

## THE INHIBITORY ON LIPASE ACTIVITY AND LD<sub>50</sub> DETERMINATION OF HERBAL FORTIFIED GREEN TEA (GAMBOENG TEA)

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### ABSTRACT

A randomized controlled trial was conducted on three formulas of an herbal drink containing green “gamboeng” tea fortified with bay leaf and cinnamon bark at various concentrations. One gram of mixed formula was brewed in hot water at 70<sup>0</sup> C-90 <sup>0</sup>C for 10–15 minutes, and the yield of each plant was determined. We also determined the phenolic content and lipase inhibition activity of each formula. We obtained a yield of each plant with green “gamboeng” tea (66.2 %), bay leaves (10.7 %), and cinnamon bark (44.8 %). Green “gamboeng” tea has the highest percentage of phenolic compound 30.25 ± 1.201 %. Inhibition lipase activity was calculated using an Elisa reader at λ =405 nm. We have three formulas with the highest lipase inhibition activity. For the three formulas, a mixture of green “gamboeng” tea, bay leaf, and cinnamon bark was F-3.2 (80.598%), F-4.1(74.306), and F-5.2 (80.299%). We also conducted acute toxicity for the formulas above using rats as an animal model. We observed the body weight, organ weight, motoric, and mortality of rats during 14 days of observation, after using the formula with the concentration of 3.75, 7.5, and 15 g/kg body weight. We also used a control group of rats in this experiment. The results of acute toxicity showed no mortality of rats from the lowest to the highest doses. We concluded that LD<sub>50</sub> of the three formulas was > 15 g/kg body weight. Between the treated groups and the control groups for organ weight and motoric activity showed no significant difference. Our conclusion is that this herbal formula drink has an activity for lipase inhibition, and is also safe for consumption by humans that suffer from obesity.

**Keywords:** Bay Leaf, Cinnamon Bark, Green Tea, Obesity.

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### INTRODUCTION

Leaves of green tea (*Camellia sinensis*) are one of the world’s popular drinks since its unparalleled healthy nature is attributable to its minimal oxidative processes. It is also used as a food supplement against disruptive diseases such as cancer, inflammation, diabetes, obesity, nerve damage, heart disease, autoimmune diseases, microbial pathogens, and osteoarthritis.<sup>1</sup> Polyphenol, especially epigallocatechin gallate (EGCG), is the main functional compound of green tea. It was reported that EGCG can protect pancreatic beta-cell damage from the effects of oxidation.<sup>2</sup> It is reported that decreased blood sugar levels may be achieved by consuming green tea drinks. By using diabetic rats as a model, we showed that rats treated with green tea had the lowest pattern of increased blood glucose over time; this shows that green tea had the best level of inhibition of blood glucose absorption, as compared to other beverage treatments. Mice treated with a mixture of green tea and mulberry leaf tea showed decreased blood glucose levels when compared to those treated with bottled drinking water, but their ability was lower when compared to those receiving green tea or mulberry leaf tea alone.<sup>3-4</sup> In our study, we used several types of plants that were reported to have bioactivity. The cinnamon leaf, or *Cinnamomum Verum* (C), is considered an antidiabetic and antioxidant,<sup>5-6</sup> while *Syzygium polyanthum*/bay leaf (SI) is high in antioxidants<sup>7</sup> and considered an anti-diabetes, anti-inflammatory, and anti-diarrhea treatment.<sup>8</sup> Tea extracts can be considered as among the alternatives to chemical food preservatives and for controlling food.<sup>9</sup> Inhibition of the lipase enzyme by green tea and some leaves such as breadfruit leaves, bay leaves, sapodilla leaves, and cinnamon leaves has

