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Phytochemical screening of water extract of gayam (*Inocarpus edulis*) Bark and its amylase inhibitor activity assay

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Abstract. The Study on Phytochemical Screening of Water Extract of Gayam (*Inocarpus edulis*) Bark and its Amylase Inhibitor Activity Assay has been carried out. The aim of this study is to identify the chemical compound of Gayam Bark by phytochemical screening and to determine its amylase inhibitor activity by using dinitrosalicylic acid methods. As much as 15.0212 g of the cut small gayam bark was put into a 250 mL extraction flask. Into the sample was added 100 mL of water and macerated for 24 hours. Then, the mixture was filtered with Whatman filter paper. The filtrate was concentrated using a rotary evaporator to give viscous extract from the Gayam bark. The results showed that the chemical compounds contained in the Gayam bark extract included terpenoids, saponins and tannins. Gayam bark displayed α-amylase enzyme inhibitor where as much as 12.64 µL (17.88 mg/mL) of the extract solution capable of reducing 50% of enzyme α-amylase activity (10 mg/mL). The results showed that the IC_{50} of extract was 75.3344 µg/mL, which was smaller than that of acarbose, indicating the extract demonstrated better inhibitor activity.
Keywords: α-amylase, gayam (*Inocarpus edulis*), bark, IC_{50}.

1. Introduction

The non-communicable diseases have become a global public health problem since 60% of the causes of death of all ages in the world are due to these diseases. One of the non-communicable diseases which gains the most attention is diabetes mellitus. Diabetes mellitus (DM) is on the 6th rank as a cause of death. In Indonesia, the number of deaths caused by DM continues to increase and is estimated to reach 21.3 million cases in 2030 or an increase of almost 300% when compared to the number of DM patients in 2000 which only reached 8.4 million.

Numbers of efforts have been conducted to reduce the risk of death caused by DM, such as traditional treatments using certain plant extracts. The results of the study prove that certain plant extracts have antidiabetic abilities, such as *Cinnamomum tamala* plants [1, 2]. In this context, the application of traditional medicines from local plants as medicinal ingredients have been widely conducted in Indonesia. Some local plants that are believed to have the ability to reduce the risk and even to cure DM include lamtoro seeds and fragrant pandan leaf extract [3].

In Indonesia, Gayam seeds are traditionally consumed mainly in the form of boiled seeds. In Maluku, Gayam plants can be used for medicinal purposes by consuming the boiled water from the bark. Gayam plants are cultivated in the lowlands (<500 m asl) of Java and Eastern Indonesia (Sulawesi and Maluku)

[4, 5]. The activity of Gayam bark extract as antidiabetic has a great potential to be developed as the candidate for anti-diabetes drug raw materials. It is presumed that there are the active ingredients in the Gayam bark extract which can inhibit the activity of amylase, which is an enzyme that hydrolyses starch to glucose and is then responsible for the increase in glucose concentration in the blood [6]. This local tradition must be proven by a scientific study. The appropriate method is required to analyse and characterize the active compounds in the Gayam bark which can inhibit amylase enzyme.

The class of chemical compounds in the Gayam bark can be determined using phytochemical screening method and the inhibition assay can be done by IC_{50} method. The phytochemical screening is a qualitative test of the chemical content to determine the class of compounds contained in a plant and IC_{50} is the concentration needed to reduce 50% of the absorbance value [7, 8].

2. Research Method

2.1. Tools

Tools employed in this study were laboratory glassware, oven (Shel Lab), hotplate (Cimarec 2), UV-Vis spectrophotometer (Apel PD-303S), micropipette (Eppendorf research) and evaporator (Buchi).

2.2. Materials

Materials used in this study were bark of Gayam (*Inocarpus edulis*), soluble starch (Merck), hydrochloric acid (Merck), Bouchardat LP (Technical grade), Mayer LP (Technical grade), diethyl ether (Merck), sulphuric acid (Merck), gelatine (Merck), $FeCl_3$ (Merck), NaCl (Merck), dinitrosalicylic acid (Bio Basic), Ka-Na-Tartrate (Merck), phosphate buffer pH 7.2 (Technical grade), pancreatin (Wako), sodium hydroxide (Merck), Whatman filter paper No. 1 and distilled water.

2.3. Procedures

2.3.1. Preparation of sample. Gayam (*Inocarpus edulis*) bark was collected from Hitu village. The sample was cleaned and cut into small pieces.

2.3.2. Preparation of extract of Gayam bark. Extraction of active substances from the Gayam bark followed the Kumanan method [2]. As much as 15.0212 g of the cut small gayam bark was put into a 250 mL extraction flask. Into the sample was added 100 mL of water and macerated for 24 hours. Then, the mixture was filtered with Whatman filter paper. The filtrate was concentrated using a rotary evaporator to give viscous extract from the Gayam bark.

