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Combined Diet of Temulawak (*Curcuma xanthorrhiza* Roxb.) and Garlic to Improve Growth and Immunity of the Catfish

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ABSTRACT

Recent research on the fishing industry has begun to use herbal supplements. The addition of herbal supplements can improve fish performance without causing negative impacts. This study aimed to determine the effect of adding a combination of Curcuma flour and garlic flour supplements to raise catfish growth and immunity. The research adopted an experimental method applying a random design, 3 treatments and 1 control, each with 4 repetitions. The results showed that the addition of mixed herbal curcuma flour and garlic flour supplements through the feed can increase weight and length growth as well as daily growth rate. Meanwhile, immunity indicators have increased though insignificantly. Thus, a combined diet of herbal curcuma flour and garlic flour is recommended for use in catfish farming.

INTRODUCTION

Over the years, catfish production in Indonesia has continued to increase in line with consumer demand. Based on data from DGT (Bureau of Aquaculture), the average increase of annual catfish production is 13.75%. For instance, in 2016 the catfish production target was 1,217 tons, while 3 years later (2019) the target was 1,770 tons. Catfish consumption is very popular in Indonesia, because it tastes good at a relatively low price, compared to other freshwater fish. To achieve production targets, technological innovations are carried out in catfish farming, including intensive cultivation through increasing the stocking density of fish (**Pratiwi** *et al.*, 2020).

Intensive catfish (*Clarias gariepinus*) cultivation uses high stocking densities and relatively large feeds. This causes competition in obtaining feed, fecal deposits and leftover food that has accumulated at the bottom of the pond causing water quality to decrease. Declining water quality can inhibit growth and immunity and are susceptible to disease. Diseases that commonly attack catfish are motil aeromonas septicemia (MAS), causing







damage to the skin, gills and internal organs, as well as bleeding in the abdomen, chest and fins, causing growth to decrease (Wati et al., 2018).

To raise the growth and immune profile, supplements of natural ingredients (herbs) are added to stimulate growth and immunity. Herbs contain active compounds that can act as immunostimulants which enhance immunity via a direct interaction with cells of the innate immune system and some of them also function to increase growth (**Putri** et al., 2017). Some natural ingredients that can be used as immunostimulants and growth promoters include temulawak (*Curcuma xanthorrhiza*) and garlic (*Allium sativum*).

Several efforts were made to streamline feeding and increase growth, one of which was by adding supplements to the feed. Some phytopharmaceuticals can function as supplements, one of which is temulawak (C. xanthorrhiza). The rhizome of curcuma contains a yellow substance (curcumin), fiber, starch, potassium oxalate, essential oil and flavonodes. These substances are useful to support metabolism and organ function. Curcuma supplements are known to increase feed consumption, survival growth rate, feed efficiency and protein efficiency ratio. Based on the analysis, the results obtained revealed that the administration of temulawak extract through artificial feed has a significant influence (P<0.05) on the growth of the body weight and the daily growth of catfish. This absolute weight gain is made possible because curcuma has antibacterial content so that it can dissolve toxins attached to the intestinal wall. This kind of condition can improve optimal nutrient absorption and at the same time promote growth (Prastito et al., 2018). Results of an experiment conducted by Xavier et al. (2021) show that a diet high in curcumin can improve the digestive process of seed and feed consumption. Whereas, feed with lower doses contributes to more consistent feeding during the development of larvae. However, both types of diets can modulate the digestive process of gilthead larvae, especially protease and proteins.

Dietary curcuma is also able to modulate oxidative activity at the beginning of larval development, increasing palatability, acceptability and food intake faster, resulting in improved growth performance in addition to immunity. This proves that feed curcumin can promote growth performance, feed intake, digestive function and has antioxidant properties in fish larvae. Similarly, curcuma extract is used to promote growth and immunity. Catfish given curcuma extract causes their appetite and growth to increase (**Purwati** et al., 2016). Temulawak contains essential oils and curcumin which can stimulate liver cells to increase bile production and secretion. Curcuma also encourages the secretion of pancreatic enzymes consisting of the enzymes protease, lipase dan amylase to improve the digestion of proteins, fats and carbohydrates and accelerate the emptying of the stomach so that hunger arises and stimulates appetite (**Prabowo** et al., 2017). Other herbs can also promote growth such as garlic even though the potential is not as strong as curcuma.

