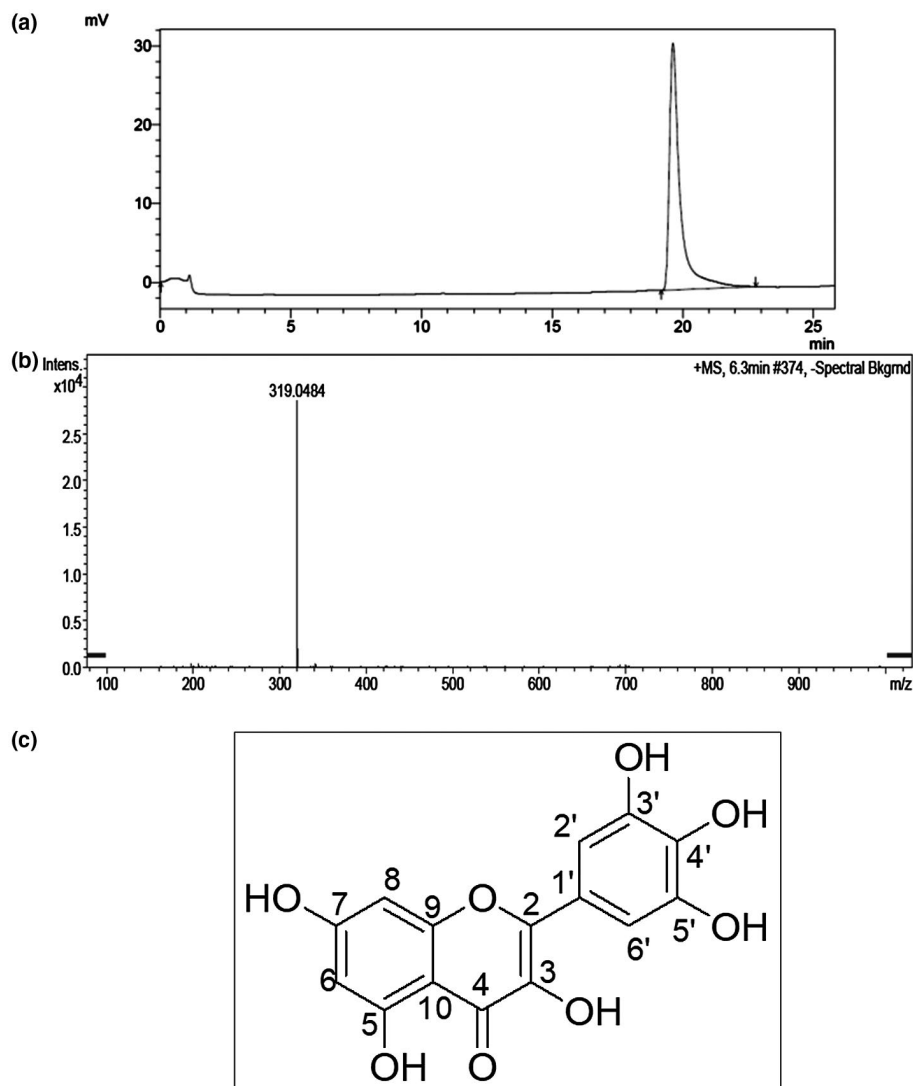


FIGURE 2 HPLC-UV-vis chromatograms (a), mass spectra (b), and chemical structures (c) of myricetin from *R. tomentosa* fruits

was reported to mitigate NF- κ B translocation, causing the diminution of TNF- α and IL-4 expression in antigen-activated RBL-2H3 cells (Kang et al., 2019). Moreover, elaeocarpusin from *Elaeocarpus sylvestris* significantly decreased TNF- α and IL-4 production via suppression of NF- κ B activation in mast cells (Kim et al., 2018). Besides, the suppressive effect of formononetin on NF- κ B activation also contributed to the attenuation of IL-1 β , IL-6, and TNF- α production (Xu & An, 2017). Hence, the suppressive effect of myricetin on IL-4 and TNF- α production was suggested due to inactivation of NF- κ B. Therefore, the regulatory role of myricetin on NF- κ B activation is further elucidated in RBL-2H3 cells.

