

The Extract of Kincung Flower (*Etlingera elatior* (Jack) R.M.Sm.) Activity to Decrease IL-4 and IgE Levels in Type I Hypersensitivity White Male Mice

Elidahanum Husni*, Relin Yesika, Yufri Aldi

Elidahanum Husni*, Relin Yesika,
Yufri Aldi

Faculty of Pharmacy, University Andalas,
INDONESIA.

Correspondence

Elidahanum Husni

Faculty of Pharmacy, University Andalas,
Indonesia

E-mail: elidahanumhusni@phar.unand.ac.id

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ABSTRACT

Introduction: Kincung Flower (*Etlingera elatior* (Jack) R.M.Sm.) is a herbal plant which contains many secondary metabolites. It showed to suppress allergic reactions by inhibiting mast cell degranulation, active cutaneous anaphylaxis and decreasing the number of basophils and eosinophils. **Aim:** The study conducted to determine the decreased IL-4 and IgE level of type I hypersensitivity male white mice using kincung flowers extract. **Material and Methods:** The maceration method used to make ethanol extract of Kincung flower (*Etlingera elatior* (Jack) R.M.Sm.). The research used 25 allergic male white mice, which made by injected 20% albumen that given on the first day 0.2 mL/20 g intraperitoneally. On the seventh day are given albumen with the same dose subcutaneously. The characteristic of the allergic mice is the redness at the injection site. It divided into five groups: the negative control group, the positive control group and three dose groups (100; 300; and 1000 mg/kg). After mice given extract for seven days, then measured IgE and IL-4 levels in the serum of mice. **Results:** The results after three dose groups (100; 300; and 1000 mg/kg) given, the negative and positive control group showed sequentially the IL-4 level was: 33.024; 27.933; 25.192; 23.130 and 41.538 ng/ mL. And IgE level in serum was 0.944; 0.629; 0.210; and 1,597 µg/ mL. **Conclusion:** It concluded that kincung flowers decreased IL-4 and IgE level significantly ($p < 0.05$). So it could use as an anti-allergic drug.

Key words: Allergies, *Etlingera elatior* (Jack) R.M.Sm, Kincung Flower, IgE, IL-4, Mice.

INTRODUCTION

World Allergy Organization (WAO) estimated that the prevalence of allergic diseases from the entire country's population ranges from 10 - 40%.¹ The incidence of allergic diseases was high enough in various parts of the world, both in developed and developing countries. United Kingdom (UK) had the highest prevalence of allergic diseases in the world; more 20% of the population had one or more allergic disorders. In asthma, Scotland had the highest prevalence with 18.4%, the United Kingdom with 15.3%, Canada with 14.1%, United States with 10.9%, while Indonesia was 1.1%.^{2,3} In rhinitis, The International Study on Asthma and Allergies in Childhood (ISAAC), the prevalence of rhinitis symptoms in children varied between 0.8% to 14.9% at 6 -7 years old and between 1.4% to 39.7% at 13 - 14 years old. Indonesia was one of the countries with low prevalence, besides Albania, Romania, Georgia and Greece. Whereas the countries with high prevalence were Australia, New Zealand and the United Kingdom.⁴

The allergic reaction or type I hypersensitivity is one of the dangerous immune system responses because it could damage tissues, so it became a severe disease and ultimately can cause death.⁵ IgE antibodies are responsible for type I hypersensitivity reactions. IgE antibodies mediate mast cell degranulation reactions, so that mast cells release allergic mediators such as

histamine, serotonin, prostaglandins, and others. If IgE production suppressed, then the antigen reaction with IgE will decrease so that the number of mediators released will decrease. In the production of IgE by plasma cells, the cytokine responsible is IL-4. Plasma cells will produce IL-4 if these cytokine levels are high in the blood and will dominate the binding of B cells. Furthermore, B cells proliferation to plasma cells which produce higher IgE.^{6,7}

During the second exposure, the antigen is immediately bound by IgE, which is on the surface of basophil cells and mast cells.⁸ The cell undergoes a degranulation process and releases allergic mediators such as histamine, serotonin, prostaglandins, and others. Mediators are responsible for the emergence of allergic reactions such as itching, redness, oedema, and impaired tissue function.^{9,10}

Recent research of kincung flower (*Etlingera elatior* (Jack) R.M.Sm.) showed it could be used as an alternative drug for anti-allergies because it had activity in inhibiting the active cutaneous anaphylactic reaction in mice.¹¹⁻¹³ It could inhibit degranulation of mice mast cells^{12,13}, which decreased basophils, eosinophils and lymphocytes. It increased neutrophils and the total number of leukocytes.¹³

Kincung flower had properties as anti-cancer and tumours.¹³⁻¹⁷ It showed anti-cancer activity against cervical cancer cells and skin cancer.^{13,16,17} Kincung flower also contained high antioxidants¹³.