Maniat et al. (2014) reported that, giving feeding garlic flour in diet can optimize the growth performance and balance of fish. Feed containing garlic powder 10 g kg⁻¹ has a good influence on the metabolism performance, body composition and balance of Benni fish. Furthermore, the results proved that 5-30 g kg⁻¹ of *A. sativum* flour in feed improves the anabolism performance, body composition and balance of Benni fish. In all treatments fed with garlic, final body weight, body weight gain and specific or daily growth rate rose significantly. Optimal growth performance is achieved in fish whose feed is supplemented with 10 g kg⁻¹ garlic in pellets. Other studies have proven that garlic powder stimulants against growth achievements and percentage of feed efficiency are influenced by fish species. Guided by achievement of optimal growth results and normal body composition can be recommended a diet of 10 g kg⁻¹ garlic powder is a natural alternative in increasing the growth triggers of fish. A similar result was reported by **Setijaningsih** et al. (2021) stating that, the addition of garlic extract of 1% or equivalent to 10g kg⁻¹ in feed of *Oreochromis*

niloticus was the optimal concentration to achieve body weight gain, specific growth rate and increased the number of erythrocytes and hemoglobin percentage.

Besides being able to increase growth, curcumin from curcuma can also increase immunity. **Amer et al.** (2022) reported an improved growth performance with the addition of a curcumin diet. Dietary curcumin can be given up to 400 mg kg⁻¹ to improve the immune response and works in growth and antioxidant capacity. Curcumin supplementation increases the resistance of *O. niloticus* fish to *Aeromonas hydrophila* infection. The study proved that curcumin can be added to tilapia feed up to 400 mg kg⁻¹ to enhance the immune response in addition to growth and antioxidant capacity. **Pakpahan et al.** (2020) postulated that, curcumin is a secondary metabolite compound that functions to boost the body's immunity against bacterial infection attacks. Curcuma administration in the feed at a concentration of 1.1 g kg⁻¹, is the best treatment that is able to increase the number of erythrocytes, hemoglobin levels of 9.4 g/dL, hematocrit level of 32.3% and survival rate. This shows that curcumin found in curcuma is able to care for fish against *A. hydrophila* infection. Such a situation is caused because curcumin can increase immunity.

In addition to curcumin from curcuma, garlic is reported to increase immunity in farmed fish commodities. Potential garlic is used to ward off disease and maintain health through increasing phagocytic activity as well as increasing antibody levels (**Agnesa** *et al.*, **2017**). Based on the study of **Samson** (**2019**), it was proved that, adding garlic powder to the red tilapia feed by 1-1.5% can increase feed utilization and fish survival. Therefore, the addition of garlic in red tilapia pellet feed is a possible alternative rather than using synthetic compounds or chemical supplements to increase growth, use of feed, as well as the survival of fish.

The report submitted by **Zorriehzahra** *et al.* (2021) showed that immune indices such as lysozyme activity and serum content of total immunoglobulins (Ig), phagocytic activity, as well as the activity of respiratory explosions increased significantly upon adding garlic flour with 100mg kg⁻¹, compared to the control. The results proved that fish fed with garlic extract of 50 and 100 mg kg⁻¹ of feed produced the optimal survival value after being challenged with *P. damselae* than the other treatments. The administration of garlic extract with a dosage of 100 mg kg⁻¹ for feed is able to improve the growth performance, immune system and resist the infection of *Mugil cephalus* seeds to *P. damselae* bacterial infections. Improved growth performance is characterized by low FCR values and increased feed efficiency. Low FCR indicates that fish are more efficient in feed utilization.

Thus, the present study aimed to determine the effect of adding a combination of curcuma and garlic flour in fish diet on catfish growth and immunity.

MATERIALS AND METHODS

Procedures

Curcuma xanthorrhiza Roxb. and garlic (Allium sativum) were bought from traditional market at Pasarwage, Purwokerto Regency, Central Java. Curcuma and garlic were cleaned, cut into small pieces, then air-dried at room temperature. Then, they were blended into flour, macerated with 100 mL distilled water kg⁻¹ feed, and then sprayed to commercially feed.

