ANTIDIABETIC AND ANTIARRHEAL ACTIVITY FROM EXTRACT OF NAMNAM (Cynometra cauliflora) LEAF

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Abstract

In addition to decorate the yard, plant namnam (Cynometra caulifora) turns out to have merit as an herbal remedy because it has biological activities such as antimicrobial, antioxidant, cytotoxic, antiviral, anti-inflammatory, antitumor and antidiabetic. It is study will add to scientific information about plants Namnam the potential to be developed as a functional food and natural herbal remedies in particular as antidiabetic agents and antiarrheal. Ability as an antidiabetic agent tested by the ability of plants Namnam inhibit α-amylase. Meanwhile, antiarrheal activity will be tested against the isolated rabbit intestine recorded on kymograph. The analysis showed that the leaf extract of plant Namnam (C. caulifora) at a concentration of 100 mg/mL, 250 mg/mL and 300 mg/mL each is able to inhibit α-amylase activity by 20.68%; 70.24% and 72.59% and IC50 of 200.67 ± 0.53 mg/mL. The study also showed that the sample tested has the ability to decrease the activity of isolated rabbit intestine in the pattern described in kymograph. Thus the plant leaves Namnam (C. caulifora) potential to be developed as antidietes agent and antiarrheal. This may partially explain the use in traditional medicine for the treatment of diarrhea. These results support their use in folk medicine.

Keywords: Antidiabetic, antiarrheal, Cynometra caulifora, Namnam

1. Introduction

Indonesia is rich in a wide range of biodiversity that have the potential to be developed as a drug or drug raw materials, particularly traditional medicines derived from plants. Plants have potential as medicines because of the presence of secondary metabolites that accompanies the existence of these plants. The compounds are secondary metabolites that are toxic can be used to treat various types of diseases in humans, such as alkaloids, flavonoids, saponins, tannins, steroids and triterpenoids [1]. flavonoids for example, has the ability to inhibit the enzyme α-amylase and α-glucosidase [2]. The existence of these flavonoids according Unnikrishnan et al. [3] allows the plants act as antidiabetic agents.

Plants that have the function of which is crop namnam (C. caulifora). This plant is a plant fruit underutilized but has medical value as traditional medicines and cultivated as an ornamental plant by rural communities [4]. Whereas namnam especially on the leaves contain active compounds such as tannins, saponins, flavonoids, terpenoids and glycosides [5]. Some studies also have shown that plants namnam (C. caulifora) is known to be rich in biological activities such as antimicrobial, antioxidant, cytotoxic, antiviral, anti-inflammatory, antitumor and antidiabetic [5,6,7,8]. In fact, the methanol extract of the fruit C. caulifora highly cytotoxic against promielositik leukemia cells HL - 60 and inhibits cell proliferation [7]. Another potential that is also often used for generations in is as antiarrheal. Scientifically researchers have linked antiarrheal function is presumably because the tannin compounds contained in plants that work as adstringens that can shrink the mucous membranes of the intestines that can reduce the occurrence of diarrhea and relieve the state of nonspecific diarrhea in mice [9,10].

Thus a great potential to be used active ingredient, however, utilization is still very low. Besides scientific information relating to other functions are still very minimal. Therefore, research on plant namnam (C. caulifora) the potential to do, especially leaf extract. This study aims to determine the effect of the methanolic extract leaf namnam by studying the inhibition of α-amylase (antidiabetic agents) and the contraction of isolated rabbit intestine (antiarrheal). The result can be used for further recommendation as antidiabetic agents, antiarrheal or other benefits that can improve plant utilization value namnam (C. caulifora).

2. Materials and Methods

2.1. Plant material

The test material used in this study are the leaves namnam (C. caulifora) obtained from Desa Cintaratu, Kecamatan Parigi, Kabupaten Pangandaran, West Java Indonesia. This plant has been identified by the Bogor-based Research Center for Biology Indonesian Institute of Sciences as C. caulifora L. All chemicals and reagents used of analytical grade.

