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PROPERTIES OF BAJAKAH TAMPALA WOOD (*Spatholobus littoralis* Hassk) ETHANOL EXTRACT AGAINST α-AMYLASE INHIBITION AS AN ANTIDIABETIC COMPOUND CANDIDATE AND ITS FORTIFICATION IN KAPOK HONEY

Rista Anggriani, Noor Harini, Afifa Husna, Sukardi Sukardi, Maureen Danysha NS

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ABSTRACT

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1. INTRODUCTION

Diabetes mellitus is categorized as a degenerative disease or non-communicable disease which is still a health concern in every country around the world. The main contributors to chronic degenerative diseases are unhealthy lifestyles such as smoking, drinking alcohol, diet and obesity, lack of physical activity, stress, and environmental pollution (Handajani *et al*., 2010). It is estimated that 143 million people in the world suffer from diabetes mellitus (DM), almost five times compared to 10 years ago, and this number will probably double by 2030. Based on the World Health Organization (WHO) report that DM is one of the biggest killers in the world. DM is a syndrome characterized by chronic high blood sugar (hyperglycemia) due to impaired insulin production, secretion, or insulin resistance. This disease is very important to note because of the complications it causes. Complications of DM are short-term and long-term. This is related to persistently high blood glucose levels. In general, the adverse effects of hyperglycemia are macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, diabetic neuropathy, and retinopathy) (Yuhelma *et al*., 2015).

The Indonesian government establishes policies in an effort to improve health services to overcome the problem of degenerative diseases. The government stipulates the Decree of the Minister of Health of the Republic of Indonesia No. 381/Menkes/SK/III/2007 in one of the subsystems of the National Health System which states that the development and improvement of clinical trial research on the use of traditional medicines are aimed at obtaining traditional medicines that are of high quality, safe, have real

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efficacy that has been scientifically tested and can be used widely, either for personal use as well as in formal health services. In addition, the government stipulates Regulation of the Minister of Health Number 9 of 2016 concerning efforts to develop health through self-care for the use of family medicinal plants and their cultivation and processing skills. Medicinal plants or what are known as herbal plants in general can be interpreted as all types of plants that contain natural chemical compounds that have pharmacological effects and important bioactivity against infectious diseases to degenerative diseases (Suryanto and Setiawan, 2013).

Natural chemical compounds that have the potential to antidiabetics are flavonoids. Flavonoids are low molecular weight phenolic compounds composed of 2-phenyl-chromone from acetic acid derivatives (Amiani *et al*., 2022; Arifin and Ibrahim, 2018). Flavonoids are classified as antioxidant compounds that can stabilize and repair damaged cells (Asfar and Yasser, 2018; Firdausya and Amalia, 2020; Fitriani *et al*., 2020; Suharto *et al*., 2019). In addition, flavonoids also have anti-inflammatory, anti-allergic, anti-thrombotic, and anti-viral effects (Halim *et al*., 2022; Omidian *et al*., 2020). As antioxidant compounds, flavonoids have a hypoglycemic effect in DM patients. Flavonoids have an important role in preventing DM and its various complications. Based on the experiments carried out to prove the hypoglycemic effect of these flavonoids, it was concluded that plants containing flavonoids can reduce blood sugar levels (Brahmachari, 2011; Eryuda and Soleha, 2016; Pasaribu *et al*., 2021).

One of the flavonoid contents is found in Bajakah Tampala wood. The Bajakah Tampala plant which has the scientific name *Spatholobus littoralis* Hassk is a plant that grows a lot in Central Kalimantan. Based on previous research, Bajakah Tampala contains phenolic, flavonoid, tannin, and saponin content (Fitriani *et al*., 2020). The flavonoid content in the yellow Bajakah wood from South Kalimantan, yellow Bajakah wood from Central Kalimantan, and red Bajakah wood from Central Kalimantan reach 83,00 mg/mL; 10 mg/mL; and 60 mg/mL (Arifin *et al*., 2021). The antioxidant activity of the phenolic and flavonoid compounds in the Bajakah wood extract is claimed to be higher and stronger in reducing free radicals than vitamin C (Fitriani *et al*., 2020). The antioxidant activity of the ethanol extract of Bajakah wood is very strong which ranges from 13.25 ppm to 27.98 ppm (Amiani *et al*., 2022). Bajakah Tampala wood ethanol extract can reduce levels of free radicals (reactive oxygen species/ROS), fat weight, and body weight in obese male Wistar rats (Novanty *et al*., 2021).