Catfish (6-7 cm) were bought from farmer's hatcheries at Dukuhwaluh village, Purwokerto regency and reared in plastic tank 100 L for 5 days acclimation. During the period of acclimation, catfish were fed with pellets alone without the addition of treatment (control). Catfish were then transferred into 16 plastic tanks. Each tank (20 L) was stocked with 15 fishes. The completely randomized design was applied, and one tank was a control. Catfish were fed at a daily dose of 3% of body weight, given twice a day in the morning and evening.

The treatment used a combination of curcuma flour and garlic based on the optimal dose of garlic 30,000 mg kg⁻¹ feed (**Lengka** *et al.*, **2013**) and curcuma 50,000 mg kg⁻¹ feed (**Insana & Wahyu, 2015**). The percentage combination of curcuma and garlic was as follows:

T1: 40% optimum dose (OD) of curcuma + 60% OD of garlic (20.000 mg +18.000 mg) kg⁻¹ feed);

T2: 50% OD of curcuma + 50% of OD garlic (25.000 mg + 15.000 mg) kg⁻¹ feed;

T3: 60% OD of curcuma + 40% OD garlic (15.000 mg + 21.000 mg) kg⁻¹ feed), and

T0: control respectively.

Hematocrit and leukocrit assays

At the end of the study test (day 50), hematological indicators were examined by taking 1ml of blood samples from the caudal veins of fish from each tank. The blood sample was then entered in a heparinization tube, further the hematocrit and leukocrit values were measured.

Data analysis

Growth process and hematology indicators were analyzed using a one-way ANOVA, and Duncan multiple range test was used to compare the average value at a significance level of 5% using SPSS software (Version 21.0).

RESULTS AND DISCUSSION

Some research results showed that administration of curcuma (*Curcuma xantorriza* Roxb) and garlic extracts can increase growth and boost immunity. The implementation of herbal diets is already widely used in fish farming. This is because the use of herbs to increase growth and immunity does not have a negative effect. Herbal diets can be used either solely or combined with other herbs to find an effective dosage.

 Table 1. Growth and immunity of catfish given curcuma and garlic flours supplement

Parameter	Experimental group			
	Т0	T1	T2	Т3
Initial weight (g)	2.40 <u>+</u> 0.09	2.49 <u>+</u> 0.04	2.48 <u>+</u> 0.39	2.49 <u>+</u> 0.09
Final weight (Wt; g)	9.43 <u>+</u> 1.83 ^a	14.39 <u>+</u> 1.38 ^b	12.62 <u>+</u> 1.13 ^b	14.82 <u>+</u> 2.26 ^b
WG (g)	7.03+1.87 ^a	11.89 <u>+</u> 1.42 ^b	12.34 ± 80.08^{b}	10.14 ± 1.122^{b}
Initial length (cm)	6.51 <u>+</u> 0.2	6.97 <u>+</u> 0.21	6.46 <u>+</u> 0.17	6.39 <u>+</u> 0.15
Final length (Lt; cm)	10.14 <u>+</u> 0.61 ^a	11.64 <u>+</u> 0.23 ^b	11.55 ± 0.28^{b}	11.15 <u>+</u> 0.33 ^b
LG (cm)	3.63 <u>+</u> 0.49 ^a	4.67 ± 0.28^{b}	$4.69 \pm 0.23^{\text{b}}$	5.16 ± 0.20^{b}
SGR	2.71 <u>+</u> 0.39 ^a	3.49 <u>+</u> 0.21 ^b	3.25 ± 0.18^{b}	3.55 ± 0.26^{b}
Feed intake (g)	352.80	373.82	357.70	375.84

Values are represented as the mean±SE. abc in a row with different superscript letters shows significant difference (p<0.05). T0: Control; T1: Combination 40% tumeric + 60% garlic in feed; T2: Combination 50% tumeric + 50% garlic in feed, and T3: Combination 60% tumeric + 30% garlic in feed.

Growth performance

Based on the analysis (Table 1), the same profile achievement was obtained in the final weight (Wt), final length (Lt), weight and length gain (WG & LG), and daily growth rate showed significant (P<0.05), compared to control. The growth values obtained (Wt, Lt, WG, LG and daily growth rate or SGR) showed the same pattern; the control group or that without treatment is quite different from groups under treatment (P<0.05). This proves that the growth obtained is strongly associated with the combination of curcuma (Curcuma Curcuma Cu

ratio (REP) also increased significantly (P<0.05) compared to controls, results of all treatments were significantly different from those of the controls.