2.3.3. Identification of alkaloid [9]. A few milligrams of the extract was dissolved in 10 mL of a mixture of distilled water and 2 N HCl (9:1). The mixture was then heated for 2 minutes on a water bath. It was then cooled and filtered. The filtrate obtained was used as an experimental solution which was then treated as follows: (a) two drops of Bouchardat LP was added to 1 mL of the filtrate. The positive results of alkaloid occurred with the formation of brown precipitate. (b) two drops of Mayer LP was added to 1 mL of the filtrate. The positive results of alkaloid occurred with the formation of white precipitate.

2.3.4. Identification of flavonoid [8]. A few milligrams of Gayam bark extract was dissolved in 10 mL of methanol and then divided into 3 test tubes. The second and third tubes were added with concentrated NaOH and H_2SO_4 then compared with the first tube as a control. Positive results if there is a Green-brown-yellow colour change.

2.3.5. Identification of terpenoid. A few milligrams of the extract were dissolved in 5 mL diethyl ether, then filtered and evaporated in a evaporating dish to dry. Into the residue was added 10 drops of anhydrous acetic acid and 5 drops of concentrated sulphuric acid. A positive result if the red-violet-blue colour is formed.

2.3.6. Identification of saponin [9]. Into a few milligrams of the extract was added 10 mL of hot distilled water, then cooled and shaken vigorously for 10 seconds. Positive results was indicated by the formation

of a stable foam for not less than 10 minutes, as high as 1 cm to 10 cm. On the addition of 1 drop of 2N hydrochloric acid the foam did not disappear.

2.3.7. Identification of tannin [7, 9]. A few milligrams of the extract were dissolved in 10 mL of distilled water. The mixture was heated for 5 min. Then, the solution was cooled and filtered. As much as 1 mL of filtrate was analysed by: (a) adding two drops of gelatine solution 10%. The positive results were indicated by the formation of white precipitate. (b) adding two drops of $FeCl_3$ solution 1%. The positive results were indicated by the formation of violet green precipitate.

2.3.8. Amylase inhibitor activity assay. Evaluation of inhibition activity of amylase was carried out by measuring the total reducing sugar based on the method of dinitrosalicylic acid (DNS) [10] by using starch as a substrate. As much as 900 µL of mixture (containing 800 µL of phosphate buffer solution pH 7.2 and 100 µL of amylase enzyme from pancreatin with activty of 30 U/mg) were incubated at 37°C for 5 min. Furthermore, 100 µL of substrate (control) was added. Incubation was continued for 3 minutes. The reaction was quenched by adding 1 mL of DNS solution. The mixture was then heated for 5 minutes and cooled in ice water. The absorbance was measured at a wavelength of 540 nm. The inhibition test of amylase activity was carried out by adding the extract of Gayam bark (5, 10, 15 and 20 µL) into the mixture. Determination of total reducing sugars resulting from amylase activity was done using glucose as standard. The % inhibition of the extract was calculated using the following equation:

$$\%\ \text{inhibition} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \text{x } 100\%$$

2.3.9. Determination of inhibitory concentration of amylase. Inhibitory concentration –50 (IC_{50}) is defined as the concentration of the sample solution required to reduce the 50% absorbance value compared to the absorbance value of the blank solution [7]. It is calculated using the linear regression equation, where the absorbance of the test is the x axis and percent inhibition (% inhibition) is the y axis. From the equation y = a + bx, IC_{50} can be calculated the following equation:

$$IC_{50} = \frac{50 - a}{b}$$

3. Results and Discussion

3.1. Preparation of extract of Gayam bark

Gayam (*Inocarpus edulis*) bark used in this study was the skin of a male Gayam tree. This Gayam is the one which never produces fruit. The male gayam tree was collected in Mamoa village, Central Maluku. The sample was not dried before extracting since the extraction process was carried out using water as a solvent. The collected sample was then cut into small pieces with the aim of increasing the surface area so that the active substance can be optimally extracted.

The extraction process was conducted by maceration technique. As much as 15.0212 g of the cut sample was put into a 250 mL Erlenmeyer flask and added with 100 mL of distilled water. Next, the sample was macerated at 50°C for 24 h while stirring using a magnetic stirrer. The crude extract was then concentrated using a rotary evaporator. The extract was obtained in 0.0894 g and was dissolved with distilled water until the volume was 5 mL (the concentration of Gayam bark extract was 17.88 mg/mL).