3.4 | The suppressive effect of myricetin on intracellular signaling molecules

The activation of Fc ϵ RI by allergen-induced crosslinking of IgE causes degranulation and cytokine generation in mast cells (Gilfillan & Tkaczyk, 2006). Indeed, the mast cell degranulation is

associated with Syk-dependent PLC γ activation (de Castro, 2011). The activation of Syk causes PLC γ activation which leads to hydrolysis of phosphatidylinositol 4,5-bisphosphate to form inositol 1,4,5-trisphosphate (IP3). Afterward, IP3 binds to its receptors on endoplasmic reticulum membrane, causing calcium release from the endoplasmic reticulum stores and degranulation (Siraganian, de Castro, Barbu, & Zhang, 2010). In this study, myricetin has been found to mitigate degranulation in the activated RBL-2H3 mast cells. In order to determine whether myricetin suppress Syk-dependent PLC γ activation, western blot analysis was conducted to measure the phosphorylation level of Syk and PLC γ molecules. It was shown that Syk and PLC γ phosphorylation in control group were markedly augmented as compared with that of blank group. Meanwhile, myricetin treatment diminished the level of Syk and PLC γ phosphorylation in a concentration-dependent manner (Figure 5a). Obviously, the inhibition of Syk kinase activity could mitigate the releases of various mediators from granule (Masuda & Schmitz, 2008). Moreover, it was evidenced that the absence

