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METHANOL EXTRACT OF *PIPER RETROFRACTUM* VAHL. POTENTIALLY MEDIATES MAST CELL STABILIZATION

NURUL JADID[*1], RISKA Y RACHMAN[1], SYLVIANA ROSYDA HARTANTI[1], NURLITA ABDULGANI[2], WIWI WIKANTA[3] AND WIRDHATUL MUSLIHATIN[1]

[1]Laboratory of Plant Sciences and Biotechnology, Department of Biology, Institut Teknologi Sepuluh Nopember Surabaya, Indonesia
[2]Zoological Laboratory, Department of Biology, Institut Teknologi Sepuluh Nopember Surabaya, Indonesia
[3]Department of Biology, Muhammadiyah University Surabaya, Indonesia

ABSTRACT

Allergy is immune reactions against foreign substances (allergens), which triggers mast cell degranulation. *Piper retrofractum* Vahl is abundantly found in Indonesia and presents high pharmaceutical value. However, no research has been reported its biological effect as an anti-allergy. This research aims to evaluate the bio-potential activity of Methanol Extract of *Piper retrofractum* Vahl (MEPR) in decreasing mast cell degranulation. This research was performed using random complete design with different treatments of MEPR (50, 100, and 200 µg/mL). While, aminophylline coronet crown® 100 µg/mL was used as positive control. Parameters measured are the percentage of mast cell degranulation and cell viability. Our result showed that 100 µg/mL of MEPR decreases effectively the mast cell degranulation (40,96%), which is significantly different compared to non-treated cells (91,39%). Moreover, it displayed slightly different percentage of mast cell degranulation compared to those treated with aminophylline coronet crown® (40,97%). Whereas, mast cells treated with 200 µg/mL of MEPR showed the best result, which possess the lowest percentage of mast cell degranulation 32,83%. In addition, the percentage of cell viability from treatments with 100 and 200 µg/mL of MEPR were 94,10% and 75%, respectively. Taken together, these results suggests that MEPR might potentially mediate mast cell stabilization.

KEY WORDS: **allergy, degranulation, mast cell, *Piper retrofractum* Vahl.**

***Corresponding author**

NURUL JADID
Laboratory of Plant Sciences and Biotechnology, Department of Biology, Institut Teknologi Sepuluh Nopember Surabaya, Indonesia

INTRODUCTION

The prevalence of allergic-based diseases is increasing significantly throughout the world. It affects more than 300 million people and is estimated to nearly double in 2025 [1,2]. Allergic reaction can be generally defined as an overreaction of the immune system against foreign substances (allergen). Many studies have reported the role of immunoglobulin, mast cells, basophils and eosinophils in allergic inflammation responses[3]. However, the principal immunologic components of immediate hypersensitivity are mast cells and immunoglobulin E (IgE)[4]. Mast cells are ovoid or irregularly elongated inflammatory cells that respond to signals of innate and adaptive immunity. The IgE will bind specifically to the epitopes of the allergen. Subsequently, IgE will bind to the high-affinity receptors FcεRI, which are omnipresent in the surface of mast cells. This cross-linking complex leads to the activation of the mast cell, which will be followed by degranulation process. This process is characterized by the release of vasoactive and proinflammatory mediators including histamine, tryptase, IL-6, IL-8 and β-hexosaminidase[3,6-7]. Plants have been considered as one of the promising target for drug development. Recently, the use of traditional plant-based medicine has arisen. However, knowledge about plant-based medicine associated with allergic reaction is extremely limited to a certain family of plants[8]. One example of the medicinal plants, possessing high economical value is pepper plant. They consist of more than thousand species and are distributed mainly in the tropical region, including Thailand, Malaysia, and Indonesia[9]. Some species, such as *Piper betle*, *Piper nigrum, Piper cubeba* L., *Piper longum* and *Piper retrofractum* Vahl. have been commonly used in many pharmaceutical and food industries. Among them, *Piper cubeba* and *Piper longum* have been reported to potentially possess anti-allergic activity[10-11] and inhibit the release of histamines[12], respectively. Meanwhile, little is known about the potential role of *Piper retrofractum* Vahl, which is originally and extensively cultivated in Indonesia, as an anti-allergy. Some previous reports showed that methanol extract of *P. Retrofractum* Vahl contains piperine, oleic acid, *N-isobutyl-2E,* 4*E*,14*Z*-eicosatrienamide and methyl piperate[13]. Our present study aims to find out the effect of methanol extract of *P. retrofractum* in decreasing mast cell degranulation.