3.2. Identification of Chemical Compounds

Identification of alkaloid compounds was carried out by adding few drops of Bouchardat and Meyer reagents to the extract of the Gayam bark which had previously been dissolved in a mixture of distilled water and hydrochloric acid. Alkaloid compounds were generally found in plants in the form of salts that are soluble in water or in alkaline form, so that alkaloids can be withdrawn using water solvents in

an acidic condition [11]. The results of the addition of Bouchardat and Meyer reagents showed that the samples did not contain alkaloids, since there was no precipitate observed.

Identification of flavonoids was performed by dissolving few milligrams of the extract into 10 mL of methanol. The solution was then divided into three parts, one of which was used as a control. The second and third tubes were added with concentrated NaOH and H_2SO_4 then compared with the first tube as a control. Positive results were indicated by the colour change [8]. Fig. 1 and Fig. 2 showed the reaction of flavonoid in sodium hydroxide (NaOH) and sulphuric acid (H_2SO_4).

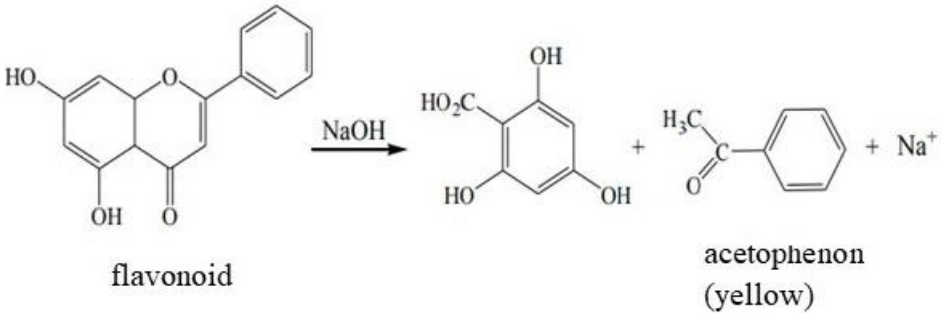

Figure 1. Reaction flavonoid in NaOH [12].

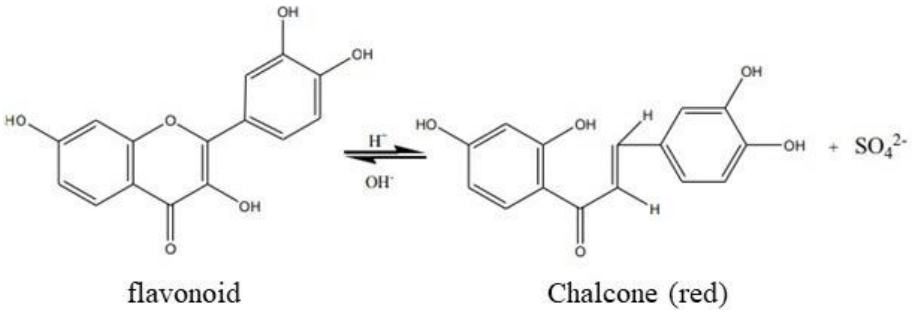

Figure 2. Reaction flavonoid in H_2SO_4 [13].

The results showed that the extract did not contain flavonoids. This can be seen from the colour of the solution that did not change when compared to the colour of the control solution. The results of this identification are contrary to those conducted by Santi [14] who isolated flavonoid compounds from gayam bark. Identification of flavonoids on the Gayam bark extract showed that the colour of solution did not change comparing to the control solution. It indicated the extract did not contain flavonoids. The results were in contrary to those conducted by Santi [14] which isolated flavonoid compounds from gayam bark.

The identification of terpenoids was carried out by dissolving a few milligrams of extract into diethyl ether. Next, a few drops of acetic acid anhydride were added and continued to add concentrated sulphuric acid drop by drop. Positive results were indicated by the formation of a red-violet-blue colour complex depending on the type of terpenoids [11]. The analysis showed the presence of terpenoids on the sample which can be proven by the formation of a red complex after the addition of acetic acid and sulphuric acid to the extract. It indicated that the terpenoids contained in the sample were triterpenoids [11]. These results also demonstrated that there was no steroid in the Gayam bark extract indicating that it was contrary to the results reported by Aditya [15] where steroid compounds were found in the Gayam bark and displayed antioxidant activity and by Krisna *et al.* [16] which tested the antioxidant activity of steroid compounds on gayam leaves.

The identification of saponin was carried out by shaking the Gayam bark extract solution in hot distilled water. The results showed that the samples positively contained saponins, which were observed through many and stable foam formed above the surface of the solution.

Identification of tannin was carried out using a colour reaction of iron (III) chloride. Phenolic moiety on tannins will react with Fe^{3+} ions from iron (III) chloride to generate coloured complex compounds. The blackish blue colour indicated the presence of hydrolysed tannins, while the brownish green colour indicated the presence of condensed tannins [17]. The results showed that the Gayam bark extract contained the condensed tannins. Based on research conducted by da Silva *et al.* [18], the condensed tannins in *Araucaria angustifolia* extract were effective in inhibiting α-amylase activity.