Prabowo et al. (2017) postulated that, using curcuma extracted from curcumin can improve the work of digestive organs by stimulating the bile wall to secrete bile fluids in helping the digestion of fat. Curcumin compounds can also stimulate the pancreas to secrete pancreatic enzymes that contains lipase, amylase and protease to improve digestion of proteins, fats and carbohydrates. These results increase efficiency absorption of nutrients by the intestinal wall, accelerating emptying of the stomach, and subsequently causing hunger and stimulating appetite. Furthermore, a similar result was reported by Rojtinnakorn et al. (2012), regarding the effect of giving curcuma extract supplements to the feed of sand goby (Oxyeletoris marmoratus) on the activity of gastrointestinal enzymes such as trypsin, lipase, amylase and chymotrypsin. Sand goby fries fed diets containing 0.3% turmeric powder witnessed a significant effect on the enzyme's increment and the food digestion, including improvement of absorption. A similar work by Mooraki et al. (2019) reported that, the addition of 0.3% turmeric through feed can increase growth, survival and condition factors even if the increase is not significant. It is necessary to consider various influential factors such as the way of administration, the dose given and the method of extraction, thus it needs further research that can affect the results.

Furthermore, **Prastito** *et al.* (2018) reported that, the administration of curcuma or *temulawak* extract (*Curcuma xanthorrhiza* Roxb.) for commercial feed has a very significant influence on weight, total feed consumption, feed efficiency, daily or specific growth rate (SGR) and protein efficiency ratio but did not differ markedly to survival. Curcuma rhizomes contain yellow dyes (curcumin), fiber, starch, potassium oxalate, essential oils and flavonoid which function to improve metabolism and organ function.

Besides being able to increase growth, specifically supplements of tumeric can also reduce FCR which shows efficient use of the feed provided. This was reported by in the study of Mukherjee et al. (2009), reporting that the consumption of turmeric by fantail guppy (Poecilia reticulate) caused the reduction of FCR and improved growth performance. Similarly, the report of Afifah et al. (2021) recorded that, the supplement of 1% turmeric extract combined with 1.5% garlic extract serves essentially as an attractant and plays a role in promoting the growth of gouramy. The addition of 1.5% garlic flour or 1% turmeric extract to gourami is effective as an attractant and can increase the growth of test fish with the highest feed consumption value in the garlic flour treatment of 1-1.5%; the lowest conversion ratio value (FCR), and the highest utilization efficiency were found in the 1.5% garlic flour or 1% turmeric treatment. According to Sanchez et al. (2019), using powdered young turmeric tubers up to a 4% merging rate improves the growth performance of the Nile tilapia. The study concluded that, young turmeric tubers can be utilized as a growth promoter feed additive and can improve the survival of the Nile tilapia. Furthermore, the study recommended utilizing a 4% incorporation rate of powdered young turmeric bulbs as a feed additive in the Nile tilapia (Oreochromis niloticus) foods to have a healthy outlook profile for consumers in addition to its main benefits of reducing feed costs and organically raising aquaculture species.

Garlic in aquaculture can encourage growth and stimulate response of eating beside enhancing immune system. The effect of garlic is associated with the presence of organosulfur compounds including allicin (Lee & Gao, 2012). Allicin can increase growth by stimulating and increasing appetite so that growth becomes better in addition to increasing immunity. Allicin in garlic provides odor stimulation which causes the response of eating fish to increase (Sartijo & Haditomo, 2017). This proves that garlic supplementation through feed can improve the growth and survival of fish. The same results were shown in tilapia farming fed a diet of garlic flour on the feed resulting in better growth. It was proved that the

addition of garlic flour and also onions is an alternative solution to improve fish farming while serving as a growth booster. Further explained, this indicates a significant improvement of growth performance, feed utilization and biochemical composition of the body of fish fed and garlic flour supplements (**Vigneshpriya & Krishnaveni, 2016**).

Hematological parameter

Catfish fed with a combination of curcuma flour and garlic increased the percentage of hematocrit and leukocrit but were not significant (P>0.05) during the 50-day study. The percentage of hematocrit in all treatments was higher than in the control, and the percentage of hematocrit increased according to the increase in dose. The highest percentage of hematocrit was achieved at treatment 3 (T3). The same pattern occurred in the percentage of leukocytes, but in T3 treatment, the percentage was lower than that of the controls in T1 & T2 (Table 2).

