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Potency of Charcoal From The Body Part Kerandang(*Channa pleurophthalma* Blkr)Fish Which Is Not Eaten As An Antiallergy. Based On In Vitro, In Vivo and LC-HRMS

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ABSTRACT

Parts of an animal's body that are usually used as traditional medicine include: meat, horns, bones, tails, feathers, nails, fat, gall, and shells. The types of animals that have the potential to be developed as medicines consist of several criteria, namely that animals are easily found, are still widely available in nature.One method to treat allergies that have been carried down for generations in Central Kalimantan is to use charcoal from the body parts of the Kerandang fish that are not eaten such as the scalp, scales and fins. This research was conducted with the aim to find out the charcoal potency of the body parts of Kerandang (*Channa pleurophthalma* Blkr) fish from Central Kalimantan as an antiallergy drug.Testing covered antihyaluronidase in vitro, in vivo in male mouse test animals, and identification of active compounds with LC-HRMS. The results obtained: the highest inhibition (IC_{50}) of hyaluronidase in ethyl acetate extract of fish caudal fins concentration 4 mg / ml, concentrate of 15 % from charcoal of fish caudal fins can pressure IgE expression in male mouse. Identification of the active components of charcoal in the Kerandang fish caudal fins with LC-HRMS found the active compound is *Hexadecanamide*.

Keywords: *Charcoal*, Kerandang fish, antiallergy

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INTRODUCTION

The potential of natural resources in Indonesia in the form of natural and traditional medicines for generations has been used as a mixture of traditional medicines and is expected to be utilized in the development of public health. The advancement of modern knowledge and technology is not able to shift the role of traditional medicine, even at this time the government is promoting treatment back to nature [1]. The use of natural ingredients has the advantage that the therapeutic effect is constructive, the side effects caused are also very small so that natural materials are relatively safer than chemicals or synthetics on the market [2].

Allergy medicine is currently using a lot of synthetic drugs. Among them are antihistamines. But it is unfortunate that these drugs have undesirable side effects. For this reason, an effort is needed to avoid or minimize unwanted side effects. People are starting to turn to treatment by using natural ingredients to treat various diseases. An allergic reaction or so-called hypersensitivity is an unnatural immunologic reaction in someone who has previously been sensitized with an antigen that causes an excessive reaction, which manifests in inflammation or tissue damage. In rapid hypersensitivity reactions or anaphylactic reactions that play a role is immunoglobulin E (IgE). This reaction is characterized by a sudden response that occurs within minutes after the body is exposed to antigens, thereby releasing mediators present in cells such as histamine, bradykinin, arachidonic acid and prostaglandins. The release of these mediators causes allergic rhinitis, asthma, atopic dermatitis, skin flushing and shortness of breath [3].

Allergy is a condition caused by a specific immunologic reaction caused by an allergen [4,5,6]. Allergens can be in the form of dust particles, plant dust, drugs or food, which act as antigens that stimulate an immune response. The term allergic reaction is used to indicate a reaction involving IgE (Immunoglobulin E) antibodies. The allergic mechanism is dominated by mast cells that get exposure to the allergen and then release the IgE antibody enzyme. The release of IgE will trigger degranulation and result in the release of histamine, leukotrienes and other mediators. Next comes an allergic reaction. Immunoglobulin E is produced in large quantities when allergens attach to B lymphocyte cells [7,8].

Kerandang fish is a phenomenal in Central Kalimantan because charcoal made from several parts of the body of Kerandang fish that are not eaten can be used as traditional medicine to treat hereditary allergic cases. The way the body parts of the fish are burned to charcoal and then smeared on the itchy part or on the bumps that arise on the skin. Many fish that inhabit peatlands have bioactive materials and some are also useful in medicine besides their function as guardians of the biodiversity and ecology of peatlands [9].

So far the potential of charcoal from some parts of the Kerandang fish that is not eaten as an antiallergy has not been studied, causing a lack of scientific information regarding the active compounds contained in charcoal from Kerandang fish waste (*Channa pleurophthalma* Blkr).

The aim of the studywas considered necessary to characterize and identify the charcoal from the body parts of Kerandang fish (*Channa pleurophthalma* Blkr) which is not eaten as antiallergy.