been carried out, and lipase enzyme inhibition was achieved by using a mixture of green tea, bay leaves, and cinnamon leaves in a ratio (4:1:1) 67.32%.<sup>10</sup> Obesity due to diet is expected to be overcome by consuming pancreatic lipase inhibitors. Currently, there are only two drugs available in the market because several other drugs have been withdrawn from the market due to side effects. Herbs could be a good natural source to find the new pancreatic lipase inhibitors that are expected to have no side effects so they are safer to consume.<sup>11</sup> One of the diseases that are related to diabetes is obesity. In developed countries such as Japan, South Korea, and countries in Europe, as well as in developing countries, obesity is a metabolic disorder with a high growth rate. Obesity increases the risk of hyperlipidemia, hypertension, arteriosclerosis, diabetes, and coronary heart disease. Compared to dietary carbohydrates, some dietary fats are more effective at increasing body fat storage. Because the key to the treatment of obesity is to inhibit the digestion and absorption of dietary fat. This can be done by pancreatic lipase inhibitors, which cause reduced dietary fat that can be absorbed by the small intestine.<sup>12</sup> The safety of products or extracts is evaluated for their safety effect by an acute toxicity study. This acute toxicity test is to determine the LD<sub>50</sub> dose, which is the dose of the sample tested which would be lethal for 50% of the animals treated. This test aims to observe the toxic effects of the compound that can occur in a short period of time after administration with a certain dose. In this test, the acute toxicity (LD<sub>50</sub>) potential was determined, and various clinical symptoms such as the spectrum of toxic effects, and the mechanism of death were observed. Statistical methods can be used to analyze dose-response data to calculate the estimated median lethal dose (LD<sub>50</sub>). One example of a statistical method commonly used is prohibited analysis. This method can also be used to analyze several measures of approximate accuracies, such as the 95% fiducial limit.<sup>13</sup> The purpose of this study was to determine in vitro the pancreatic lipase inhibitory activity in several tea formulas enriched with herbal extracts for their potential anti-obesity activity as well as acute toxicity testing of these tea formulas in rats.

## EXPERIMENTAL

### Materials

Green tea leaves or “Gamboeng tea” was obtained from the Research Center of Tea and Quinine (P2TK), West Java. Salam leaves (*Syzygium polyanthum*) and Cinnamon leaves (*Cinnamomum verum*) were obtained from the Puspipstek Garden, Serpong, Banten Province, Indonesia. The leaves were determined at the Research Centre for Biology LIPI. Lipase enzyme was obtained from Sigma Aldrich. The rats were used Sprague Dawley rats.

### Ethical Clearance

The Experimental methods on rats in lipase activity research activities and determination of LD<sub>50</sub> herbal fortified green tea (gamboeng tea) were carried out with the approval of the Ethics Commission from the Faculty of Medicine, University of Indonesia, and Number: KET-1225/UN2.F1/ETIK/PPM.00.02/2019.

### Sample Preparation

*Syzygium polyanthum*/bay leaves (Sl) and *cinnamon* bark (K) were dried at room temperature, crushed, and then sieved with a 20 mesh sieve. Green “gamboeng” tea was also sieved with a 20 mesh sieve each of the above tea powders was mixed with a total weight of 1.5 g and put into a tea bag. Furthermore, the tea bag is brewed in 100 ml of mineral water at a temperature of 70-90°C for 10 minutes. Each sample was weighed by the following weight ratios: Formula 3.2 comprised 60% green “gamboeng” tea, 25% bay leaves, and 15% cinnamon bark per 3 grams. Formula 4.1 comprised 70% green “gamboeng” tea, 10% bay leaves, and 20% cinnamon bark per 3 grams. Formula 5.2 comprised 80% green “gamboeng” tea, 15% bay leaves, and 5% cinnamon bark per 3 grams. Each formula was brewed in hot water at 90°C for 10 minutes, and then filtered. Each tea formula infusion was stored in a Petri dish and frozen in a refrigerator at -80°C for 1x24 hours, and then dried with a freeze dryer at a temperature of -110°C for 1x24 hours. The dried extract of the formula was used for an acute toxicity test.

### Pancreatic Lipase Activity Test

Pancreatic lipase activity was tested by measuring *p*-nitrophenol resulting from the hydrolysis of *p*-nitrophenyl butyrate (*p*-NPB), as previously reported.<sup>13</sup> 20 µL of the sample was added to 20 µL of lipase enzyme in morpholine propane sulfonic acid 10 mM (MOPS) and 1mM Ethylenediamine tetraacetic acid

(EDTA), pH 6.8, and added with tris buffers (100 mM Tris-HCl and 5 mM CaCl<sub>2</sub>, pH 7.0) to 200  $\mu$ L later with pH of 6.8. It was later incubated for 15 minutes at 37°C and then added to 5  $\mu$ L *p*-nitrophenyl butyrate (*p*-NPB) /DMF 10 mM. It was then incubated again for 30 minutes at 37°C and then measured with a wavelength of 405 nm using a microplate/ELISA reader.<sup>14</sup> In this test, orlistat was used as a positive control. Pancreatic lipase pastime was decided through a standard method in the literature by measuring *p*-nitrophenol resulting from the hydrolysis of *p*-nitrophenyl butyrate (*p*-NPB).<sup>13</sup> Relative pancreatic lipase activity (%) was calculated as follows:

$$\text{Inhibitory Activity} = (\% I) = \frac{A-B}{A} \times 100 \%$$

Where, I is the inhibitory lipase activity, A is the blank lipase activity (reaction solution without the sample addition), and B is the lipase activity of the sample (reaction solution containing the sample with various concentrations). Measurement of lipase activity was carried out three times. The IC<sub>50</sub> value was determined by plotting the percentage inhibition versus the sample concentration.