Tabel 2. Hematocrit and leucocrite of catfish juveniles fed with curcuma and garlic

Tuestment	Hematology parameters			
Treatment	Hematocrite	Leucocrite		
T0	46.95 <u>+</u> 16.48 ^a	2.58±2.03 ^a		
T1	47.92 <u>+</u> 4.39 ^a	3.19 ± 1.42^{a}		
T2	49.39 <u>+</u> 6,04 ^a	3.16 ± 1.30^{a}		
T3	51.22+7.91 ^a	2.38 ± 0.67^{a}		

Diet of combined curcuma and garlic extract led to an increase in the percentage of hematocrite and leucocrite though not significant. Research conducted by **Riauwaty** *et al.* (2019) showed that, the turmeric diet in milkfish infected with pathogenic bacteria *Aeromonas hydrophyla* can increase the percentage of lymphocytes from 77% (control) to 83% (0.7 g kg⁻¹ feed), an increase in the percentage of hematocrit 2.67% compared to control, and an increase in the total number of erythrocytes including leucocytes or white blood. It clearly shows that whitefish given that the turmeric enriched pellets can increase immunity and be able to overcome infections against the bacteria *A. hydrophila* infection.

Curcuma xanthorrhiza is known to affect immunity as reported by Behera et al. (2011), and a supplement of curcuma has promoted immune non specific response of fish Labeo rohita as an effective compound. A similar opinion was also reported by Sahu et al. (2008) stating that the effectiveness of turmeric powder on immunity response of Labeo rohita to Aeromonas hydrophila and white shrimp (Litopenaeus vannamei), respectively. As supported by Nan et al. (2015), the supplementation of curcuma in feed increases percentage of hematocrit in fish, though insignificantly. The use of natural herbs as immunostimulants in fish is more beneficial than antibiotics that can cause side effects for fish, the environment and consumers. Research on the use of herbs by Hassan et al. (2018) proves that turmeric flour diets containing curcumin are known to increase hematocrit and leukocrite levels significantly compared to the control group, while between the treatments they do not differ significantly. Based on the findings, a turmeric diet containing curcumin is recommended for use in minimizing deaths caused by some pathogens.

Research conducted by **Anene** *et al.* (2022) on catfish (*Clarias gariepinus*) showed that, the addition of herbal flour supplements containing curcumin through feed encouraged an increase in aquaculture productivity through immune optimization and physiological improvements related to fish resistance. The study was conducted to assess the effect of various doses of turmeric flour on the response of hematological and biochemical indicators. Research proved that supplementation of turmeric flour through feed on catfish *C. gariepinus* at various concentrations can improve hematological conditions, biochemical indicators and cellular defenses of fish body systems including phagocytic cells.

Beside *Curcuma xanthorrhiza*, garlic (*Allium sativum*) is one of the medicinal plants known as immunostimulants. The study documented that garlic supplementation of 0.5g kg-1 has significantly increased the number of leukocytes, respiratory burst, phagocytic activity, phagocytic index and lysozyme activity, which indicates the immunostimulant properties of garlic in juvenile hybrid tilapia (**Fall & Ndong, 2011**). Thus, it was reported that the addition of *A. sativum* to the diet of fish increases the number of erythrocytes, hemoglobin concentration, hematocrit values, leukocytes and platelets (**Naqi et al., 2019**). *A. sativum* has several constituents that may play a role in the stimulation of the immune system and in the functioning of organs related to the formation of blood cells such as the thymus, spleen and bone marrow (**Fazlolahzadeh** *et al.*, **2011**). A further similar opinion reported that alisin in garlic can increase by inducing phagocytosis of granulocyte neutrophil cells and induce respiratory burst in bacterial infection of fish (**Purnomo, 2012**). Supplementation of garlic at a dosage of 0.5 g kg⁻¹ has significantly increased the number of leukocytes, respiratory burst, phagocytic activity, phagocytic index and lysozyme activity, which indicates the immunostimulant properties of garlic in juvenile hybrid tilapia (**Fall & Ndong, 2011**).