MATERIALS AND METHODS

(i) Plant Material and mice

The fruit of the *P. retrofractum* Vahl. were collected from Sumenep Region, Madura Island, Indonesia. The plant was identified following dichotomous key based on *Atlas Tumbuhan Obat*, Flora and *Farmakope Herbal Indonesia*[14-16]. The fruits were washed with tap water and dried under shade for a week[17]. The dried fruits were ground in a waring blender and used subsequently for extraction. 2-3 months of male Balb/c mice were obtained and approved by the University of Airlangga, Indonesia. The average weight of the mice is about 20-30 gr. Mice were acclimatized for 7 days before treatments.

(ii) Preparation of methanolic extract

150 g fruit powder was soaked in 400 ml methanol in a beaker glass and left to macerate in the dark, at ambient temperature for 48 h[18]. The extract was then placed and agitated in erlenmeyer flask for 1 h. The extract was then filtered and subjected to multiple maceration processes (three times per 24 h). Finally, the extract was evaporated using rotary evaporator[19].

(iii) Preparation of sensitized mast cell suspension

Healthy mice with 20-30 gr of weight were injected intraperitoneal with 0.3 ml ovalbumin (OVA). Subsequently, the treated mice were subjected to 0.05 ml of OVA, which was injected intraplantar. Following those treatments, mice were fasted for 18 hours in the day tenth. Subsequently, mice were sacrificed by servical dislocation and injected immediately with 10 ml PBS (Phosphate Buffer Saline) mixed with 0.1% of gelatin and 50 µg/mL *via* the intraperitoneal route. The intraperitoneal fluids were extracted after the intraperitoneal administration and subjected to 15 min of centrifugation 3000 rpm. The supernatant obtained were discharged and the pellets were washed twice with PBS solution.

(iv) Cell viability test

Cell viability test was conducted to analyse that the tested cells would remain intact or not after treatment. We used trypan blue staining method by which the intact cell would be colorless, while degranulated mast cell would be colored (blue). 50 µL of mast cell suspension was placed in the test tube and then homogenized with 200 µg/mL methanol extract. The mixture was then incubated at 37°C during 1 hour. After that, 100 µL of mixture was taken and added with 100 µL of 0,4% trypan blue. The mixture obtained were then dripped on hemocytometer and counted under microscope. The percentage of survival cells were calculated using[20]

$$\% VC = \left[1 - \frac{UVC}{TCC}\right] X\ 100\ \%$$

VC = Viable Cell
UVC = Unviable Cell
TCC = Total Cell Count

(v) Negative and positive control preparation

50 µL of mast cell suspension was mixed with 40 µL of PBS and 10 µL of tolouidin blue (for negative control) and mixed with 30 µL of 1% DMSO, 10 µL OVA and 10 µL of toloudine blue. The mixture was then incubated at 37°C and then dripped on hemocytometer and counted under microscope.

(vi) Treatment of mast cell with aminophylline (coronet crown®)

50 µL of mast cell suspension was mixed with 30 µL of aminophylline 100 µg/mL (coronet crown®), 10 µL OVA and 10 µL of tolouidin blue. The mixture was then incubated at 37°C and then dripped on hemocytometer and counted under microscope.

(vii) Treatment of mast cell with methanol extract of* P. retrofractum Vahl*. fruit

50 µL of mast cell suspension was mixed with 30 µL of extract (50 µg/mL, 100 µg/mL, 200 µg/mL), 10 µL OVA and 10 µL of tolouidin blue. The mixture was then incubated at 37°C for 30 min and then dripped on hemocytometer and counted under microscope. The

percentage of mast cell degranulation was calculated using the formula (below).

$$\% \text{ Mast Cell Degranulation} = \left[\frac{p - s}{p} \right] X\ 100\ \%$$

Note
p = total mast cell before treatment
s = total mast cell after treatment

RESULTS AND DISCUSSION

(i) Effect of methanol extract of* P. retrofractum Vahl. *fruit in decreasing cell mast degranulation in Mus musculus

50% of Chicken-derived OVA was administered as its 10 % of proteins act as an immunogenic allergen[21]. Data from the previous reports, showed that 50% chicken-derived OVA could potentially induce cell mast degranulation[22]. Cell mast suspension was extracted following treatment of chicken-derived OVA.