### Determination of Tea's LD<sub>50</sub>

10 -week-old male Sprague-Dawley rats, weighing 20-230 g, were purchased from Litbangkes Laboratory Ministry of Health Indonesia (Jakarta, Indonesia). Acclimatization due to laboratory conditions was carried out on all rats for a period of seven days prior to the experiment. The rats were housed in an air-conditioned room at 23 °C on a 12/12 h light-dark cycle with steady humidity.<sup>15</sup> Each group of rats were given feeding and water *ad libitum*. The Ethics Committee from the Faculty of Medicine, University of Indonesia has given approval for the experimental animal protocol for lipase activity and the determination of the LD<sub>50</sub> for herbal fortified green tea (Gamboeng tea) with the approval number: Ket-1225/UN2.F1/ETIK/PPM.00.02/2019. The protocol for determining LD<sub>50</sub> was according to Clinical Toxicity Guidelines published by the National Agency of Drug and Food Control Indonesia. The rats were grouped into four. Three groups received tea formulation with doses of 3.75; 7.5; 15 g/body weight respectively and one group as a control. All efforts were made to minimize the suffering and distress of the rats. One-way ANOVA method was used to analyze all of the experimental data.

## RESULTS AND DISCUSSION

Obesity agents have been extensively studied and evaluated the efficacy of their natural products using pancreatic lipase assays. Pancreatic lipase plays an important role in lipid metabolism.<sup>14</sup> herein, we tried to find a natural product obesity agent that can inhibit pancreatic lipase. We used green tea because it has been reported to have acted as a lipase inhibitor. The herbal extract test has not shown any significant activity when compared with the standard extract itself, namely, Orlistat, which has a 70% inhibitor activity at a concentration of 100 ppm. However, our previous study showed that green tea and brewing herbs (bay leaves and cinnamon bark) have a higher activity<sup>1</sup>. In a previous study on alpha-glycosidase inhibitors,<sup>16</sup> a mixture of green tea, bay leaves, and cinnamon leaves formula with a ratio of 4:1:1 had the highest inhibition (95.35%) compared to different ratios. A similar pattern was also observed in the lipase inhibitory activity. The formulation results related to the best activity that can inhibit the activity of the lipase enzyme are Formula 3.2 (F-3.2), Formula 4.1 (F-4.1), and Formula 5.2 (F-5.2).

Table-1: Inhibition of Pancreatic Lipase Activity and Formula of Green Tea Fortified with Bay Leaves and Cinnamon

| Formula | Green tea (%) | Bay leaves (%) | Cinnamon (%) | % Inhibition |
|---------|---------------|----------------|--------------|--------------|
| F-1     | 30            | 30             | 30           | 67.233       |
| F-3.1   | 60            | 15             | 25           | 59.587       |
| F-3.2   | 60            | 25             | 15           | 78.326       |
| F-3.3   | 60            | 30             | 10           | 77.918       |
| F-4.1   | 70            | 10             | 20           | 91.909       |
| F-4.2   | 70            | 20             | 10           | 84.304       |
| F-4.3   | 70            | 25             | 5            | 84.223       |
| F-5.1   | 80            | 5              | 15           | 77.589       |
| F-5.2   | 80            | 15             | 5            | 80.299       |
| F-5.3   | 80            | 10             | 10           | 66.869       |

Table-1 showed lipase inhibition of the formula of green tea fortified with bay leaves and cinnamon. For comparison, lipase inhibition by ECGC (74.95%) and Orlistat (46.79%) as the known lipase inhibitors in slimming tea (22.16%) act as commercial herbs for anti-obesity.<sup>12</sup> Higher lipase inhibitory activities were found in formulas containing a much higher green tea content in the F-3.2 (78.326%), F-4.1 (91.909), and F-5.2 (80.299%) such as Formula 3.2 (Gt:Bl:Cb= 60:25:15), Formula 4.1 (Gt:Bl:Cb =70:10:20), and Formula 5.2 (Gt:Bl:Cb = 80:15:5). Formulas that contain bay leaves have an inhibition below 70%.