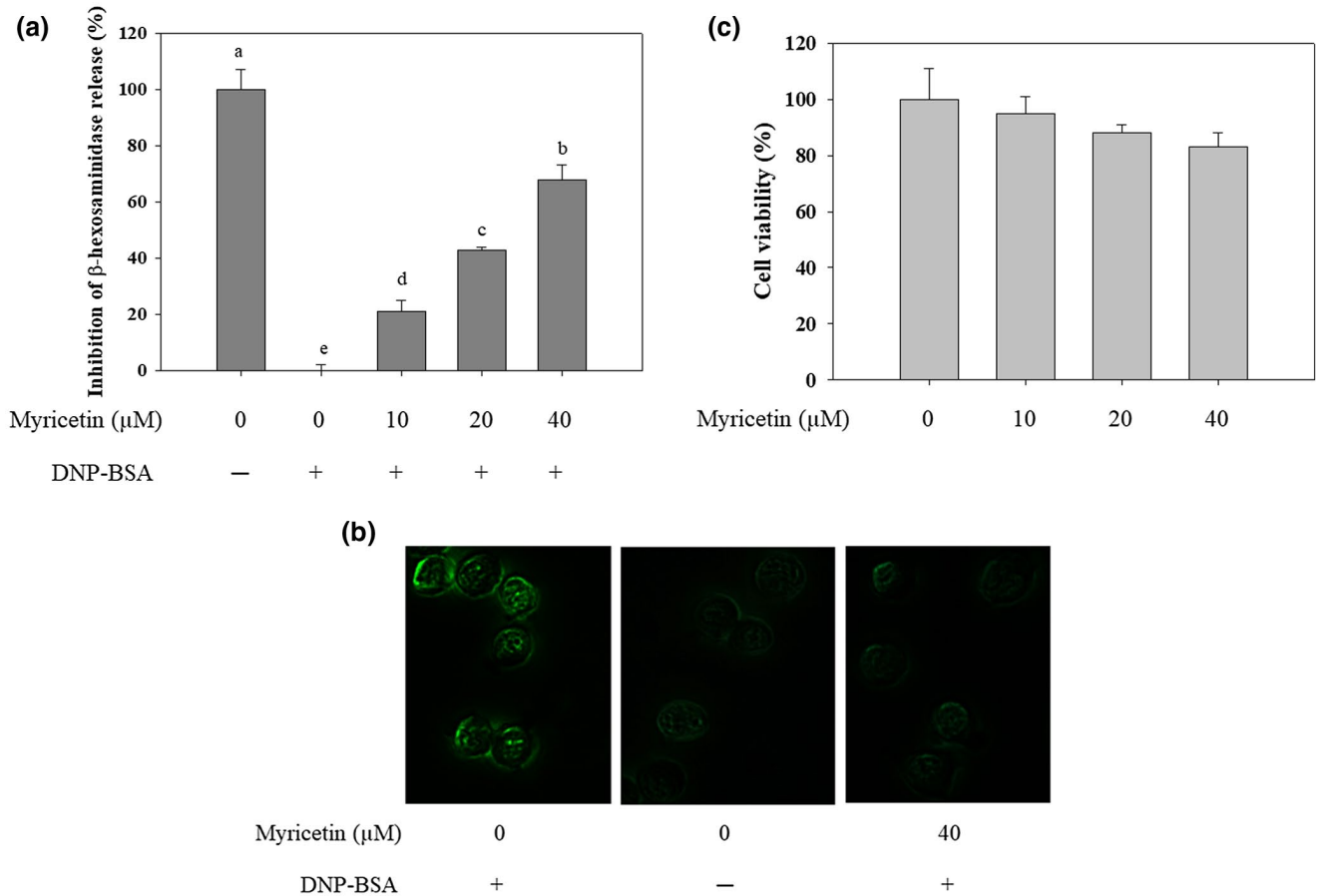


FIGURE 3 The inhibitory effect of myricetin on mast cell activation in Fc ϵ RI-mediated RBL-2H3 cells and the cytotoxic effect of myricetin on RBL-2H3 cells. (a) The cells were pretreated with myricetin and sensitized with DNP-specific IgE antibody before stimulation of antigen DNP-BSA. The levels of β -hexosaminidase releases were measured via a spectrofluorometric assay. (b) RBL-2H3 cells were pretreated with myricetin and sensitized with DNP-specific IgE antibody. The cells were then incubated with Fura-3/AM before stimulated with antigen DNP-BSA. The level of intracellular Ca^{2+} elevation was monitored by a light microscope with 20 $\times$ magnification. (c) RBL-2H3 cells were treated with myricetin for 24 hr. Cell viability was assessed by MTT method, and the results were expressed as percentage of surviving cells over blank cells. Each determination was made in three independent experiments, and the data are shown as means $\pm$ SD. Different letters a–e indicate significant difference among groups ($p < .05$)

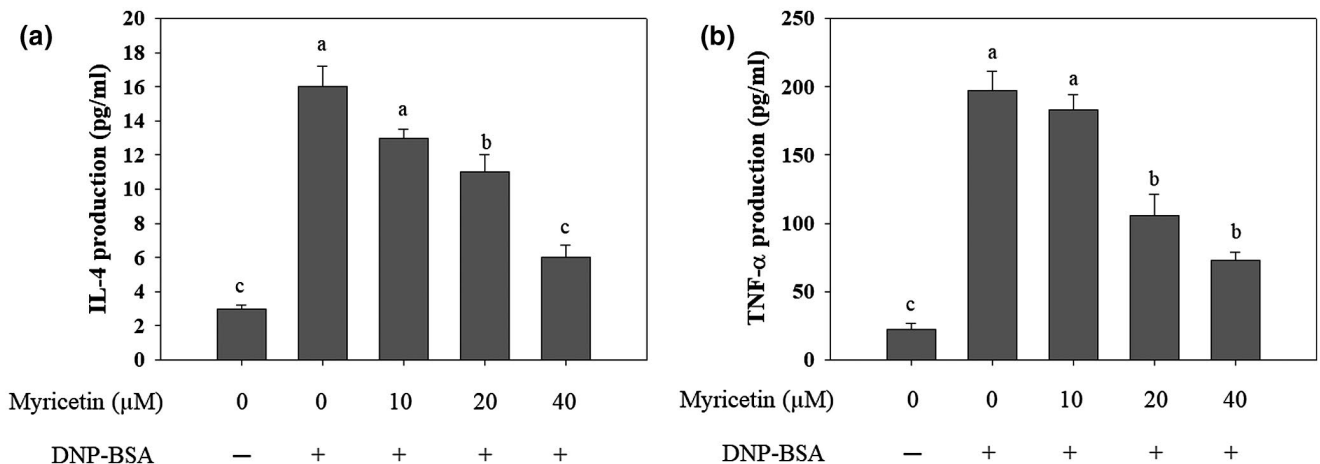


FIGURE 4 Suppressive effect of myricetin on IL-4 and TNF- α production from Fc ϵ RI-mediated RBL-2H3 cell activation. The cells were pretreated with myricetin and sensitized with DNP-specific IgE antibody before stimulation of antigen DNP-BSA. The production levels of IL-4 (a) and TNF- α (b) were quantified in culture media using commercial ELISA kits. Each determination was made in three independent experiments, and the data are showed as means $\pm$ SD. Different letters a–c indicate significant difference among groups ($p < .05$)

of Syk completely abolished degranulation and cytokine release in RBL-2H3 cells (Zhang, Berenstein, Evans, & Siraganian, 1996). These results suggested that myricetin down-regulated Syk activation, leading to suppression of PLC γ activation and subsequent inhibition of calcium elevation and degranulation.

Besides, NF- κ B is well-known to play a key role in the control of gene expression in inflammatory responses. The activation