Research conducted by **Pashaki** *et al.* (2018) showed that feeding a garlic diet with 5 g kg⁻¹ through feed on goldfish fry can increase white blood cell count (WBC), lymphocytes, lysozyme and IgM significantly (P<0.05), compared to controls. Based on the results of experiments, the administration of garlic extract supplements to the feed by 5 g kg⁻¹ can increase some parameters of blood, immunity, including the growth of goldfish fry. Furthermore, **Naqi** *et al.* (2019) reported that, a diet of garlic flour in tilapia can promote a better immune response than control. The results of this study showed a significant difference (P<0.05) to hematological parameters among all treatment groups at different dietary doses of garlic. Therefore, it can be concluded that, the addition of garlic flour through feed can be used as an immunity stimulant which, in turn, can improve growth performance.

Conclusions from the results of research conducted by Fall and Ndong (2011) prove that an 0.5 g kg⁻¹ garlic supplement significantly increases white blood cell count, explosive gas exchange of O₂ with CO₂, phagocytic activity, phagocytic index and lysozyme activity. This suggests that garlic serves as an immunostimulant in the cultivation of juvenile hybrid tilapia. A considerable range of pressure both pathogenic and environmental on the immune system of fish requires the presence of additional nutrients and supplements from garlic given through feed. This work provides a new perspective for the use of medicinal plants as adjuvant therapies added to fish feed to prevent disease. Further studies including the determination of the required dosage and mechanism of action of garlic need to be focused on. Then, based on the results of Pashaki et al. (2018) study, it was proven that the garlic diet at the optimal dose of 5 g kg⁻¹ can increase several hematological and immune parameters in addition to growth in spawn goldfish. This is because in garlic there are bioactive compounds, especially allicin which can increase immunity, including antimicrobials and growth stimuli. In addition to allicin, garlic contains phosphate compounds, alkaline enzymes, peroxidase. The parameters of increased immunity with a garlic diet increased white blood cells (WBC), lymphocytes, lysozyme activity and IgM in goldfish fry.

Isroni et al. (2020) reported that, garlic extract added to commercial feed can improve non-specific immune responses, with an increase in total leukocytes and phagocytic activity of goldfish. Garlic is a natural plant that contains active ingredients, such as sulfur compounds: allicin, disulfide, trisulfide; enzymes: alliinase, pectinase; and amino acids: arginine and minerals such as selenium. Garlic extract has potential as an immunostimulant agent in goldfish (*C. carpio*).

The observed water quality parameters are temperature and pH in accordance with the Indonesian national standard (SNI) for catfish enlargement. Water quality is managed by replacing fresh water by 20-30%. The results of water quality measurements in this study

were water temperatures ranging from 26-28°C and pH 7.3-7.9. The temperature and pH are still classified as feasibility for catfish cultivation according to SNI, namely a temperature of 27-30°C and a pH of 6.5-8.5 (Rachmawati et al., 2015). Temperature is one of the things that affects the growth and graduation of livelihoods in fish farming. Good temperatures result in high growth and dilution rates. Temperatures below the optimum and above the optimum cause slow fish growth, caused by relatively low feed consumption. Optimum temperature results in absolute weight and length, specific growth rate and higher dilution rate than control (Tang et al., 2019). In addition to temperature, pH also affects cultivation productivity. According to research on turbot fish conducted by Shuang-Yao et al. (2018), water pH plays an important role on survival, growth performance, energy budget and oxidative stress in turbot fish. At a time when the environmental pH ranges from 6.8 to 7.8, turbot fish are able to grow heavier and faster with an energy efficient for growth increase. Based on this, the pH range of water ranging from 6.8 to 7.8 is recommended to optimize the growth and productivity of fish farming.

CONCLUSION

Research empowering a combination of herbal supplements of *temulawak* (*Curcuma xanthorrhiza*) and garlic flour for catfish resulted in significant growth and raised (though insignificant) immunity as well. Growth increased with respect to the parameters of weight and length growth as well as the daily growth rate. For the parameters of immunity, both hematocrit and leukocrit increased though insignificantly. Based on this study, the combination of herbal supplements of curcuma and garlic flour is recommended for use in fish farming, in this case for catfish growth and immunity. Further studies to examine the opportunities of significant effect of the natural ingredients for similar purpose is warranted.

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