### Determination of LD<sub>50</sub> and Toxicity Observation

We investigated the potential toxicity effect of the three formulas of herbal fortified green tea on the manifestation of the body weight of rats during the experiment. Body weight was closely related to the amount of feed consumption that was one of the quantitative criteria for toxic effects. Figure-1 shows the mean body weight of rats in the control groups and the treated groups. Monitoring of weight showed no significant changes ( $p > 0.05$ ) during the experiment, as seen between the control group and the treated group with varied concentrations of Formula F-3.2, F-4.1, and F-5.2. Neither female rats nor male rats showed significant changes in the amount of food and fluid consumption. (data not shown)

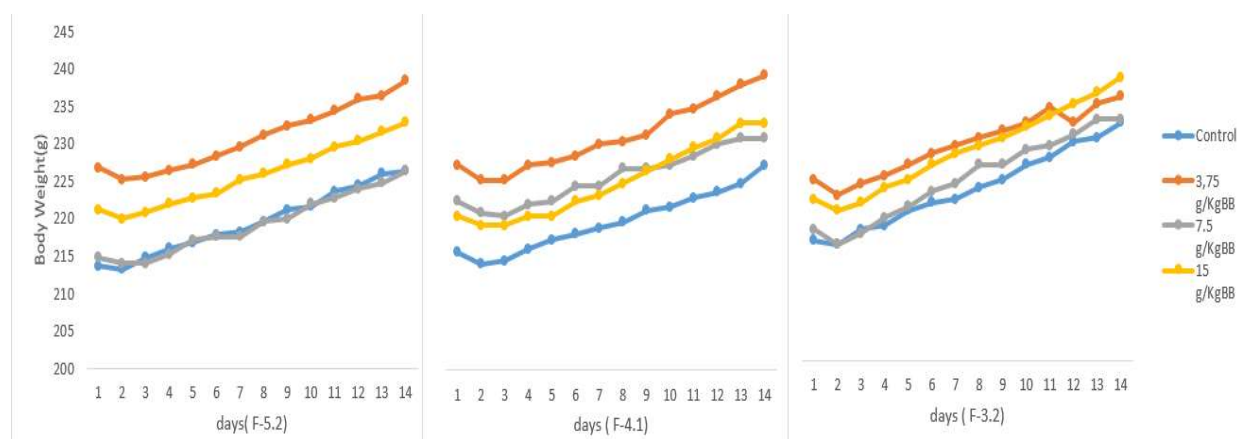


Fig.-1: Male Rat Body Weight Changes during Acute Toxicity Study of Formula F-3.2, Formula F-4.1, and Formula F-5.2. (No significant changes ( $p > 0.005$ ))

Cardiovascular, respiration, somatomotor, skin, and fur condition of each animal in each group were observed one hour after being given the extract of the tested formula each day for 14 days (Table-2). During the 14-day observation period, the motoric condition of the animals in each group showed no significant changes and appeared to be normal for both the control group and the dose group up to the highest dose of 15 g/kg BW. This observation is important because, in acute toxicity, the drug dose was given in a very high dose, supposing there would be effects on the neuromotor and cardiovascular functions of the animals.

Table-2: Observation of Rats during the 14-Day Experiment

| Observation    | Male    |              |             |            |
|----------------|---------|--------------|-------------|------------|
|                | Control | 3.75 g/kg bb | 7.5 g/kg bb | 15 g/kg bb |
| Formula F-3.2  |         |              |             |            |
| Cardiovascular | NL      | NL           | NL          | NL         |
| Respiration    | NL      | NL           | NL          | NL         |
| Somatomotor    | NL      | NL           | NL          | NL         |
| Skin and Fur   | NL      | NL           | NL          | NL         |
| Formula F-4.1  |         |              |             |            |
| Cardiovascular | NL      | NL           | NL          | NL         |
| Respiration    | NL      | NL           | NL          | NL         |
| Somatomotor    | NL      | NL           | NL          | NL         |
| Skin and Fur   | NL      | NL           | NL          | NL         |
| Formula F-5.2  |         |              |             |            |
| Cardiovascular | NL      | NL           | NL          | NL         |

|              |    |    |    |    |
|--------------|----|----|----|----|
| Respiration  | NL | NL | NL | NL |
| Somatomotor  | NL | NL | NL | NL |
| Skin and Fur | NL | NL | NL | NL |