2.2. Phytochemical studies

Phytochemical analyses of the extract were performed according to the methods of Harborne [11]. The extract was screened for the presence of alkaloids, saponins, tannins, flavonoids, quinones and steroids/triterpenoids.
2.3. Preparation of methanol extract

Leaves Namnam (C. cauliflora L.) washed, sorted and dried in the sun for 30 hours until the moisture content 9-10%. Then milled in a blender (Arte, Indonesia), in order to obtain a fine powder. Namnam leaf powder of 100 gram soaked with 100 mL of methanol (JT Baker, USA) and macerated for 24 hour. After the results of the marinade filtered with Whatman filter paper no.4 (Merkmilipore, Germany), in order to obtain the first filtrate. Residu Namnam leaves macerated again with methanol (JT Baker) for 9 hours, thus obtained filtrate evaporated with the second rotary. Filtrat evaporator (Heidolph 4000 Laborata, USA) at a temperature of 49°C to obtain a thick extract. The extraction process is done five times. Samples ready to be tested next.

2.4. Organt isolated for antidiarrheal test

Rabbit abdomen cut open with scissors. Then, the rabbit intestine removed and put into a large petri dish containing Tyrode solution of 37°C. With a 20 cc syringe, containing Tyrode solution of 37°C, the intestinal contents slowly sprayed out thoroughly. Furthermore, the small intestine is isolated from the proximal to distal direction, namely the duodenum, jejunum and ileum. Then the third part of the intestine is cut along each 2 cm. Then cuts the small intestine is tied with the thread (made in a glass beaker smaller containing a solution of Tyrode 37°C), one end of the intestine attached to the ends of the glass tube aerator, and the other end tied to the kymograf who are in it such that part of the intestine in a state capable of sufficiently stretchable. Finally observations began in the normal contraction. Then intestine given namnam plant leaf extract, then the observed changes. This is done repeatedly and compared.


α-amylase inhibition testing done on methanolic extract. α-amylase inhibition is determined by calculating the reducing sugars produced in experimental condition. Testing is done by preparing the extract solution with a concentration of 100-350 mg/mL (w/v in DMSO) in a test tube. Each of these test tubes containing 1 mL of sample solution, then added 1mL enzyme solution 5μ/mL, homogenized, and then incubated for 30 minutes at a temperature of 37°C. After that, add 1 mL substrat1 % w/v in phosphate buffer pH 6.9 to each tube containing extract solution. Furthermore, homogenized and then incubated for 10 minutes. The resulting solution incubation, add 1 mL of 1 % DNS solution, then heated in boiling water bath for 10 minutes until it forms a brownish red color. The solution was cooled to room temperature and then added 6 mL of distilled water.

Absorbance of the solution was measured using a UV - VIS spectrophotometer at a wavelength of 540 nm (λ max 3-amino-5-nitrosalisilic acid). The amount of maltose formed is proportional to the 3-amino-5-nitrosalisilic acid measured. The more maltose is formed, the higher the acid absorption 3-amino-5-nitrosalisilat measured. The percentage inhibition of α-amylase is determined using the following equation:

\[
\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%
\]

IC50 can be calculated using the linear regression equation \( y = bx + a \):

\[
\text{IC}_{50} = \frac{50 + a}{b}
\]

3. Results and Discussion

3.1. Phytochemical analysis

Medicinal plants are known to possess various phytochemical principles, which are responsible for their pharmacological activities and some side effects. Phytochemical analysis of the extract revealed the presence of saponins, steroids/triterpenoid, flavonoids, tannins, dan quinons while alkaloids were however not detected (Table 1) which could be responsible for its pharmacological actions.

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Methanol extract</th>
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<tbody>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids/triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
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<tr>
<td>Quinons</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
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3.2. In vitro α-amylase inhibition study

At various concentrations tested showed positive results, which may inhibit α-amylase. The higher the concentration of the compound Namnam (C. cauliflora) then the higher the α-amylase inhibitory (Table 2). Rais et al. [12], also with sambiloto plant have stated that the increase in the concentration of effect on amylase inhibitory activity, but the effect of the increase is not significantly. The amount of α-amylase inhibitory work due namnam leaves contain active compounds which act as inhibitors compounds as shown in Table 1. In this study also showed
that the greater the concentration, the amount of phytochemical compounds that act as inhibitors higher (Table 2).