In previous studies, 70% ethanol extract from Bajakah wood was considered capable of exhibiting various functional properties such as antioxidants (Iskandar and Warsidah, 2020), antibacterial (Saputera *et al*., 2019), speeding up wound healing time (Saputera and Ayuchecaria, 2018), and reduce body fat (Novanty *et al*., 2021). The previous extraction was carried out by Ayuchecaria *et al*. (2020) using the maceration method with ethanol solvent with a concentration of 90%. The difference in solvent concentration was applied to determine the efficiency point of taking the compound on the plow wood. The higher the concentration of the solvent used, the higher the phenol content obtained, while the lower the flavonoid content (Suhendra *et al*., 2019).

So far, Bajakah Tampala wood has been analyzed for flavonoids both qualitatively and quantitatively. While Azzahra *et al*. (2022) only postulated based on the antidiabetic effect of the phytochemicals of Bajakah wood lowering glucose levels by inhibiting phosphodiesterase and reducing oxidative stress. For this reason, it is necessary to test the inhibitory activity of α-amylase to reveal the antidiabetic effect of the Bajakah Tampala wood extract.

In addition, the extract of Bajakah wood has never been fortified into other foods, for example in honey. In fact, many studies have combined honey with various plant extracts that have functional compounds. Honey fortification with herbal plants has been widely carried out, including adding phenolic extracts from Spirulina platensis (Guldas *et al*., 2022); coumarin from Melilotus flowers (Sowa *et al*., 2019); herbal oil from *Taraxum officinale*, *Echinacea angustifolia*, *Urtica dioica*, *Calandula officinalis*, *Plantago lenceolata*, and *Arnica* (Pohorecka, 2004); as well as various herbal plant extracts such as nettle, hawthorn, pine, chokeberry, aloe, lavender flower, lemon leaf, peppermint leaf, and ginger *(Dżugan et al*., 2016). The addition of coumarin extract from Melilotus flower into honey is known to increase antioxidant activity (FRAP, DPPH, and PCL) and prove that this herbal honey can be used in the prevention of blood-related diseases (Sowa *et al*., 2019). Similar results were also reported by Guldas *et al*., (2022), Pohorecka (2004), and Dżugan *et al*., (2016) who reported that there was an increase in antioxidant activity in honey fortified with bioactive compounds from various types of herbs. Thus, this study aim to determine the levels of TPC, TFC, antioxidant activity (DPPH

scavenging activity), and inhibitory activity against α-amylase from BTWEE at various solvent concentrations. In addition, this study investigate the effect of adding BTWEE to Kapok honey on its bioactivity according to TPC, TFC, antioxidant activity (DPPH scavenging activity), and α-amylase imhibition.

2. MATERIAL AND METHODS

Materials

The raw materials used in the research were Bajakah Tampala wood in the form of stems which are reddish brown in color, purchased from Palangkaraya City, Central Kalimantan, and kapok honey from Malang which is light brown in color and tends to be clear. The chemicals used were ethanol p.a, quercetin p.a, gallic acid p.a, aquades, methanol p.a, diphenyl picrylhydrazin (DPPH) p,a , $AlCl_3$, Follin Ciocallteu, Na_2CO_3, α-amylase, amilum, and 3,5 dinitrosalicyclic acid (DNS).

The tools used in this study include digital scales (Solechan MT), hotplate, rotary evaporator, UV-Vis spectrophotometer BEL photonics.

Research Methods

This research phase begins with the extraction of Bajakah Tampala Wood with various concentrations of ethanol (70%, 80%, and 90%). Then, 1% of BTWEE was added into Kapok honey. This second stage is applied with triplo. Last, Kapok honey fortified 1% BTWEE was compared to Kapok honey without the addition of 1% BTWEE.