MATERIAL AND METHODS

Sample preparation

The raw material used in this study is from some parts of the Kerandang fish that is not eaten in the form of scalp, scales, dorsal fins, pectoral fins, ventral fins, anal fins and caudal fins obtained from collecting fishermen in Sebangau Kereng Bengkirai Lake in Central Kalimantan.

The parts of body the Kerandang fish that is not eaten each collected, cleaned, then dried for 2-3 days, then burned to charcoal using a normal oven with a maximum temperature of 200^{o}C. After becoming charcoal, it is weighed until it reaches the weight of each sample of 500 grams.The extraction which is modified at a temperature of no more than 4°C. Extraction of maceration method that will be carried out using ethanol, ethyl acetate and chloroform solvent. Dry samples used each of 100 grams, put in 3 pieces of 1000 ml erlenmeyer then added as much as 500 ml of solvent, allowed to stand for 24 hours, filtered using a vacuum filter. The filtrate is then dried in a vacuum rotary evaporator. The obtained supernatant is a crude extract that will be tested further [10].

Antihyaluronidase test (*In vitro*)

Antihyaluronidase activity testing with a slight modification [11,12].The sample solution (1, 2 and 4 mg / ml) was dissolved in a mixed solvent (5% DMSO in ethanol). 50 µl bovine hyaluronidase (7900 units / ml) was dissolved in 0.1 M acetate buffer (pH 3.5) mixed with 100 µl of each sample solution, and then incubated for 20 minutes in a water bath at 37 ° C. 100 µl 12.5 mM calcium chloride was added to the reaction mixture, and then the mixture was incubated for 20 minutes in a water bath at 37 ° C. Bovine hyaluronidase activated by Ca2 + is reacted with 250 µl sodium hyaluronate (1.2 mg / ml) dissolved in 0.1 M acetate buffer (pH 3.5), and then incubated in a water bath at 37 ° C for 40 minutes . 100 µl 0.4 N sodium hydroxide and 100 µl of 0.4 M potassium borate were added to the reaction mixture, and then incubated in a bath of boiling water for 3 minutes. After cooling to room temperature, 1.5 µl dimethylaminobenzaldehyde (DMAB) (4 g dimethylaminobenzaldehyde was dissolved in 350 µl of 100% acetic acid and 50 µl of 10 N hydrochloric acid) was added to the reaction mixture, and then incubated in a water bath at 37 ° C for 20 minutes. Optical density (OD) in the reaction mixture was measured using a 585 nm spectrophotometer. The percentage of inhibition is calculated by the following equation:

$$\% \text{ Inhibitors} = [(ODc - ODs) / ODc] \times 100$$

Note: ODc is the optical density of the control, ODs is the optical density of the sample being tested. IC_{50} values that cause 50% inhibition are determined by linear regression analysis.

Immunoglobulin E (IgE) test (*In Vivo*)

The highest charcoal antihyaluronidase activity was applied topically in a cream form in the ovalbumin allergy mouse group for 7 (seven) days with negative control, positive control (hydrocortisone cream), concentrates are 10%, 15%and 20%, then the specific IgE was measured on the day 8^{th}. Measurement of specific IgE in blood serum of mouse using the Mouse OVA sIgE ELISA marker kit.

LC-HRMS

Sample preparation before being injected into LC-HRMS. Extract samples (powder/paste/liquid) are diluted according to the solvent (polar). Dilution is done by looking at the thickness of the sample (not too thick and not too thin) with the final volume of 1300 ul. Vortex for about 1 minute. Spindown for approximately 2 minutes. Take the supernatant then filter it using a 0.22 um syringe filter and put it in a vial. Samples in vials are ready to be inserted into the autosampler and then injected into the LC-HRMS according to the desired injection method. The resulting data is converted to NetCDF format to make it easier to process data with mzcloud. mzcloud data processing consists of several steps, namely creating a sample chromatogram, reducing noise, identification based on molecular weight, and compiling data.

Analysis of Data

Antihyaluronidase test data and specific IgE results produced were analyzed using the Factorial Complete Randomized Design of single factor (One Way) ANOVA (Analysis Of Variant) using the SPSS 23 program. LC-HRMS qualitatif analyzed according database mzcloud and compared with PubChem, Lipinski and swissADME bioinformatic method.