Gowri et al. [13] in Teucrium species plants extract that its inhibitory activity may be as a result of the existence of various phytochemicals like flavonoids, tannins, saponins, anthraquinone, steroid, phlobatannin, terpenoid, in them.

Table 2. The inhibitory effect of the methanolic extract of leaf C. cauliflora on α-amylase

<table>
<thead>
<tr>
<th>CONCENTRATION (mg/mL)</th>
<th>inhibition (%)</th>
<th>IC50 (mg/mL)</th>
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<tbody>
<tr>
<td>100</td>
<td>20.68</td>
<td>200.67±0.53</td>
</tr>
<tr>
<td>250</td>
<td>70.24</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>72.59</td>
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</tr>
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</table>

This research has also shown the presence of flavonoids and tannins are successfully detected in phytochemical analysis to leaf extracts Namnam (C. cauliflora) (Table 1). Previous research studies on α-amylase inhibitors identified from medicinal herbs recommend that a number of capability inhibitors belong to flavonoid class that has features of inhibiting α-amylase activities [14]. Kazem et al. [15] has also concluded in his research on aqueous extract of Morinda lucida that the best inhibitory activity on α-amylase because the presence of phytochemicals like flavonoids, saponins, and tannins in the plant extract. This active ingredient has been widely believed to have a major role inhibit the activity of α-amylase. Flavonoid compounds such as polyphenols capable of inhibiting carbohydrate hydrolyzing enzymes because of their ability to bind with proteins [16, 17].

The action mechanism proposed for inhibitory capacity of flavonoids correlated the potency of inhibition of these compounds with the number of hydroxyl groups on the B ring of the flavonoid skeleton with the formation of hydrogen bounds between the hydroxyl groups of the polyphenol ligands and the catalytic residues of the binding site of the enzyme. Lo Piparo et al. [18] investigated the interactions between flavonoids and human α-amylase in order to understand the molecular requirement for enzyme inhibition. They showed that the potency of inhibition is correlated with the number of hydroxyl groups on the B ring of the flavonoid skeleton. The interaction occurs with the formation of hydrogen bonds between the hydroxyl groups in position R6 or R7 of the ring A and position R4’ or R5’ of the ring B of the polyphenol ligands and the catalytic residues of the binding site and formation of a conjugated π-system that stabilizes the interaction with the active site [18].

Tannin is also one example of the potential of polyphenols in the α-amylase inhibitory. Tannins could cause several effects on the biological system because they are potential metal ion chelators and protein precipitation agents forming insoluble complexes with proteins, as well as biological oxidants [19]. The main inhibitory effects of the tannins is related with its the ability to strongly bind to carbohydrates and proteins. However, Kandra etal. [20] suggested that the interaction between tannins, such galloylated quinic acid, and human α-amylase is also correlated with free OH groups in the tannin, that are able to participate in hydrogen bonding [20].

Inhibitors of these enzymes delay carbohydrate digestion, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial increase of plasma glucose [21]. The mechanism of action is through the inhibition of the last step in carbohydrate digestion, namely the conversion of disaccharide to monosaccharide (glucose) and a consequent decrease in the rate of entry of glucose into the systemic circulation [22]. Important constituents for the inhibitory activity against α-amylase are mainly polyphenolic compounds [23]. Inhibition of these α-amylase holds of carbohydrate digestion and extend the total carbohydrate digestion time, leading to a decrease in the rate of glucose absorption and therefore reducing the postprandial plasma glucose rise [24]. The outcomes of this study show that the administration of leaf extract namnam (C. cauliflora) may possibly control the postprandial blood glucose ranges and confirm the use of these herbs suggested as a treatment of diabetes Type-2.