of NF- κ B leads to the translocation of p50/p65 dimers into the nucleus. Subsequently, it binds to the DNA template and induces cytokine expression, such as TNF- α and IL-4 (Liu, Zhang, Joo, & Sun, 2017). Therefore, the downregulation of NF- κ B activity may attenuate the production and expression of inflammatory cytokines. In this study, western blot analysis was conducted to measure the inhibitory effect of myricetin on NF- κ B translocation in the activated RBL-2H3 cells. Figure 5b showed that the activation of RBL-2H3 cells caused a significant release of I κ B- α and a remarkable increase of p50/p65 subunits. Conversely, myricetin was found to reduce I κ B- α and p50/p65 releases at a dose-dependent manner. It indicates that myricetin was able to inactivate NF- κ B, leading to the inhibition of I κ B- α release and p50/p65 nuclear translocation. Obviously, several polyphenols have been determined to inhibit phosphorylation of kinases, thus preventing the subsequent degradation of I κ B subunit (Karunaweera, Raju, Gyengesi, & Münch, 2015). It was reported that flavonoids could suppress NF- κ B- and Akt-related signaling pathways in murine intestinal epithelial cells (Ruiz & Haller, 2006). Romier and colleagues have evidenced that chrysin could suppress NF- κ B activity by blocking the phosphorylation of I κ B- α subunit (Romier, Van De Walle, During, Larondelle, & Schneider, 2008). Moreover, epigallocatechin gallate and theaflavins were determined to block NF- κ B activity and subsequently attenuate cytokine production (Lagha & Grenier, 2016). As the result, the inactivation of myricetin on NF- κ B activity may contribute to the suppression of IL-4 and TNF- α production in the activated RBL-2H3 cells.

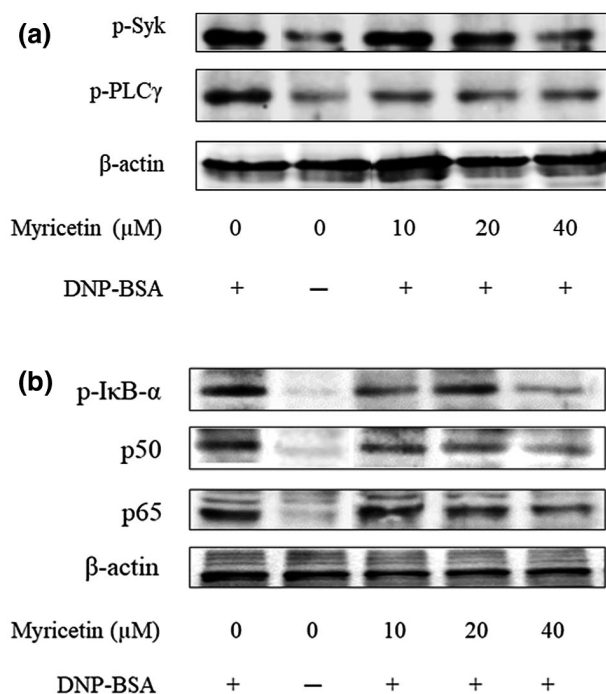


FIGURE 5 The down-regulative effect of myricetin on signaling molecules in Fc ϵ RI-mediated RBL-2H3 cells. The cells were pretreated with myricetin and sensitized with DNP-specific IgE antibody before stimulation of antigen DNP-BSA. The protein expression level was assessed by Western blotting. β -actin was used as internal controls. Each determination was made in three independent experiments

3.5 | Free radical scavenging activity of myricetin

Free radical is a reactive atom containing an unpaired electron in an atomic orbital (Rao, Kalva, Yerramilli, & Mamidi, 2011). It was reported to associate with the upregulation of mast cell activation and degranulation (Mannaioni & Masini, 1988). Especially, the blockade