\*NL = Normal

We also observed other findings (organ weight and gross necropsy). Accordingly, it seems likely that the three herbal formulas of fortified green tea were not toxic in the studied doses. LD<sub>50</sub> calculation results, based on mortality data (Table-3), showed that the LD<sub>50</sub> value in the group of males and females is apparent; this means that the three herbal formulas of fortified green tea are practically nontoxic (apparent LD<sub>50</sub> > 15 gr/kg BW). Gamboeng tea is a green tea that is usually consumed and considered safe for humans. But in this study, we fortified green tea with three herbs, investigating whether the interaction between the herbs and the interaction with green tea could have a toxic effect. But from the results of mortality data, we can conclude that herbal fortified green tea with varying formulas is non-toxic.

Table-3: Rat Mortality and LD<sub>50</sub> Value in the Group of Male Rats

| Formula       | Dose (g/KgBW) | The rat's average body weight (g) | Number of deaths/number of experimental animals |
|---------------|---------------|-----------------------------------|-------------------------------------------------|
| Formula F-3.2 | control       | 218                               | 0/5                                             |
|               | 3.75          | 224                               | 0/5                                             |
|               | 7.50          | 219                               | 0/5                                             |
|               | 15.00         | 222                               | 0/5                                             |
| Formula F-4.1 | control       | 211                               | 0/5                                             |
|               | 3.75          | 222                               | 0/5                                             |
|               | 7.50          | 218                               | 0/5                                             |
|               | 15.00         | 216                               | 0/5                                             |
| Formula F-5.2 | control       | 214                               | 0/5                                             |
|               | 3.75          | 227                               | 0/5                                             |
|               | 7.50          | 216                               | 0/5                                             |
|               | 15.00         | 221                               | 0/5                                             |

Another method to measure sample toxicity is the measurement of organ weight. Organ abnormalities such as heart, liver, lungs, and kidneys are closely related to the presence of toxic metabolites. Substances that enter the human body undergo metabolism in the liver, kidneys and intestines. Figure-2 shows no significant changes in body weight in the liver, kidneys, heart, spleen, brain, lungs, and intestines. Table-3 shows that there was no significant change in weight in either the control rats or the treated rats. The results of statistical data analysis showed  $p > 0.05$ , there was no significant change in body weight. Based on the data obtained, it was found in the control and treatment groups.

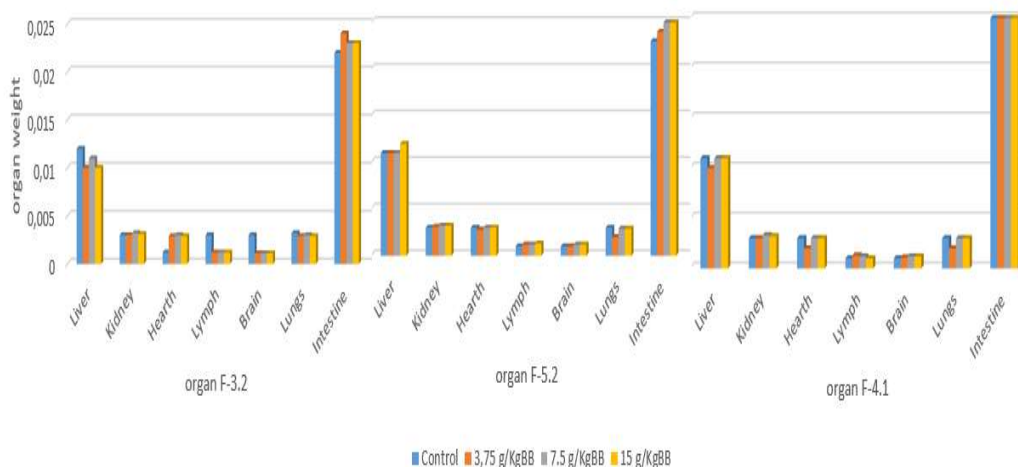


Fig-2: Organs Weight of Rat after Treatment with Formula F-3.2, Formula F-4.1, Formula F-5.1

## CONCLUSION

The formula of green “gamboeng” tea, bay leaves, and cinnamon bark is suitable for use as an inhibitor of the lipase enzyme, which can reduce obesity with the highest inhibition of 91.509% for F4.1 (Green tea: Bay leaves: Cinnamon bark = 70: 20: 10), and the lowest inhibition of 59.59% for F-3.1 (Green tea: Bay leaves: Cinnamon bark = 60: 15: 25). These findings confirm that Formula 4 has the highest inhibition compared to the other formulas. Administration of the three formulas of fortified herbal gamboeng tea F-3.2, F-4.1, and F-5.2, up to the highest dose of 15 g/kg BW, did not have any toxic effect both in terms of parameters of body weight, neuromotor functionality, organ weight, and the LD50 value > 15 g/kg BW is considered as practically non-toxic.