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¹⁸⁻²⁰ and anti-bacterial to *Bacillus cereus*, *Micrococcus luteus*, and *Staphylococcus aureus*.^{13,20,21} Kincung flower also had anti-diabetic and anti-inflammatory,^{13,22} whitening and antiaging.^{14,23}

Kincung flower contains many secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoid.^{13, 24-26} Identification with GC-MS, kincung flowers had compounds such as 1-dodecanol, β -pinene²⁷, 17-pentatriacontane^{13,28} cyclododecane dan 1,1-dodecanediol diacetate.^{13,29,30}

Last research showed the activities of extract of kincung flower inhibit hypersensitivity of active cutaneous, inhibited mast cell degranulation, and decreased eosinophils and basophils. Based on that explanation, the researchers are interested in continuing research related to the activity of kincung on IgE and IL-4 levels in allergic white male mice.

MATERIAL AND METHOD

Place and time

The research conducted in four months in April-September 2019. Preparation and extraction of *Etingera elatior* (Jack) R.M.Sm. Also determining the characterisation from kincung flower's extract were conducted in three weeks at Central Laboratory in Faculty of Pharmacy Universitas Andalas.¹³

Tools and materials

The tools in this study were the Evaporator (Buchi® R-210 Rotavapor), UV-vis spectrophotometer (Thermo Scientific GENESYS 10S UV-Vis), Bio-rad spectrophotometer, beaker glass (Pyrex), Erlenmeyer (Pyrex), digital analytical balance (Ohaus), Silica gel 60 F254 (Merck), desiccator, spatula, dark bottle, TLC vessel, sonde instrument, surgical instrument, filter paper, animal cage, centrifuge.

The materials in this study were kincung flower (*Etingera elatior* (Jack) R.M.Sm.), aqua dest (Bratachem), ethanol 70%, ethanol p. a (Merck), formic acid (Merck), ethyl acetate (Merck), kit Mouse IL-4 Platinum ELISA, kit Mouse IgE ELISA, rutin (Merck), methanol (Merck), ethanol 80%, Aluminum chloride (Merck), Sodium acetate (Merck), ovalbumin.¹³

Extracting Kincung Flower (*Etingera elatior* (Jack) R.M.Sm)

Kincung flowers as much as 2 kg was dried until it became dry Simplicia, then it made powder and sifted with sieve number 60. The powder (250 grams) macerated using 70% ethanol solvent (1:10) for 24 hours. Twice repetition using the same type and amount of solvent. It filtered with filter paper, collected the filtrate and evaporated in a rotary evaporator until it became a semi-solid extract (35.276 grams).³¹

The TLC and total flavonoid test

TLC test: The extract dissolved in methanol P and the comparison (rutin) dissolved in ethanol P. Spot them on TCL plate (Silica gel 60 F25). The chromatography solvent (ethyl acetate P, formic acid P and water (100: 15:17) put into a chromatography vessel. Dry the TLC plate and Place it under an ultraviolet light. Moreover, Calculate the retardation factor (Rf).^{13,32}

Total flavonoid: The extract and rutin dissolved in ethanol 80%. Then pipette 0.5 mL supernatant and rutin solution, add each of them 1.5 mL of ethanol, 0.1 mL of AlCl₃10%, 0.1 mL of Na acetate 1 M and 2.8 mL aqua dest. Measure the absorption at the maximum absorption in wavelength 418nm.^{13,32}

Prepare the mice serum for calculation Of IgE and IL-4 levels

After 24 hours of the last extracts are given, mice blood taken by the guillotine method. The blood centrifuged for 30 minutes and 3000rpm until obtained serum. Then the serum was used for testing IgE and IL-4 levels by the ELISA method.³³

Ethical test

In the research used the mice, the subject of study, so it required an ethical test that qualifies to mitigate the treatment gave an adverse impact on a human subject in this research. The ethical test conducted by the Commission of Ethics Faculty of Medicine Universitas Andalas, Padang, Sumatera Barat.¹³

RESULTS AND DISCUSSION

Kincung flower extract was a semi-solid extract with characteristic characterisation odour, brown-black colour, and sour taste. The yield percentage was 14.11%, and according to Indonesian Pharmacopoeia Herbal, the yield percentage of kincung extract was not less than 9.86%.¹³ The shrinkage of dried kincung flower extract was 6.65%, and according to Indonesian Pharmacopoeia Herbal, it was not more than 10%.¹³ The total ash content of kincung flower extract was 4.57%, and according to Indonesian Herbal Pharmacopoeia, it was not more than 7.5%.