(viii) Data analysis

Statistical analysis was carried out using SPSS 16.0 software (version 10.0, SPSS Inc., USA). All data were expressed as mean ± standard deviation. The Tukey's test was used to assess the differences between means. P value of < 0.05 was considered to be statistically significant.

Subsequently, percentage of cell mast degranulation was calculated after the inclusion of several concentration of MEPR (Table 1). The average yield of mast cell degranulation on negative control and positive control are 0% and 91, 39%, respectively. Meanwhile, percentage of mast cell degranulation of the treatments with 50, 100, dan 200 µg/mL MEPR and aminophylline coronet crown® 100 µg/mL are 52,79%; 40,96%; 32,83%; 40,97%, respectively. Zero percent of mast cell degranulation in negative control was normal since they were not treated with the chicken-derived OVA.

Table 1
Percentage of cell mast degranulation

Treatments	Cell mast degranulation (%)		Mean ± SD*
	Repetition		
	1	2	
K1	0	0	0^{d} ± 0
K2	91,02	91,75	$91,39^{a}$ ± 0,52
K3	54,46	51,11	$52,79^{b}$ ± 2,37
K4	40,82	41,09	$40,96^{c}$ ± 0,19
K5	31,43	34,23	$32,83^{c}$ ± 1,98
K6	36,63	45,30	$40,97^{c}$ ± 6,13

Note:K1: Negative control (without allergen and MEPR); K2: Positive control (with allergen); K3: Allergen + 50 µg/mL MEPR; K4: Allergen + 100 µg/mL MEPR; K5: Allergen + 200 µg/mL MEPR; K6: Allergen + 100 µg/mL aminophylline coronet crown® ; *Means followed by the same letter are not significantly different (p-value < 0,05) using Tukey's test

Dramatic augmentation of percentage of mast cell degranulation was shown by the positive control treatment. Interestingly, we observed decline of the percentage of mast cell degranulation in treatment with several type of MEPR concentration (p-value<0,05). The best response was showed by treatment with 200 µg/mL of MEPR, which displayed about 32,83% of decline percentage of mast cell degranulation, compared with treatment using 100 µg/mL and 50 µg/. Unexpectedly, treatment with 100 µg/mL aminophylline coronet crown® showed bigger percentage of mast cell degranulation compared with the application of MEPR in 200 µg/mL. This showed that MEPR at 200 µg/mL was better than aminophylline coronet crown® 100 µg/mL, which is known to be mast cell stabilization agent. The overall results showed that higher concentration of MEPR would potentially decrease the percentage of mast cell degranulation (figure 1). It means that MEPR would potentially stabilize mast cell and prevent release of histamine as allergic mediator.