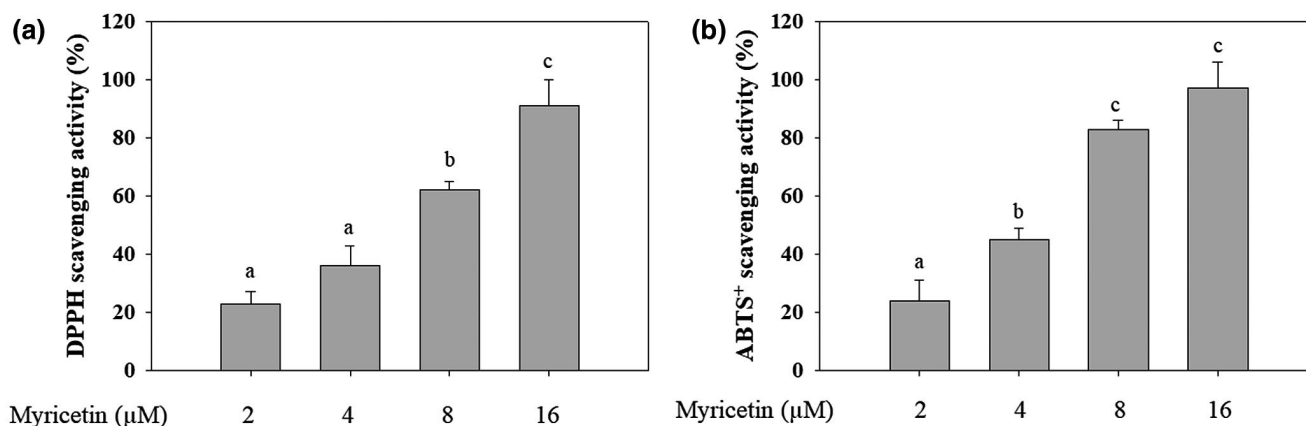


FIGURE 6 DPPH and ABTS⁺ radical scavenging activities of myricetin. (a) Myricetin was incubated with DPPH radical in the dark at room temperature for 30 min and the absorbance of mixture was then measured at 490 nm. (b) Myricetin was incubated with ABTS⁺ radical in the dark at room temperature for 15 min and the absorbance of mixture was then measured at 734 nm. Each determination was made in three independent experiments, and the data are shown as means $\pm$ SD. Different letters a–c indicate significant difference among groups ($p < .05$)

of ROS generation and the scavenging of ROS could impair Ca^{2+} mobilization, thus alleviating mast cell degranulation (Suzuki et al., 2003). Therefore, antioxidants blocking free radical generation as well as scavenging free radicals may contribute to the attenuation of mast cell activation. Herein, the antioxidant activity of myricetin was examined via DPPH and ABTS⁺ radical scavenging assays. Notably, myricetin significantly scavenged DPPH and ABTS⁺ radicals in a concentration-dependent manner (Figure 6). Myricetin scavenged DPPH and ABTS⁺ radicals up to 91% and 97% at the concentration of 16 μM , respectively. Evidently, flavonoid chemically acts as single electron donors, meanwhile it serves as derivative of conjugated ring structures and hydroxyl groups in in vitro cell culture (Gupta, Kumria, Garg, & Gupta, 2010). Its antioxidant activity was found due to rapid donation of a hydrogen atom to radicals and increase in production of intracellular antioxidative enzymes (Gupta et al., 2010). Hence, the high antioxidant activity of flavonoids, especially myricetin, may partly contribute to the suppression of mast cell activation and degranulation in RBL-2H3 cells.

4 | CONCLUSION

In conclusion, the present study has successfully isolated myricetin from *R. tomentosa* fruits and its anti-allergic activity has been evaluated in FcεRI-mediated RBL-2H3 cells. The inhibitory effect of myricetin on mast cell activation was determined via decreasing β-hexosaminidase release, alleviating intracellular Ca^{2+} elevation, and suppressing IL-4 and TNF-α production. The action mechanism of myricetin is suggested due to stabilizing mast cell membrane, blocking NF-κB activation, and scavenging free radicals, thus attenuating mast cell degranulation. However, the further studies using other mast cell lines as well as in vivo models are necessary to confirm the anti-allergic activity as well as the action mechanism of myricetin. Therefrom, the potential application of myricetin derived from *R. tomentosa* fruits as an anti-allergic agent could be proposed reliably.

ACKNOWLEDGMENTS

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106-NN.02-2016.68.

CONFLICTS OF INTEREST

The authors have declared no conflicts of interest for this article.