The identification of chemical compounds carried out in this study should be further investigated since there were still many differences comparing to the similar study. In fact, according to Venkataraman [19] based on the taxonomical approach that the plant in the same genus or family will contain the same chemical compounds. It raised the question of why there are differences in the components of chemical compounds in the same plant.

From here, the authors presumed that environmental factors also affect the chemical content. It was also probably because the gayam used was the male gayam which never produces fruit. It still cannot be proven with certainty, therefore, further research is highly required

3.3. Amylase inhibitor activity assay

In the α-amylase inhibitory activity assay, there are two types of samples tested, namely sample solutions and controls. The sample solution was solution of extract with a concentration of 17.88 mg/mL with the variation of volume, 5, 7.5, 10, 12.5, and 15 µL. The principle of the inhibitory activity of α-amylase assay is to measure the total reducing sugar based on the method of dinitrosalicylic acid (DNS) [10] with starch as a substrate. The starch used is water-soluble starch, because the Gayam bark extract is prepared using water as solvent. Fig. 3 showed the reaction of DNS with glucose.

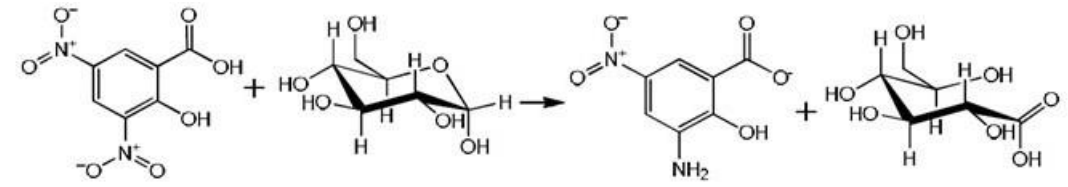

Figure 3. Reaction DNS with glucose [10].

The measurement showed that the more the amount of extract added led to the decrease of the amount of reducing sugar hydrolysed by the α-amylase enzyme. In other words, the absorbance of the sample solution decreased. This can be seen from table 1.

Table 1. Effect of increasing volume of the extract on the absorbance of sample.

Increasing volume of the extract (µL)	Absorbance (λ=540 nm)
0 (Control)	0.819
5	0.709
7.5	0.626
10	0.482
12.5	0.377
15	0.339

The absorbance which showed 50% inhibition was 0.409. Therefore, the IC_{50} value was 12.64 µL or 75.3344 µg/mL. Many studies have been conducted to determine the inhibition of α-amylase by certain plant extracts usually acarbose as positive control. This is because the acarbose is an oligosaccharide produced by microorganism fermentation which is also an active ingredient in hypoglycemic drugs that are active in carbohydrate catabolism inhibitors, including α-amylase inhibitors [20, 21].

Poongunran *et al.* [22] evaluated several medicinal plants as α-amylase inhibitors including the bark of *Ficus racemosa,* all parts of *Phyllanthus debilis,* fruit of *Phyllanthus emblica,* sap of *Pterocarpus marsupium* also acarbose as a comparison with IC_{50} values of 19.73; 973; 397.67; 2.97 and 262.54 µg/mL, respectively. Among the four samples, two of them have better IC_{50} values than acarbose. There are also a number of studies that do not include acarbose as a comparison as in Nickavar *et al.* [23]. Nickavar *et al.* [23] performed the α-amylase inhibition assay towards six *Salvia* species. Two of the six species, *S. verticillata* and *S. virgata* displayed the IC_{50} values of 18.34 and 19.73 mg/mL, respectively. This value was certainly still far comparing to the acarbose obtained by Poongunran *et al.* [22].

The IC_{50} value of acarbose obtained Poongunran *et al.* [22] was quite high compared to the IC_{50} value of the Gayam bark obtained in this study. This is quite surprising considering that acarbose is widely known as α-amylase enzyme inhibitor. The reason why the Gayam bark extract, in this study, has a better potential than acarbose because the material used in this study is an extract which can contain various kinds of active compounds. These compounds can be synergistic in inhibiting the α-amylase enzyme.

4. Conclusions

Based on the results of the research, it can be concluded that the chemical compounds contained in the water extracts of Gayam (*Inocarpus* edulis) bark included terpenoids (triterpenoids), saponins, and tannins. The water extract from the Gayam (*Inocarpus edulis*) bark has the ability as an inhibitor of the α-amylase enzyme with an IC_{50} of 75.3344 µg/mL, which was obtained by adding 12.64 µL of extract (17.88 mg/mL) into the test solution with a total volume of 3 mL.

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