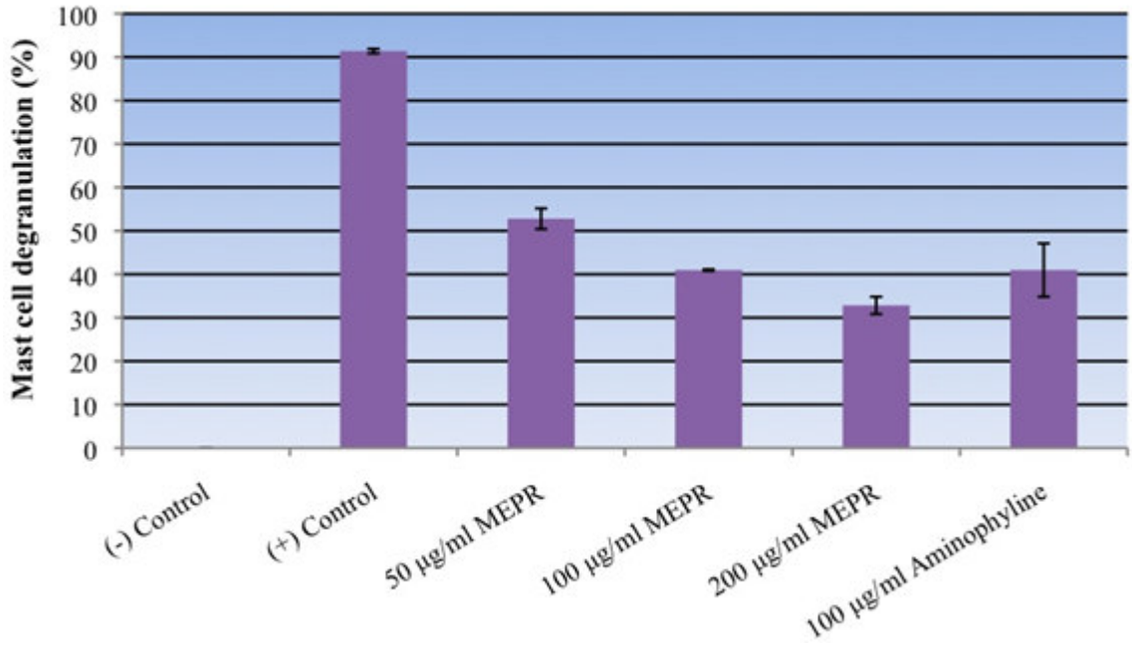

Figure 1

Percentage of mast cell degranulation. The percentage of mast cell degranulation is significantly decreasing in a dose-dependent manner (p-value < 0,05), where 200 µg/mL MEPR performed the best result in decreasing mast cell degranulation (32.83 %) compared to the other tretaments and to the mast cell stabilizing agent (Aminophyline coronet crown®)

(ii) Mast cell viability test

Mast cell viability test was conducted in order to evaluate the toxic effect of the MEPR against mast cell. In this present study, we have chosen 100 µg/mL and 200 µg/mL as the two highest concentration of MEPR. This kind of test is important to compare their toxicity. The result showed that the highest percentage of cell viability was obtained in the treatment with 100 µg/mL MEPR (94.1%). However, treatment with 200 µg/mL also showed good result (75%) (Table 2).

Table 2
Percentage of cell viability

MEPR Concentration	Total cell			% of cell viability	
	Viable	Non viable	Total	non *viable*	*viable*
100 µg/mL	303	19	322	5,9	94,10
200 µg/mL	15	5	20	25	75

Based on the phytochemical test with UV-Vis spectrophotometer, 100 mg of MEPR contains 16.55% alkaloids, 15.03% phenol, 7.11% terpenoids and 2.08% piperidine. Alkaloid was predominant in the MEPR. An example of alkaloids, which is predominantly present in the Piperaceae plants is piperine. Some reports showed that piperine possess an anti-inflammatory activity[23]. Other reports also showed that other alkaloid such as plumerianine, which is found in *Plumeria acutifoliai*[24], Veratroylzygadenine found in *Veratrum nigrum*[25] and dehydrocorydaline found in *Corydalis turtschaninovii*[26] have been reported to inhibit allergic reaction.

CONCLUSION

Our result showed that 200 µg/mL of MEPR is effective in decreasing mast cell degranulation percentage (32.83 %) compared to 100 µg/mL of MEPR and aminophylline coronet crown®, which showed 40,96 % and 40.97 % mast cell degranulation, respectively. In addition, mast cells treated with 200 µg/mL of MEPR showed lower percentage of cell viability (75 %) compared to 100 µg/mL of MEPR (94,1 %).

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REFERENCES

1. Beausoleil JL, Fiedler J, Spergel JM. 2007. Food Intolerence and Childhood Asthma: What is the Link. Pediatr Drugs. 2007 May; 9(3): 157-163.

2. Julia V, Macia L, Dombrowicz D. The Impact of Diet on Asthma and Allergic Diseases. Nat. Rev. Immunol. 2015 May; 15(5): 308-22.