In comparison, the ash insoluble in the acid of kincung flower extract was 0.02%, and according to Indonesian Pharmacopoeia Herbal, it was not more than 0.1%.¹³ The Determination of total flavonoid used rutin as a standard which tested in UV-vis Spectro, and it obtained wavelength was 418nm. The linear regression of the rutin calibration curve for the calculation of total flavonoid levels was $y = 0.0046x - 0.0562$ with $R^2 = 0.998$. The result of the total flavonoid kincung flower extract was 1.564%, and according to Indonesian Pharmacopoeia Herbal, the total flavonoid content of kincung flower extract was not less than 0.58% which calculated as rutin.^{13,32}

The UV spectrum of rutin wavelength shown in Figure 1.

The results of Rf values extract obtained as can be seen in Figure 2.

On the first day, the mice sensitized by injecting albumin 20% 0.2 mL/20gBB intraperitoneally. Then the seventh day, the allergic was boosted by injected them subcutaneously. It carried out to increase the formation of IgE antibodies, so the allergic reactions of mice will get worse. Allergic mice used for further treatment, except for the negative control group used healthy mice. The allergic mice will be shown redness to red spots or bumps around the body and injection site. Kincung flower extracts given to mice for seven days, and then the mice take the serum.

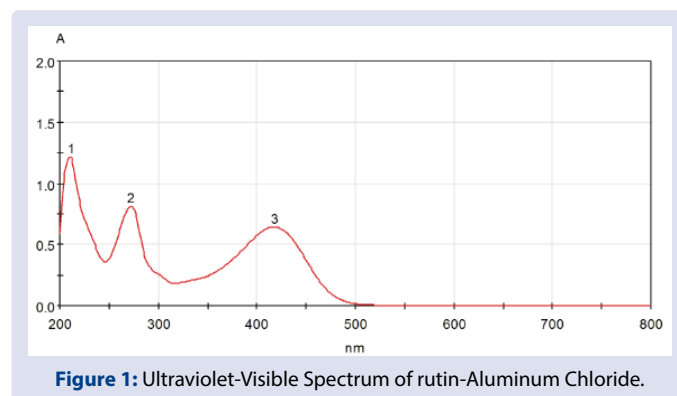


Figure 1: Ultraviolet-Visible Spectrum of rutin-Aluminum Chloride.

IL-4 level male mice type 1 hypersensitivity showed in Table 1. One-way Anova analysis of IL-4 level after kincung flowers extract at doses of 100, 300, and 1000 mg/kg BW in type I hypersensitivity mice showed a significant decreased ($p < 0.05$). The result of decreased IL-4 level showed in Table 1. Duncan test results showed that extracts with doses of 1000 mg/kg BW, doses of 300 mg/kg BW and 100 mg/kg BW differed in reduced IL-4 levels and the effects produced by doses of 1000 mg/kg BW and 300 mg/kg BW could reduce IL-4 levels such as healthy mice without allergies.

In type 1 hypersensitivity reactions, the role of cytokines is enormous, especially the process of IgE formation. Interleukins played a role in this regulation was cytokines produced by Th2 cells in the form of IL4 and IL10. IL-4 produced by T cells, mastocytes, basophils and eosinophil cells. The primary function of IL-4 are triggering the proliferation of B lymphocytes, IL-4 could induce MHC class II expression on the surface

of resting B lymphocytes and encourage the production of antibodies especially IgE and IgG1 from B lymphocytes.^{5,33-35}

In several studies showed that the flavonoids could inhibit IL-4 and IL-13 by activation of basophil cells.³⁶ Rutin had an anti-allergic activity because there was an effect on mast cell activity mediated by IgE.³⁷ It had an anti-allergic inflammatory effect and protected against allergic rhinitis by reducing levels of inflammatory cytokines.³⁸

Kincung flowers are rich in flavonoids that could inhibit IL-4 and IL-13 by activation of basophil cells.³⁶ It had an anti-allergic inflammatory effect and protected against allergic rhinitis by reducing levels of inflammatory cytokines.³⁸

It suspected that the main compound flavonoids on kincung flower is rutin; it can suppress IL-4 production by macrophage cells. IL-4 level was reducing, the proliferation and differentiation of b cells to become plasma cells which production IgE also decreased. IL-4 has receptors in plasma cells in producing IgE, the more IL-4 that is bound to plasma cells, the higher the production of IgE.^{6,35}

Thus the decrease in IL-4 will have a direct impact on the decline in IgE production. With the decrease in IgE or the cessation of IgE production, the degranulation process of mast cells and basophils will decrease or not occur at all.^{7,35}

IgE levels in serum white male mice showed in Table 2. After one-way Anova analysis on the IgE levels after kincung flower extract given at doses of 100, 300, and 1000 mg/Kg BW in type I hypersensitivity mice showed that there was a decrease significantly (< 0.05). Duncan test results showed that the effects produced by doses of 1000 mg/kg BW could reduce IgE levels such as healthy mice without allergies.