RESULTS AND DISCUSSION

The results of the calculation of the activity of antihyaluronidase (IC_{50}) extracts of Kerandang fish charcoal samples based on solvent concentration (1, 2 and 4 mg / ml) and inhibition of sample extracts as in **Figure 1.**

Figure 1 shows that the IC_{50} value of 7 (seven) types of charcoal parts of the body of fish that are not eaten extracted with 3 (three) types of solvents (chloroform, ethanol, ethyl acetate) based on solvent concentration (mg/ml) and extract inhibition (%) caused inhibition 50% of hyaluronidase activity with IC_{50} values of 0.06-12.54 mg / ml. Data of inhibition / inhibition (IC_{50}) hyaluronidase (in vitro) was obtained based on the linear regression analysis equation with the equation $Y = aX + b$. IC_{50} values represent the extract values of samples needed to inhibit 50% of hyaluronidase activity determined based on linear regression analysis [10].

IC_{50} calculation results show that charcoal parts of the body of fish that are not eaten Kerandang fish which has the highest antihyaluronidase activity is caudal fins charcoal extracted with ethyl acetate concentration of 4 mg/ml that

can inhibit 50% of hyaluronidase activity by 0.06 mg/ml. Charcoal which has the lowest activity is charcoal from Kerandang fish pectoral fins which is extracted with 4 mg / ml chloroform solvent that is equal to 28.6 mg /ml.

Hyaluronidase is one of the important enzymes found in allergic / inflammatory reactions so that hyaluronidase becomes one of the target enzymes that play a role in the process of mast cell degranulation [13,14,15]. This enzyme is known to be involved in the effects of allergies, cancer migration, inflammation, and also increased vascular system permeability in the extracellular matrix of connective tissue, found both in organs (testes, spleen, skin, eyes, liver, kidneys, uterus and placenta) and body fluids (tears, blood and sperm) [16,17,18].

It was reported that hypo-allergies and anti-inflammatories are strong inhibitors for hyaluronidase activity [15], so hyaluronidase enzyme inhibitory activity is used as one of the parameters of hypo-allergic testing [19,20].

A description of the specific IgE levels in the group of mouse before sensitization ovalbumin and after sensitization ovalbumin as in **Table 1**.

Graph of the average levels of specific IgE in the male mouse group after administering therapeutic concentrates of charcoal from Kerandang fish caudal fins as in **Figure 2.**

The average level of immunoglobulin E (IgE) specific to male mouse in the control group (-) was 404.63 $\pm$ 4.57. In the group given hydrocortisone cream (control (+)), the average specific immunoglobulin E (IgE) level was 284.76 ± 34.26. Then the group given a charcoal concentrate of 10% (group 1) had an average specific level of Immunoglobulin E (IgE) of 216.17 ± 17.13. Furthermore, the group that was given a charcoal concentrate of 15%(group 2) had an average specific immunoglobulin E (IgE) level of 97.33 ± 1.52. And the group given a charcoal concentrate of 20%(group 3) had an average level of Immunoglobulin E (IgE) of 107.37 ± 3.0. Of the five treatment groups, the group given 15%of caudal fins charcoal treatment (group 2) had the lowest average specific IgE level, meaning that the treatment concentrate given as much as 15%was able to reduce specific IgE levels in the blood of male mouse. Whereas in the group without treatment (control (-)) had the highest level of specific immunoglobulin E (IgE), which means that in the group of male mouse that were not treated did not decrease IgE levels. In the 3 treatment groups of tail fin charcoal concentrates, the group given a concentrate of 15%had the lowest average specific immunoglobulin E (IgE) level, while the group given a concentrate of 10%had the average specific immunoglobulin E (IgE) level the highest. This means that the treatment of a 10%concentrate has not had a significant effect on reducing IgE levels in male mouse. The treatment that gave a significant effect was on the treatment of 15%concentrate (group 2), where there was a decrease in IgE levels in male white mouse.

Specific immunoglobulin E (IgE S) levels in male mouse before sensitization ovalbumin and after sensitization ovalbumin that indicates increased. This indicates that an allergic reaction was suspected in the group of male mouse. Immunoglobulin E (IgE) values in the bloodstream are very small, usually measured less than 1 U / ml (1 U = 2.4 ng). IgE levels in normal individual serum ranged from 0.1-0.4 ng/ml. Individual IgE levels below 48 ng / ml indicate that individuals do not occur allergic. IgE levels between 48-240 ng/ml indicate that allergy is still questionable, while IgE levels above 240 ng/ml indicate the individual is definitely experiencing an allergy [5].