3. Stone KD, Prussin C, Metcalfe DD. IgE, Mast Cell, Basophils, and Eosinophils. J. Allergy Clin. Immunol. 2010 Feb; 125(2 Suppl 2): S73-S80.
4. Gohil PV and Mehta AA. Evaluation of Mast cell Stabilizing and Anti-Anaphylactic Activity of polyherbal Formulation. Adv. Biol. Res. 2011; 5(6): 304-308.
5. White MV. The Role of Histamine in Allergic Diseases. J. Allergy Clin. Immunol. 1990 Oct; 86(4 Pt 2): 599-605.
6. Cheong H, Choi EJ, Yoo GS, Kim KM, and Ryu SY. Desacetylmatricarin, An Anti-Allergic Component from *Taraxacumplatycarpum*. Planta Med. 1998 Aug; 64(6): 577-78.
7. Theoharides TC and Kalogeromitros D. The Critical Role of Mast Cells in Allergy and Inflammation. Ann. N.Y. Acad. Sci. 2006 Nov; 1088: 78-99.
8. Tewtrakul S, and Subhadhirasakul S. Anti-allergic Activity of Some Selected Plants in The Zingiberaceae family. J. Ethnopharmacol. 2007 Feb; 109(3): 535-38.
9. Chaveerach A, Mokkamul P, Sudmoon R, Tanee T. Ethnobotany of the Genus *Piper* (Piperaceae) in Thailand. Ethnobot. Res. App. 2006; 4: 223-231.
10. Choi EM and Hwang JK. Investigations of Anti-inflammatory and Antinociceptive Activities of *Piper cubeba*, *Physalisangulata* and *Rosa hybrida*. J. Ethnopharmacol. 2003 Nov; 89(1): 171-5.
11. Nahak G, and Sahu RK. Phytochemical Evaluation and Antioxidant activity of *Piper cubeba* and *Piper nigrum.* J. Appl. Pharma. Sci. 2011 Oct;1(8): 153-157.
12. Choudhary GP. Mast cell stabilizing activity of *Piper longum* Linn. Indian J. Asthma Allergy and Immunol. 2006; 20(2): 116-20.
13. Fabarani NP. Phytochemicals and antibacterial activity of *Piper retrofractum* Vahl. And *Piper arborescens* ROXB [thesis]. Universiti Teknologi Malaysia; 2012.
14. Dalimartha S. Atlas TumbuhanObat. Jakarta: Trubus Agriwidya; 1999.
15. Steenis CGGJ, Hoe GD, Bloembergen S, Eyme PJ. Flora untuk Sekolah di Indonesia. Jakarta: BalaiPustaka; 2013.
16. Departemen Kesehatan RI. Farmakope Herbal Indonesia. Jakarta: Departemen Kesehatan Republik Indonesia; 2015.
17. Mary LH, Joseph J, Sudarsanam D. Antiproliferative activity of methanolic fruit extract from *Cucurbita digitata*. IJPBS. 2014 Oct; 5(4): (B) 542-547.
18. Lachumyl SJT, Zuraini Z, Sasidharan S.Antimicrobial activity and toxicity of methanol extract of *Cassia fistula* seeds. RJPBCS. 2009 Oct; 1(4): 391-398.
19. Harborne AJ. Phytochemical methods A Guide to Modern Techniques of Plant Analysis. 3rd ed. Netherland: Springer;1998.
20. Alvianti F, Mukhtar R, Marianne. Penghambatan degranulasi mastosit tersensititasi aktif oleh *Curcuma mangga* Val. & Zijp pada mencit secara in vitro. J. Pharm. Pharmacol. 2012 1(1): 44-54.
21. Stevens L. Egg White Proteins. Comp. Biochem. Physiol. B. 1991; 100 (1): 1-9.
22. Gui Y, Li Z, Lin H, Samee H, Khalid J. Studies on The Specific Degranulation of Mast Cell Sensitized by Several allergens *In Vitro*. Cell Mol. Immunol. 2009 Apr; 6(2): 149-53.
23. Ahmad N, Fazal H, Abbasi BH, Farooq S, Ali M, Khan MA. Biological role of *Piper nigrum* L. (Black pepper): A review. Asian Pac. J. Trop. Biomed. 2012 Dec; 2(3): S1945-S1953.
24. Vijayalakshmi A, Ravichandiran V, Velraj M, Hemalatha S, Sudharani G, Jayakumari S. Anti-anaphylactic and anti-inflammatory activities of a bioactive alkaloid from the root bark of *Plumeriaacutifolia*Poir. Asian Pac. J. Trop. Biomed. 2011 May 10; 1(5): 401-405.
25. Cholakova M, Bratanov M, Christov V, Kostova N, Gantcheva M, Nikolova E. The veratrum alkaloid, veratroylzygadenine, suppresses contact dermatitis in mice. J. Med. Plants Res. 2009 Dec; 3(12): 1109-1112.
26. Rìos JL, Bas E, Recio MC. Effect of Natural Products on Contact Dermatitis. Curr. Med. Chem-Anti-Inflammatory & Anti-Allergy Agents 2005 Feb; 4(1): 65-80.