Bajakah Tampala Wood Ethanol Extraction

The Bajakah Tampala woods were sorted to be separated from undesirable materials, then shredded using knife. Bajakah Tampala woods are weighed 300 g, and put into a glass jar. Ethanol was added with a concentration of 70%, 80%, and 90% respectively until the woods were completely submerged, using ratio 1:3 of Bajakah Tampala wood : ethanol. The maceration process was carried out for 3 x 24 hours, with stirring every 8 hours at room temperature. The macerate was separated from the pulp by filtering using kertas Whatman no 40, then the filtrate was concentrated using a rotary evaporator at 78°C for about 3 h. The BTWEE concentrate was characterized with brown in color and concentrated. The BTWEE was further analyzed for TPC, TFC, antioxidant activity (DPPH scavenging activity), and α-amylase inhibition. After that, BTWEE was added to the Kapok honey.

Fortification of Kapok Honey with BTWEE

Kapok honey was weighed as much as 99 g in a 100 mL plastic bottle and added 1 g of BTWEE. Then stirred until homogeneous.

Analysis of BTWEE and Fortified Kapok Honey

a. The BTWEE
   The BTWEE were tested for TPC, TFC, antioxidant activity, and inhibition test of α-amylase
b. Fortified honey with 1% BTWEE
   The Kapok honey with and without 1% BTWEE were analyzed for for TPC, TFC, and antioxidant activity. The inhibition test of α-amylase was carried out on the 1% BTWEE-fortified Kapok honey which has highest antioxidant activity to determine the correlation between antioxidant activity and α-amylase inhibition.

Total Phenolic content (TPC)

Using gallic acid as a reference phenolic component, the total phenolic content of the BTWEE and BTWEE fortified Kapok honey was calculated using the Folin-Ciocalteu reagent method (Orak, 2007). Ten milliliters of 96% ethanol were combined with twenty grams of BTWEE. 1500 ppm of this mixture was diluted. The Folin-Ciocalteu reagent (1.5 mL) was then added and well mixed. The mixture was let to stand for thirty minutes following the addition of three milliliters of Na_2CO_3. At 765 nm, the absorbance was measured. Using an equation derived from the standard gallic acid graph, the content of total phenolic compounds in the sample was calculated as micrograms of gallic acid equivalent.

Total Flavonoids Content (TFC)

TFC method was modified from the procedure reported by Chang *et al.* (2002). The standard flavonoid compound was quercetin. Ten milliliters of 96% ethanol were used to dissolve fifty milligrams of BTWEE and BTWEE fortified Kapok honey. One mililiter of mixture was mixed, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. Following a 30-minute incubation period at room temperature, the reaction mixture's absorbance at 415 nm was determined. The concentration of all flavonoid components in the sample was calculated using an equation derived from the standard quercetin graph, expressed as micrograms of quercetin equivalent.

Antioxidant Activity

In a methanolic DPPH solution, scavenging free radical potentials were examined (Burda and Oleszek, 2001). The added substance's scavenging efficiency is indicated by the degree of decolorization in the solution. Methanol 96% was used to dilute one milliliter of BTWEE and BTWEE fortified Kapok honey. Next, 4 mL of DPPH solution (50 µM) was mixed with 1 mL of the mixture. The absorbance was measured at 517 nm thirty minutes later. The antiradical activity was calculated as a percentage of DPPH decoloration using the following equation:

Antiradical activity = 100 x (1-abs sample/abs of reference)

The Inhibition Test of α-Amylase

The method to test of α-amylase inhibition refered to Thalapaneni *et al*. (2008). After adding 25 µl of samples (1000 µg/ml) to 475 µl of α-amylase, the mixture was incubated for 10 minutes at 25°C. Five hundred microliters of a 0.5% starch solution were added to each tube. Then, the reaction mixtures were incubated for ten minutes at 25°C. Two mililiters of 3,5 dinitrosalicylic acid was used to stop the reaction. After five minutes of incubation in a boiling water bath, the test tubes were allowed to cool to room temperature. The absorbance of reaction mixture was measured at 540 nm.