Skin allergy is a body reaction induced by allergen substances, when the skin becomes red, bumpy, scaly, itchy or swollen. There are various types of allergies in Southern African countries, people suffer from skin problems because of skin flora, culture and diversity[20,21]. Understanding the role of IgE in allergic reactions has become a major breakthrough in the field of allergies. The sequence of events in allergic reactions consists of the production of IgE antibodies in response to allergens, binding of IgE receptors to Fc mast cells, IgE crosslinking bound by allergens on repeated exposure, and mast release. cell mediators such as histamine, lipid mediators and cytokines. Some mast cell mediators cause a rapid increase in vascular ability and smooth muscle contraction, producing many symptoms [22].

Immunoglobulin E, like other immunoglobulins, is produced by B cells and plasma cells (usually) in response to an antigenic stimulus. The presence of interleukin IL-4 and IL-13 induce immunoglobulin class switching from other isotypes to IgE[23,24,25]. These 2 cytokines interact with receptors on the surface of B cells to initiate a signaling cascade mediated by Janus kinase 3 (JAK3) and signal transducer and activator of transcription 6 (STAT6). A second signal is required for class switching to IgE to occur, and this involves CD40 on the B cell interacting with CD40 ligand on the T cell. Once IgE is produced by allergen-specific B cells, it is released into the circulation[26,27,28].

Histamine plays a very important role in pathogenesis through the regulation of differentiation of CD4 + Th cell lymphocytes [29]. The mechanism of histamine inhibition by antihistamines (AH) is by occupying histamine receptors so that histamine can no longer occupy it or by expelling histamine that has occupied the receptor [30]. With inhibition of mast cell degranulation, the secretion of vasoactive amines, such as histamine, lipid mediators and cytokines that play a role in the inflammatory process in allergic events will also be reduced. Antihistamines have long been prescribed for atopic dermatitis as adjunctive therapy with topical agents that can block the action of histamine on the skin [31].

Topical nasal antihistamines, such as azelastine, are also available and are recommended for nasallimited mild disease and for on-demand treatment. To augment the efficacy of oral antihistamines in allergic rhinitis for those who continue to have symptoms, the preferred topical therapy is a corticosteroid nasal spray. These sprays should be considered first-line treatment in moderate to severe allergic rhinitis[32,33].

Allergy treatment can be donein various ways including:increase IgE soIncoming antigens can be destroyedthrough the complement system, givingdrugs that have anti-histamine effects,reduce IgE levels so that the bondsbetween antigens with IgE caninhibited, preventing the entry of antigensinto the body, but it also candone by inhibitingmastocyte degranulationso that no release occurspossible chemical mediatorsstimulate reactionhypersensitivity[34,35,36].In other case antiallergies work by inhibitingdegranulation of RBL-2H3 cells (RatBasophilic Leukemia) withusing β-hexosaminidaseas a biomarker, *Actinomycete nesterenkonia flava,* at the epidermis–environment interface[37,38].

Identification of charcoal ethyl acetate extract from Kerandang fish caudal fins using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) shown in **Figure 3**.

The spectrum results show that from the ethyl acetate extract of the Kerandang fish caudal fins obtained 8 (eight) peaks at retention time of 1.06; 12.12; 12.64; 16.57; 17.74; 18.08; 20.10 and 26.39 minutes. LC-HRMS data were analyzed using the mzCloud MS/MS Library data software and identification of the structure of chemical compounds detected in the LC-HRMS. The results of the analysis of ethyl acetate extract of the Kerandang fish caudal fins using LC-HRMS Best Match produced 49 compounds and compounds whose mass spectrum were identified and based on mzCloud the highest value above 90 was the Hexadecanamide compound.