ORCID

Thanh Sang Vo  <https://orcid.org/0000-0002-6726-3257>

REFERENCES

- Aketani, S., Teshima, R., Umezawa, Y., & Sawada, J.-I. (2001). Correlation between cytosolic calcium concentration and degranulation in RBL-2H3 cells in the presence of various concentrations of antigen-specific IgEs. *Immunology Letters*, 75(3), 185–189. [https://doi.org/10.1016/S0165-2478\(00\)00311-4](https://doi.org/10.1016/S0165-2478(00)00311-4)
- de Castro, R. O. (2011). Regulation and function of syk tyrosine kinase in mast cell signaling and beyond. *Journal of Receptors and Signal Transduction*, 2011, 1–9. <http://dx.doi.org/10.1155/2011/507291>
- Fischer, M. J., Paulussen, J. J., Nico, J., & Janssen, L. H. (1998). Dual effect of the anti-allergic astemizole on Ca^{2+} fluxes in rat basophilic leukemia (RBL-2H3) cells: Release of Ca^{2+} from intracellular stores and inhibition of Ca^{2+} release-activated Ca^{2+} influx. *Biochemical Pharmacology*, 55(8), 1255–1262. [https://doi.org/10.1016/S0006-2952\(97\)00600-X](https://doi.org/10.1016/S0006-2952(97)00600-X)
- Fukuishi, N., Murakami, S., Ohno, A., Yamanaka, N., Matsui, N., Fukutsuji, K., ... Akagi, M. (2014). Does β-hexosaminidase function only as a degranulation indicator in mast cells? The primary role of β-hexosaminidase in mast cell granules. *The Journal of Immunology*, 193(4), 1886–1894. <https://doi.org/10.4049/jimmunol.1302520>
- Galli, S. J., & Tsai, M. (2012). IgE and mast cells in allergic disease. *Nature Medicine*, 18(5), 693–704. <https://doi.org/10.1038/nm.2755>
- Galli, S. J., Tsai, M., & Piliponsky, A. M. (2008). The development of allergic inflammation. *Nature*, 454(7203), 445–454. <https://doi.org/10.1038/nature07204>
- Gilfillan, A. M., & Tkaczuk, C. (2006). Integrated signalling pathways for mast-cell activation. *Nature Reviews Immunology*, 6(3), 218–230. <https://doi.org/10.1038/nri1782>
- Gupta, V. K., Kumria, R., Garg, M., & Gupta, M. (2010). Recent updates on free radicals scavenging flavonoids: An overview. *Asian Journal of Plant Sciences*, 9(3), 108–117. <https://doi.org/10.3923/ajps.2010.108.117>
- Gurish, M. F., & Austen, K. F. (2001). The diverse roles of mast cells. *The Journal of Experimental Medicine*, 194(1), F1–F6. <https://doi.org/10.1084/jem.194.1.F1>
- Hamid, H., Mutazah, S., & Yusoff, M. M. (2017). *Rhodymyrtus tomentosa*: A phytochemical and pharmacological review. *Asian Journal of Pharmaceutical and Clinical Research*, 10(1), 10–16. <https://doi.org/10.22159/ajpcr.2017.v10i1.12773>
- Jutel, M., Blaser, K., & Akdis, C. (2005). Histamine in chronic allergic responses. *Journal of Investigational Allergology and Clinical Immunology*, 15(1), 1–8.
- Kalesnikoff, J., & Galli, S. J. (2008). New developments in mast cell biology. *Nature Immunology*, 9(11), 1215–1223. <https://doi.org/10.1038/ni.f.216>
- Kang, B.-C., Kim, M.-J., Lee, S., Choi, Y.-A., Park, P.-H., Shin, T.-Y., ... Kim, S.-H. (2019). Nothofagin suppresses mast cell-mediated allergic inflammation. *Chemico-Biological Interactions*, 298, 1–7. <https://doi.org/10.1016/j.cbi.2018.10.025>
- Karunaweera, N., Raju, R., Gyengesi, E., & Münch, G. (2015). Plant polyphenols as inhibitors of NF-κB induced cytokine production—A potential anti-inflammatory treatment for Alzheimer's disease? *Frontiers in Molecular Neuroscience*, 8, 24.
- Kim, D. E., Min, K.-J., Kim, M.-J., Kim, S.-H., & Kwon, T. K. (2019). Hispidulin inhibits mast cell-mediated allergic inflammation through down-regulation of histamine release and inflammatory cytokines. *Molecules*, 24(11), 2131. <https://doi.org/10.3390/molecules24112131>
- Kim, K., Kim, Y., Kim, H. Y., Ro, J. Y., & Jeoung, D. (2008). Inhibitory mechanism of anti-allergic peptides in RBL2H3 cells. *European Journal of Pharmacology*, 581(1–2), 191–203. <https://doi.org/10.1016/j.ejphar.2007.11.033>
- Kim, M.-J., Kim, Y.-Y., Choi, Y.-A., Baek, M.-C., Lee, B., Park, P.-H., ... Kim, S.-H. (2018). Elaeocarpusin inhibits mast cell-mediated allergic inflammation. *Frontiers in Pharmacology*, 9, 591. <https://doi.org/10.3389/fphar.2018.00591>
- Ko, C.-H., Shen, S.-C., Lee, T. J., & Chen, Y.-C. (2005). Myricetin inhibits matrix metalloproteinase 2 protein expression and enzyme activity in colorectal carcinoma cells. *Molecular Cancer Therapeutics*, 4(2), 281–290.
- Ko, Y.-J., Kim, H.-H., Kim, E.-J., Katakura, Y., Lee, W.-S., Kim, G.-S., & Ryu, C.-H. (2013). Piceatannol inhibits mast cell-mediated allergic