% inhibition = (abs control-abs extract)/abs control

Data Analysis

The data obtained from the test of Kapok honey with 1% BTWEE were analyzed by one-way analysis of variance (ANOVA) using SPSS version 25.0. If the test results show that the calculated F is greater than or equal to the F table, then a further test with DMRT at the 5% level is carried out to determine the difference in the effect of each treatment. Meanwhile, to find out the difference between Kapok honey without and with the addition of 1% BTWEE, the T-paired test was carried out.

3. RESULTS AND DISCUSSION

Phytochemical Characteristics of BTWEE

Based on Table 1, it is suspected that the concentration of ethanol used to extract Bajakah Tampala wood affects the total phenols and flavonoids detected. This is in line with (Suhendra *et al*., 2019) who states that the higher the concentration of the solvent used, the higher the phenol content obtained. Meanwhile, flavonoids are the largest group of polyphenols synthesized in a plant (Ghasemzadeh *et al*., 2010). Therefore, the total phenol content often has a direct ratio to the total flavonoid content in a plant.

In this study, 70% ethanol yielded BTWEE with the highest phenol and flavonoid content because flavonoid compounds are generally in the form of polar glycosides so they must be dissolved in polar solvents. The phenol content derived from this study was almost equivalent to other studies which also stated that BTWEE was around 12.33 mgGAE/g at a sample concentration of 500 g/mL and 350 mgGAE/g at a sample concentration of 10 mg/mL (Ayuchecaria *et al*., 2020; Iskandar *et al*., 2022). The flavonoid content of BTWEE has a value similar to that of the yellow Bajakah in Central Kalimantan of around 10 mg/mL (*Arifin et al*., 2021). However, BTWEE's flavonoid content was lower than that of the red Bajakah leaf, the yellow Bajakah leaf, and the red Bajakah fruit, which had flavonoid levels of 140.17 g/mL; 94.25 mg/mL; 86.50 mg/mL, respectively (Istiqomah and Safitri, 2021).

This indicates that the synthesis of flavonoids in the leaves is more than in the stems. According to (Ghasemzadeh *et al*., 2010), synthesis of flavonoids depends on the quantity and speed of photosynthesis. Leaves are vegetative organs of plants that function as a place for photosynthesis.

Table 1. Total Phenol and Flavonoid Content of BTWEE

Sample	TPC (mg GAE/g)	TFC (mg QE/g)
BTWEE 70%	36.14	11.70
BTWEE 80%	33.33	10.75
BTWEE 90%	30.99	9.32

GAE = gallic acid equivalent
QE = quercetin equivalent
BTWEE = ethanol extract of Bajakah Tampala Wood
TPC = Total Phenolic Content
TFC = Total Flavonoid Content

Functional Properties of BTWEE
Antioxidant Activity

The antioxidant activity of BTWEE at various solvent concentrations was evaluated using the DPPH method based on free radical scavenging mechanisms. The DPPH test is based on the reduction of the purple color of the DPPH solution in which a hydrogen atom transfer reaction occurs between the antioxidant and peroxyl radicals (Wootton-Beard *et al*., 2011) at a wavelength of 517 nm resulting in a decrease in absorbance.

Table 2 shows the same phenomenon as in Table 1 that the concentration of ethanol in the extraction affects the detected antioxidant activity. Antioxidant activity decreases with increasing concentration of ethanol used. This is consistent

with the total phenols and flavonoids contained in each extract (Table 1). The value of antioxidant activity in this study was lower than in similar studies. According to Iskandar *et al*. (2022) Bajakah Tampala wood has an antioxidant activity of 50% at 198.76 g/mL; 349.89 g/mL; 2.17 g/mL for hexane, water, and ethyl acetate fractions. This difference in value is due to differences in the samples analyzed. In this study, only the results of ethanol extraction were used, while in the research of Iskandar *et al*. (2022) the sample is the result of fractionation after being extracted with ethanol. Thus, it is assumed that the fractionated compound is in accordance with its polarity

Table 2. Antioxidant Activity of BTWEE

Sample	DPPH scavenging activity (%)
BTWEE 70%	41.88
BTWEE 80%	39.32
BTWEE 90%	36.39

BTWEE = ethanol extract of Bajakah Tampala Wood

Although fractionation was not carried out in this study, the antioxidant activity could be attributed to the detected phenols and flavonoids. Phenolic compounds have been proven to act as protectors against the harmful effects of free radicals (Foti, 2007). While flavonoids as a group of phenolic compounds that are widely found in plant tissues can act as antioxidants. The antioxidant activity of flavonoids stems from their ability to donate hydrogen atoms or through their ability to chelate metals (Redha, 2010).