Hexadecanamide is found in Palmitoylethanolamide (PEA). Other names are: n-(2-hydroxyethyl) hexadecanamide, n-hexadecanoylethanolamine, palmidrol PEA, palmitylethanolamide, palmitoylethanolamide, n-(2-hydroxyethyl)hexadecanamide, n-(2-hydroxyethyl)-hexadecanamide palmid. Hexadecanamide formula which is a derivative of palmitic acid is $C_{16}H_{33}NO$ [39].
Hexadecanamidehas been shown to have anti-inflammatory, anti-nociceptive, nervous,anticonvulsant, and antifungal properties[40,41].

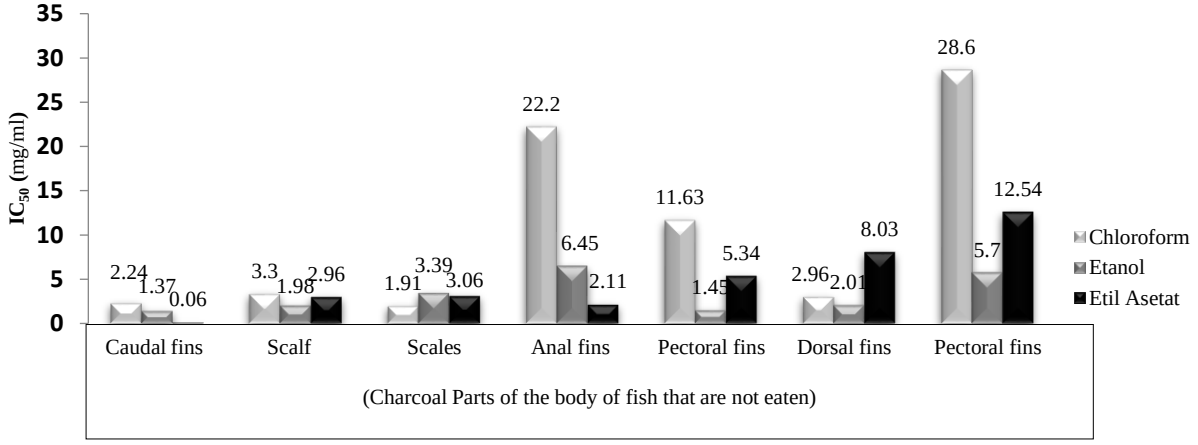

Figure 1. The results of the calculation of the activity of antihyaluronidase (IC_{50}) extracts of charcoal parts of the body of fish that are not eaten samples based on solvent concentration (1, 2 and 4 mg / ml) and inhibition of sample extracts

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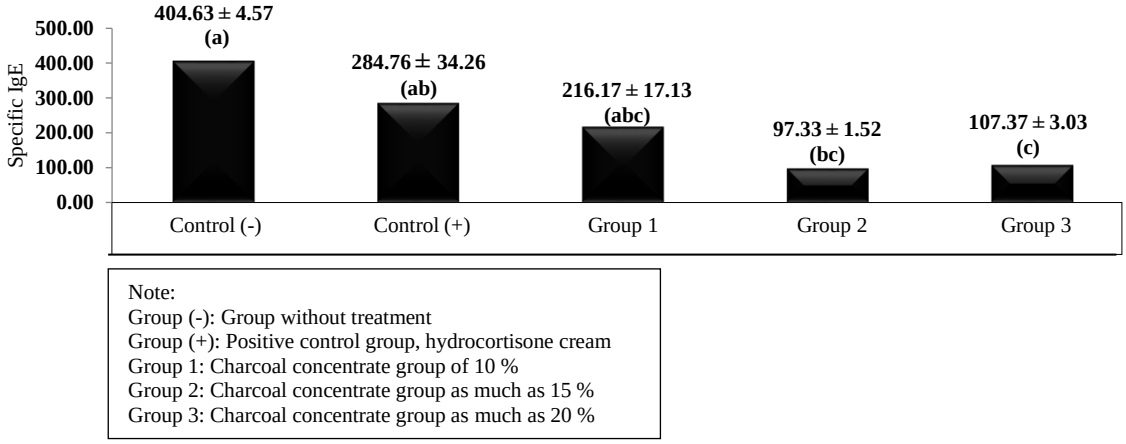

Note:
Group (-): Group without treatment
Group (+): Positive control group, hydrocortisone cream
Group 1: Charcoal concentrate group of 10 %
Group 2: Charcoal concentrate group as much as 15 %
Group 3: Charcoal concentrate group as much as 20 %