Flavonoids contained in kincung flowers is rutin, which known had an anti-allergic activity because there was an effect on mast cell activity mediated by IgE.³⁷ In the type 1 hypersensitivity reactions, IgE was antibodies which played an important role, IgE would be produced more in response to allergies. Furthermore, specific IgE bound to mast cells and basophil cells, and they would degranulate and release oxidative products. The reduction levels of IgE, it reduced IgE bound to mast cells or basophils thereby reduced the process of degranulation and reduced to release of mediators, one of them was the histamine which was a cause of symptoms of type I hypersensitivity reactions.³⁵

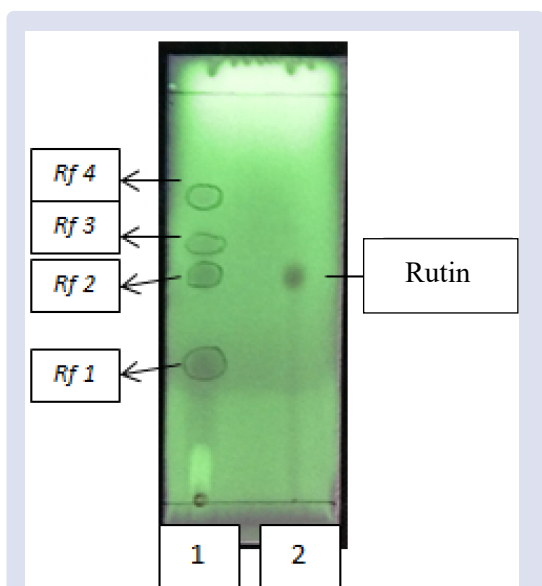


Figure 2: TLC kincung flower extract with eluent ethyl acetate P: formic acid P: water (100:15-17) and silica gel 60 F254. Description: 1. TLC kincung flower extract 2. Rutin standard. There are four marks, and the rutin showed in Rf2 with the value of 0.446. The extract has suitable for Indonesian Pharmacopoeia Herbal.¹³

Table 1: IL-4 Levels in white male mice’s serum after kincung flower (*Etilingera elatior* (Jack) R.M.Sm.) extract given for six days.

Group	IL-4 Levels (ng/L)					Mean ± SD
	I	II	III	IV	V	
I. Negative Control group	23.571	22.226	24.915	23.571	22.226	23.130 ± 1.36
II. Positive Control group	33.656	51.809	37.690	38.277	54.910	41.538 ± 7.33
III. 100 mg/kgBW	34.929	36.814	32.311	37.690	39.522	33.024 ± 2.94
IV. 300 mg/kgBW	26.260	26.932	37.690	29.622	27.960	27.933 ± 1.39
V. 1000 mg/kgBW	24.915	26.260	26.932	22.226	26.260	25.192 ± 1.80

Table 2: IgE Levels in white male mice’s serum after kincung flower (*Etilingera elatior* (Jack) R.M.Sm.) extract given for six days.

Group	IgE Levels (µg/L)					Mean ± SD
	I	II	III	IV	V	
I. Negative Control group	0.213	0.120	0.251	0.180	0.105	0.173 ± 0.06
II. Positive Control group	1.527	1.790	1.703	1.527	1.440	1.597 ± 0.14
III. 100 mg/kgBW	0.835	1.105	0.730	0.992	1.060	0.944 ± 0.15
IV. 300 mg/kgBW	0.884	0.636	0.630	0.452	0.544	0.629 ± 0.16
V. 1000 mg/kgBW	0.188	0.202	0.218	0.257	0.188	0.210 ± 0.02

CONCLUSION

Kincung Flower (*Etingera elatior* (Jack) R.M.Sm.) could decrease IgE and IL-4 levels on allergic mice so it could use as an anti-allergic drug.