Inhibition Activity of α-amylase

There is no previous research to test the inhibition of α-amylase in BTWEE. Azzahra *et al*. (2022) only postulated the antidiabetic effect of the phytochemicals of Bajakah wood to lower glucose levels by inhibiting phosphodiesterase and reducing oxidative stress. The inhibition test of α-amylase was conducted to determine the decrease in α-amylase activity in breaking down starch. The more maltose produced from starch, the more starch is hydrolyzed into maltose and glucose. The principle of the test is to detect the reaction between maltose and glucose with DNS (3,5-dinitosalicyclic acid) to produce a color. Incubation was carried out at 37°C because it was the temperature for the α-amylase to work. Heating at 95°C for 5 minutes to stop the reaction because the enzyme is denatured (Pambudi *et al*., 2021).

The BTWEE has a tendency that the increase in the concentration of ethanol for the extraction, the lower the α-amylase inhibitory activity. There is a direct correlation between flavonoid levels and α-amylase inhibitory activity. According to Kaushik *et al*. (2015), one of the compounds that can inhibit α-amylase activity is flavonoids. At 70% BTWEE showed almost 50% α-amylase inhibition at a concentration of 10000 g/mL. The α-amylase which inhibited its activity in breaking down starch is correlated with the effect of lowering blood glucose.

Table 3. Inhibition Activity of α-amylase of BTWEE

Sample	Inhibition activity of α-amilase (%)
BTWEE 70%	45.38
BTWEE 80%	36.96
BTWEE 90%	29.01

BTWEE = ethanol extract of Bajakah Tampala Wood

In another study, testing of α-amylase inhibitory activity was carried out on leaf and fruit samples. For example, noni juice has an α-amylase inhibitory activity of about 27% (Anggriani *et al*., 2022). Meanwhile, in Tithonia leaves with a concentration of 500 g/mL inhibited α-amylase 20.53% for hexane extract; 29.394% for ethyl acetate extract; and 17.282% for methanol extract (Fitrianingsih *et al*., 2016). The high value of inhibition of α-amylase activity in leaves was influenced by the flavonoid content. Leaves are organs where photosynthesis occurs, where flavonoids are synthesized.

Characteristics of Kapok Honey fortified with 1% BTWEE

Characteristics of Kapok honey that added 1% BTWEE based on total phenol, total flavonoid, and antioxidant activity can be seen in Table 4. The levels of phenol, flavonoid, and antioxidant activity in unfortified Kapok honey ranged from 140.85 mg GAE/g, 16.23 mg QE/g, and 26.60% respectively. The phenols and flavonoids in the Kapok honey are contributed from the nectar of the kapok tree which is sucked by the honey bees. Honey is composed of mostly phenolic compounds in various types, such as gallic acid, cinnamic acid, pinocembrin, chrysin, and coumarin *(Hussein et al*., 2011). After Kapok honey was fortified with 1% BTWEE, the phenol content and antioxidant activity tended to increase except for flavonoids. The increase in the value of phenol and antioxidant activity indicates a synergistic interaction between Kapok honey and BTWEE, while the antagonistic interaction between them is on flavonoids.