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## REFERENCES

1. Megawati, N. Artanti, H. Mulyani, A. Darmawan, H. Syahrian, P.D. N. Lotulung, E. Supriadi, G. Widiyarti, R. T. Dewi, L. Meilawati, T. Ernawati, I. D. Dewijanti and Minarti, *The Indonesian Journal of Nutrition*, **9**(1), 48(2020), <https://doi.org/10.14710/jgi.9.1.48-52>
2. S. Shiyan, T. Hertiani, R. Martien and A.K. Nugroho, *Rasayan Journal of Chemistry*, **12**(3), 1098(2019), <http://dx.doi.org/10.31788/RJC.2019.1235276>
3. R. Johnson, S. Bryant and A.L. Huntley, *Maturitas*, **73**(4), 280(2016), <https://doi.org/10.1016/j.maturitas.2012.08.008>
4. S. Shiyan, Fitrya, Arimia and G. Pratiwi, *Rasayan Journal of Chemistry*, **13**(3), 1472(2020), <http://dx.doi.org/10.31788/RJC.2020.1335755>
5. P. Ranasinghe, S. Perera, M. Gunatilake, E. Abeywardene, N. Gunapala, S. Premakumara, K. Perera, D. Lokuhetty and P. Katulanda, *Pharmacognosy Researches*, **4**(2), 73(2012), <https://doi.org/10.4103/0974-8490.94719>
6. M.B. Hossaina, N.P. Bruntonb, C. Barry-Ryana, A.B. Martin-Dianaa and M.Wilkinson, *Rasayan Journal of Chemistry*, **1**(4), 751(2008)
7. M. D. Hidayati, T. Ersam, K. Shimizu and S. Fatmawati, *Indonesian Journal of Chemistry*, **17**(1), 49(2017), <https://doi.org/10.22146/ijc.23545>
8. A. Hamad, M.G.P. Mahardika, I. Yuliani and D. Hartanti, *Rasayan Journal of Chemistry*, **10**(2), 564(2017), <http://dx.doi.org/10.7324/RJC.2017.1021693>
9. O. Y. Olnrewaju, E. A. Ekundayo, F. Akharaiyi and D. Ewhenodere, *Tropical plant Research an International Journal*, **4**(3), 376(2017), <https://doi.org/10.22271/tpr.2017.v4.i3.049>
10. S-H. Eom, M-S. Lee, E-W. Lee, Y-M. Kim and T. H. Kim, *Phytother. Res*, **27**(1), 148(2013), <https://doi.org/10.1002/ptr.4694>
11. I. Kamzi, M. Afzal, S. Rahman, M. Iqbal, F. Imam and F. Anwar, *European Journal of Pharmacology*, **709**(1–3), 28(2013), <https://doi.org/10.1016/j.ejphar.2013.02.032>
12. B. Bolon, W. Hazchek, R. Ochoa, C. Rousseaux and M. Wallig, *Handbook of Toxicologic Pathology (Second Edition)*, Academic Press, **1**, 307(2002)
13. Y. S. Kim, Y. M. Lee, H. Kim, J. Kim, D.S. Jang, J. H. Kim and J.S. Kim, *Journal of Ethnopharmacology*, **130**(3), 621(2010), <https://doi.org/10.1016/j.jep.2010.05.053>
14. T. T. Liu, X-T. Liu, Q-X. Chen and Y. Shi, *Biomedicine & Pharmacotherapy*, **128**, 110314(2020), <https://doi.org/10.1016/j.biopha.2020.110314>
15. R. Pariyani, I. S. Ismail, A. A. Azam, F. Abas, K. Shaari, and M.R.Sulaiman, *Biomed Research International*, ID742420, **8** (2015), <https://doi.org/10.1155/2015/742420>
16. Megawati, T. Ernawati, L. Meilawati, I. D. Dewijanti and E. Supriadi, *AIP Conference Proceedings*, **2175**, 020013-6(2019), <https://doi.org/10.1063/1.5134577>

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