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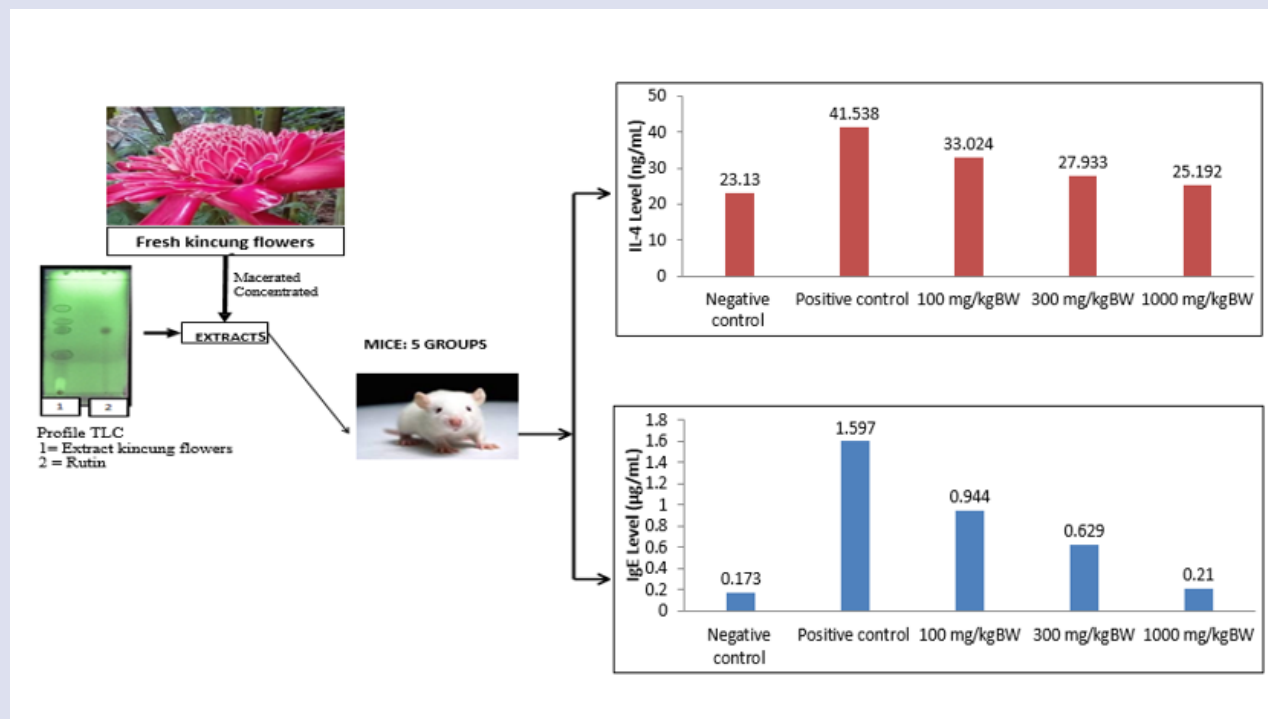
CONFLICTS OF INTEREST

The author(s) declare(s) that there is no conflicts of interest regarding the publication of this article.

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GRAPHICAL ABSTRACT



SUMMARY

The research on the activity of kincung flower extract (*Etilingera elatior* (Jack) R.M.Sm.) on IL-4 and IgE Levels carried out. The semi-solid kincung flower extract had standardised according to Indonesian herbal pharmacopoeia. The doses of 100, 300, 1000 mg/kg BW of kincung flower extract given to male white mice for seven days. Kincung flower extract could decrease IL-4 and IgE levels significantly ($p < 0.05$).

ABOUT AUTHORS

- **Dr. Elidahanum Husni, M.Si, Apt.:** Currently, as a lecturer at the Faculty of Pharmacy, University Andalas. Graduated from Faculty of Pharmacy Universitas Andalas in 1986, then Master Program in 1995 at School of Pharmacy Institut Teknologi Bandung (ITB Bandung) and Doctoral Program in Department Biomedical, Faculty of Medicine, University Andalas in 2015. The research and expertise is Pharmacognosy.
- **Relin Yesika, Apt:** Graduate student in Faculty of Pharmacy Universitas Andalas who involved in assisting the research and collecting data.
- **Prof. Dr. Yufri Aldi, M.S.i., Apt.:** Currently, as Professor at the Faculty of Pharmacy, University Andalas. Graduated from Faculty of Pharmacy Universitas Andalas in 1989, then Master Program in 1994 at School of Pharmacy Institut Teknologi Bandung (ITB Bandung) and Doctoral Program in Department Biomedical, Faculty of Medicine, University Andalas in 2013. The research and expertise are Pharmaco-Immunology. Currently working as the Head of the Department Doctoral Programme of Pharmacy in Faculty of Pharmacy, Universitas Andalas.

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