- inflammation. *International Journal of Molecular Medicine*, 31(4), 951–958. <https://doi.org/10.3892/ijmm.2013.1283>
- Kumar, V., & Sharma, A. (2010). Mast cells: Emerging sentinel innate immune cells with diverse role in immunity. *Molecular Immunology*, 48(1–3), 14–25. <https://doi.org/10.1016/j.molimm.2010.07.009>
- Lagha, A. B., & Grenier, D. (2016). Tea polyphenols inhibit the activation of NF- κ B and the secretion of cytokines and matrix metalloproteinases by macrophages stimulated with *Fusobacterium nucleatum*. *Scientific Reports*, 6, 34520. <https://doi.org/10.1038/srep34520>
- Liu, T., Zhang, L., Joo, D., & Sun, S.-C. (2017). NF- κ B signaling in inflammation. *Signal Transduction and Targeted Therapy*, 2, 17023. <https://doi.org/10.1038/sigtrans.2017.23>
- Mannaioni, P. F., & Masini, E. (1988). The release of histamine by free radicals. *Free Radical Biology & Medicine*, 5(3), 177–197. [https://doi.org/10.1016/0891-5849\(88\)90080-9](https://doi.org/10.1016/0891-5849(88)90080-9)
- Masuda, E. S., & Schmitz, J. (2008). Syk inhibitors as treatment for allergic rhinitis. *Pulmonary Pharmacology and Therapeutics*, 21(3), 461–467. <https://doi.org/10.1016/j.pupt.2007.06.002>
- Matsuo, N., Yamada, K., Shoji, K., Mori, M., & Sugano, M. (1997). Effect of tea polyphenols on histamine release from rat basophilic leukemia (RBL-2H3) cells: The structure-inhibitory activity relationship. *Allergy*, 52(1), 58–64. <https://doi.org/10.1111/j.1398-9995.1997.tb02546.x>
- Mohammed, A.-E.-S.-I. (2015). Phytoconstituents and the study of antioxidant, antimalarial and antimicrobial activities of *Rhus tripartita* growing in Egypt. *Journal of Pharmacognosy and Phytochemistry*, 4(2), 276–281.
- Nabeshima, Y., Hiragun, T., Morita, E., Mihara, S., Kameyoshi, Y., & Hide, M. (2005). IL-4 modulates the histamine content of mast cells in a mast cell/fibroblast co-culture through a Stat6 signaling pathway in fibroblasts. *FEBS Letters*, 579(29), 6653–6658. <https://doi.org/10.1016/j.febslet.2005.09.104>
- Olejnik, A. K., & Brzezińska-Błaszczak, E. (1998). Tumor necrosis factor alpha (TNF- α) modulates rat mast cell reactivity. *Immunology Letters*, 64(2–3), 167–171. [https://doi.org/10.1016/S0165-2478\(98\)00106-0](https://doi.org/10.1016/S0165-2478(98)00106-0)
- Rao, P. S., Kalva, S., Yerramilli, A., & Mamidi, S. (2011). Free radicals and tissue damage: Role of antioxidants. *Free Radicals and Antioxidants*, 1(4), 2–7. <https://doi.org/10.5530/ax.2011.4.2>
- Romier, B., Van De Walle, J., During, A., Larondelle, Y., & Schneider, Y.-J. (2008). Modulation of signalling nuclear factor- κ B activation pathway by polyphenols in human intestinal Caco-2 cells. *British Journal of Nutrition*, 100(3), 542–551. <https://doi.org/10.1017/S0007114508966666>
- Ruiz, P. A., & Haller, D. (2006). Functional diversity of flavonoids in the inhibition of the proinflammatory NF- κ B, IRF, and Akt signaling pathways in murine intestinal epithelial cells. *The Journal of Nutrition*, 136(3), 664–671. <https://doi.org/10.1093/jn/136.3.664>
- Siebenhaar, F., Redegeld, F. A., Bischoff, S. C., Gibbs, B. F., & Maurer, M. (2018). Mast cells as drivers of disease and therapeutic targets. *Trends in Immunology*, 39(2), 151–162. <https://doi.org/10.1016/j.it.2017.10.005>
- Siraganian, R. P., de Castro, R. O., Barbu, E. A., & Zhang, J. (2010). Mast cell signaling: The role of protein tyrosine kinase Syk, its activation and screening methods for new pathway participants. *FEBS Letters*, 584(24), 4933–4940. <https://doi.org/10.1016/j.febslet.2010.08.006>
- Sugiura, Y., Matsuda, K., Yamada, Y., Nishikawa, M., Shioya, K., Katsuzaki, H., ... Amano, H. (2007). Anti-allergic phlorotannins from the edible brown alga, *Eisenia arborea*. *Food Science and Technology Research*, 13(1), 54–60. <https://doi.org/10.3136/fstr.13.54>