3.3. Effect of methanolic extract leaf of Cynometra cauliflora on isolated rabbit ileum

Methanolic extract leaf Cynometra cauliflora significantly reduced intestinal transit time as observed by the decrease in intestinal motility of isolated rabbit jejunum as shown in figure 1 and 2. It also revealed that the effects of the extract on the isolated rabbit ileum were dose related and the extract relaxed the spontaneous contraction of the rabbit ileum (figure 1and 2).
Antidiarrheal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars [25]. This activity could be linked to the presence of tannins and flavonoids found during phytochemical screening of the extract. Clinically diarrhea may result from disturbed bowel function, in which case there is impaired intestinal absorption, excessive intestinal secretion of water and electrolyte, and a rapid bowel transit [26].

Mechanism of antidiarrheal activity can be predicted based on research on Punica granatum peels extract could be due to several mechanisms: (1) an increase in the water and NaCl reabsorption, (2) reduced mucosal secretion, and (3) inhibition of prostaglandin release from intestinal mucosa [27]. The extract may increase the reabsorption of water and NaCl by decreasing intestinal motility. This is supported by observation that the intestinal motility was significantly reduced in treated animals compared with control. Tannates are known to reduce mucosal secretion and make the intestinal mucosa more resistant [28, 29]. This allegation is getting stronger because the phytochemical analysis results indicate the presence of tannin in extract methanolic leaf namnam (Cynometra cauliflora) (Table 1).

Punica granatum peels aqueous extract caused a concentration-dependent relaxation of rat ileal smoothmuscle. The contractions of smooth muscles are known to depend on the concentration of intracellular Ca$^{2+}$ [30,31]. Because plant extract caused an inhibition of the contractions of the ileum, this implies that this extract decreased the cytosolic calcium, either by inhibiting Ca$^{2+}$ influx or by inhibiting Ca$^{2+}$ release from intracellular stores, or both. The relaxant effect could also have resulted from other mechanisms such as a decrease in the sensitivity of contractile apparatus to existing concentrations of Ca$^{2+}$ or inhibition of the binding of Ca$^{2+}$ to the contractile proteins [32]. Further studies are required to ascertain the precise mechanism of action of aqueous extract on ileal smooth muscles.

The spasmogenic and spasmolytic effect of a particular medicinal plant extract on the isolated ileum depends on predominant phytochemical constituents. Phenolic compounds exhibit spasmolytic activity while saponins are responsible for the spasmogenic activities of many plant extract preparation. From the phytochemical analysis of the extract of Cynometra cauliflora, the extract contains content of phenolic. However, the result obtained in this study
indicated that the crude extract of *Cynometra cauliflora* also contains other active ingredients with spastic effect higher than the anticholinergic effect of the phenolics.

Earlier studies showed that anti dysenteric and anti diarrhea properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids and sterols [25,33]. Hence, tannins, sterols, alkaloids may be responsible for the mechanism of action of extract leaf *C. cauliflora* anti-diarrhea activity. The anti-diarrhea activity of this extract may also be due to the presence of denatured proteins, which form protein tannates. Protein tannates make the intestinal mucosa more resistance and hence, reduce secretion. This can be due to the fact that the extract increased the reabsorption of by decreasing intestinal motility in isolated rabbit ileum [34].

Other than that tannin act as an astringent, as astringent tannins mechanism is to shrink intestinal surfaces or substances that are protective against intestinal mucosa and can agglomerate protein. Hence tannin can help stop diarrhea [34]. This was also confirmed by Aliyu and Chedi [35] in a study of the ethanolic stem bark extract of *pterocarpus erinaceus* por (Fabaceae) on some isolated smooth muscles which states that flavonoids and tannins identified during phytochemical screening of the extract may be the biologically active components responsible for the gastrointestinal.

### 4. Conclusion

Inhibitor of α-amylase activity qualitatively showed that the substrate concentration of 100 mg/mL, 250 mg/mL and 300 mg/mL each is able to inhibit α-amylase activity by 20.68%; 70.24% and 72.59% and IC₅₀ of 200.67 ± 0.53 mg/mL. Namnam leaf extract (C. caulifora) has the ability to decrease the activity of isolated rabbit intestine. The methanol extract of namnam leaves (*Cynometra cauliflora*) has the potential to be developed as anti diabetic agent through the mechanism inhibition of α-amylase and anti diarreal property so that needs to be preserved.

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### References