Fortification 1% BTWEE to Kapok honey resulted in higher phenol levels than forest honey

with 1.5% temulawak and ginger, rambutan honey with Namnam leaf extract (1:1), calliandra honey with Katuk leaf methanol extract (1:1) and trigona honey with *F. carica* extract ethanol 70% and *O. stamineus* water extract (1:1:1) which has a phenol content of 17.09±3.09 g GAE/mL; 145,01 g GAE/mL; 8.01 mg GAE/g and 127.65 g GAE/mL, respectively (Fathoni *et al*., 2020; Rashidi *et al*., 2020; Septiana *et al*., 2019; Sumarlin *et al*., 2018). The same thing also happened to the flavonoids in Kapok honey with the addition of 1% BTWEE which had a higher value than rambutan honey with Namnam leaf extract (1:1) and calliandra honey with Katuk leaf methanol extract (1:1) which had flavonoid levels of 6.565 g QE /mL and 5.574 mg QE/g, respectively (Fathoni *et al*., 2020; Sumarlin *et al*., 2018).

Table 4. Characteristic of Kapok Honey fortified with 1% BTWEE

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH scavenging activity (%)
Kapok Honey	140.85	16.23	26.60
Kapok Honey + 1% of BTWEE 70%	261.36^{b}	9.08^{c}	77.18^{a}
Kapok Honey + 1% of BTWEE 80%	152.52^{a}	8.09^{b}	78.56^{a}
Kapok Honey + 1% of BTWEE 90%	263.90^{b}	5.99^{a}	74.81^{a}

GAE = gallic acid equivalent
QE = quercetin equivalent
BTWEE = ethanol extract of Bajakah Tampala Wood
TPC = Total Phenolic Content
TFC = Total Flavonoid Content

Meanwhile, the antioxidant activity of honey with ginger extract 1.5% with a concentration of 20000 ppm and Trigona honey with the addition of *F. carica* extract ethanol 70% and *O. stamineus* water extract ranged from 54% and 57.09%, respectively (Rashidi *et al*., 2020). Both of these antioxidant activity values were lower when compared to Kapok honey with the addition of 1% BTWEE. Therefore, the Kapok honey indicates a strong activity on the inhibition of DPPH as free radicals.

Comparison of Inhibition Activity of α-amylase between Kapok honey with and without 1% BTWEE

The α-amylase inhibitory activity was tested on Kapok honey without and with 1% BTWEE 80% to determine whether there was an effect of adding BTWEE. The selection of raw honey with 1% BTWEE to be tested for α-amylase inhibitory activity based on the highest antioxidant activity value. Farsi *et al*. (2011) reported that there is a correlation between antioxidant activity and α-amylase inhibitory activity.

Table 5. Comparison Inhibition Activity of α-amylase between Kapok honey with and without 1% BTWEE

Sample	Inhibition activity of α- amilase (%)
Kapok Honey	0.19
Kapok Honey + 1% of BTWEE 80%	0.27
T-paired test result	No.sig

BTWEE = ethanol extract of Bajakah Tampala Wood

Based on Table 5 shows that the T-test results do not show any difference between Kapok honey without and with 1% BTWEE 80%. However, in terms of value, there was an increase in α-amylase inhibitory activity in Kapok honey. This indicates a correlation between antioxidant activity and α-amylase inhibitory activity. The higher the antioxidant activity, the greater the inhibition of α-amylase.

Other study, Trigona honey with the addition of *F. deltoidea* extract from the boiled method and *O. stamineus* water extract (1:1:1) showed 6.49% of α-amylase inhibition (Rashidi *et al*., 2020). This shows there is a possibility if BTWEE is added with a ratio of one-third of honey, then the value of α-amylase inhibition will be high. The inhibition of α-amylase in Kapok honey with 1% BTWEE 80% indicates that Kapok honey has the potential as antidiabetic functional honey. Generally, people with metabolic disorders, including diabetics, assume that honey has high sugar content. Thus, Kapok honey with 1% BTWEE can be a good idea as a breakthrough in antidiabetic functional honey.

4. CONCLUSION

The difference in ethanol concentration in the extraction of Bajakah Tampala wood affects its bioactivity. The higher the ethanol concentration, the lower the TFC, antioxidant activity, and α-amylase inhibition activity. Ethanol 70% is recommended for extraction of the Bajakah Tampala wood. BTWEE has the potential as an antidiabetic candidate that contributes to the functional properties of Kapok honey.

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