- Suzuki, Y., Yoshimaru, T., Matsui, T., Inoue, T., Niide, O., Nunomura, S., & Ra, C. (2003). Fc ϵ RI signaling of mast cells activates intracellular production of hydrogen peroxide: Role in the regulation of calcium signals. *The Journal of Immunology*, 171(11), 6119–6127. <https://doi.org/10.4049/jimmunol.171.11.6119>
- Tan, J.-Y., Chen, X.-Q., Kang, B.-J., Qin, Z.-X., Chen, J.-H., Hu, R.-D., & Wu, L.-C. (2018). Myricetin protects against lipopolysaccharide-induced disseminated intravascular coagulation by anti-inflammatory and anticoagulation effect. *Asian Pacific Journal of Tropical Medicine*, 11(3), 255. <https://doi.org/10.4103/1995-7645.228443>
- Taur, D., & Patil, R. (2011). Mast cell stabilizing and antiallergic activity of *Abrus precatorius* in the management of asthma. *Asian Pacific Journal of Tropical Medicine*, 4(1), 46–49. [https://doi.org/10.1016/S1995-7645\(11\)60030-8](https://doi.org/10.1016/S1995-7645(11)60030-8)
- Turner, H., & Kinet, J.-P. (1999). Signalling through the high-affinity IgE receptor Fc ϵ RI. *Nature*, 402(6760supp), 24–30. <https://doi.org/10.1038/35037021>
- Vo, T. S., Kim, Y.-S., Ngo, D.-N., & Ngo, D.-H. (2019). The role of *Rhodomyrtus tomentosa* (Aiton) Hassk. Fruits in downregulation of mast cells-mediated allergic responses. *BioMed Research International*, 2019, 1–7. <https://doi.org/10.1155/2019/3505034>
- Vo, T. S., Kong, C. S., & Kim, S. K. (2011). Inhibitory effects of chitoooligosaccharides on degranulation and cytokine generation in rat basophilic leukemia RBL-2H3 cells. *Carbohydrate Polymers*, 84(1), 649–655. <https://doi.org/10.1016/j.carbpol.2010.12.046>
- Vo, T. S., Le, P. U., & Ngo, D. H. (2018). The increased gamma-aminobutyric acid content by optimizing fermentation conditions of bacteria from kimchi and investigation of its biological activities. *EurAsian Journal of BioSciences*, 12(2), 369–376.
- Vo, T. S., & Ngo, D. H. (2019). The health beneficial properties of *Rhodomyrtus tomentosa* as potential functional food. *Biomolecules*, 9(2), 76. <https://doi.org/10.3390/biom9020076>
- Vo, T. S., Ngo, D. H., & Kim, S. K. (2012). Gallic acid-grafted chitoooligosaccharides suppress antigen-induced allergic reactions in RBL-2H3 mast cells. *European Journal of Pharmaceutical Sciences*, 47(2), 527–533. <https://doi.org/10.1016/j.ejps.2012.07.010>
- Vo, T. S., Ngo, D. H., Ngo, D. N., & Kim, S.-K. (2019). Investigation of the biological activities of Phu Quoc Sim fruits *Rhodomyrtus tomentosa* (aiton) hassk. *EurAsian Journal of BioSciences*, 13(1), 49–55.
- Weng, Z., Patel, A. B., Panagiotidou, S., & Theoharides, T. C. (2015). The novel flavone tetramethoxyluteolin is a potent inhibitor of human mast cells. *Journal of Allergy and Clinical Immunology*, 135(4), 1044–1052, e1045. <https://doi.org/10.1016/j.jaci.2014.10.032>
- Xu, N., & An, J. (2017). Formononetin ameliorates mast cell-mediated allergic inflammation via inhibition of histamine release and production of pro-inflammatory cytokines. *Experimental and Therapeutic Medicine*, 14(6), 6201–6206. <https://doi.org/10.3892/etm.2017.5293>
- Xu, R., Zhang, Y., Ye, X., Xue, S., Shi, J., Pan, J., & Chen, Q. (2013). Inhibition effects and induction of apoptosis of flavonoids on the prostate cancer cell line PC-3 in vitro. *Food Chemistry*, 138(1), 48–53. <https://doi.org/10.1016/j.foodchem.2012.09.102>
- Zhang, J., Berenstein, E. H., Evans, R. L., & Siraganian, R. P. (1996). Transfection of Syk protein tyrosine kinase reconstitutes high affinity IgE receptor-mediated degranulation in a Syk-negative variant of rat basophilic leukemia RBL-2H3 cells. *The Journal of Experimental Medicine*, 184(1), 71–79. <https://doi.org/10.1084/jem.184.1.71>

How to cite this article: Vo TS, Le TT, Kim S-Y, Ngo D-H. The role of myricetin from *Rhodomyrtus tomentosa* (Aiton) Hassk fruits on downregulation of Fc ϵ RI-mediated mast cell activation. *J Food Biochem*. 2020;00:e13143. <https://doi.org/10.1111/jfbc.13143>