Figure 2. Average graph and standard deviation of specific IgE levels in male mouse group after giving charcoal concentrates from kerandang fish caudal fins

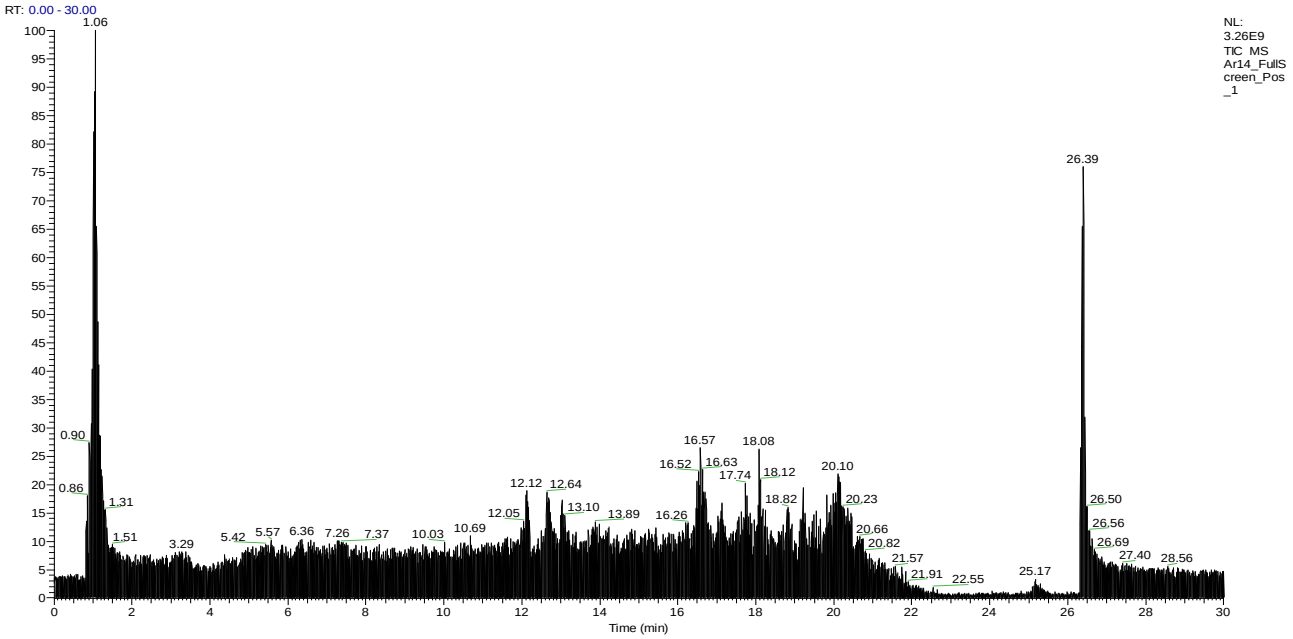

Figure 3. LC-HRMS chromatogram results

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Table 1. IgE spesifik results on male mouse group before sensitization ovalbumin and after sensitization ovalbumin

Group	Result Specific IgE (ng/ml)	
	Pre-Sensitization	After sensitization
I	95,7	434,6
	91,2	412,3
	89,9	430,9
II	92,1	591,2
	101,1	475,8
	98,7	466,6
III	102,3	1121,8
	99,1	908,7
	102,3	1147,7
IV	99,2	647,3
	112,4	789,6
	90,9	709,4
V	96,7	749,1
	102,9	736,2
	92,9	549,9

Note:
Group I : Negative control group
Group II : Positive control group
Group III : Treatment 1 group
Group IV : Treatment 2 group
Group V : Treatment 3 group

CONCLUSION

IC_{50} calculation results show that Kerandang fish caudal fins charcoal extracted with ethyl acetate concentration of 4 mg / ml can inhibit 50% of hyaluronidase activity by 0.06 mg/ml (in vitro).The concentrate of Kerandang fish caudal fins charcoal as much as 15 % was able to pressure of the specific lgE expression in the blood of male mouse (in vivo), so that Kerandang fish caudal fins charcoal potentially as antiallergy. Found *Hexadecanamide*active compound in Kerandang fish caudal fins charcoal as potential as antiallergy based on LC-HRMS.

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