

Assessing the Antimetabolite Activity of Anthocyanins in Cantigi Fruits from Two Conservation Sites in Indonesia

Ana Yulyana^{1,2}, Chaidir Amin^{1,3}, Partomuan Simanjuntak^{1,3}, Syamsudin Abdillah¹, Abdul Rohman^{4,5*} and Eko Mugiyanto⁶

1. Doctoral Program of Pharmaceutical Sciences, Faculty of Pharmacy, Pancasila University, Jl. Raya Lenteng Agung, Srengseng Sawah, 12630, Jakarta, Indonesia
2. Faculty of Pharmacy, Institut Sains dan Teknologi Nasional (ISTN), Jl. Moh Kahfi II, Srengseng Sawah 12640, Jakarta, Indonesia
3. Research Center for Pharmaceutical Ingredient and Traditional Medicine, National Research and Innovation Agency (BRIN), KST Sukarno Cibinong 16911, Indonesia
4. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Skip Utara 55281, Yogyakarta, Indonesia
5. Halal Center, Universitas Gadjah Mada, Skip Utara Yogyakarta 55281 Indonesia
6. Study Program of Pharmacy, Universitas Muhammadiyah Pekajangan Pekalongan, Central Java, Pekalongan, 51173, Indonesia

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*Corresponding author
Abdul Rohman

Email:
abdul_kimfar@ugm.ac.id

ABSTRACT

The objective of the current study was to evaluate the antimetabolite activity of anthocyanins in Cantigi fruits from two Indonesian conservation areas. Cantigi (*Vaccinium varingiaefolium*) is a native fruit species known for its rich anthocyanin content associated with various health benefits. This study collected Cantigi fruits from two conservation sites in Indonesia, Tangkuban Perahu (CTP) and Papandayan (CPP) Mountain, and the antimetabolite activity was evaluated using enzymatic assays. The results demonstrated significant antimetabolite activity of CTP, particularly in inhibiting α -Glucosidase ($53.72 \pm 1.98 \mu\text{g/ml}$), pancreatic lipase ($110.48 \pm 2.13 \mu\text{g/mL}$), and angiotensin-converting enzyme ($27.32 \pm 1.24 \mu\text{g/ml}$). Furthermore, our analysis using Liquid Chromatography High Resolution Mass Spectrometer (LC-HRMS) revealed the presence of three anthocyanin compounds, namely delphinidin, malvidin, and peonidin which are believed to contribute to the observed antimetabolite activities of Cantigi. These findings provide valuable insights into the specific compounds responsible for the bioactivity of Cantigi and further support for its potential natural source of bioactive substances. Future research should focus on elucidating the molecular mechanisms underlying the effects of these anthocyanins on the targeted enzymes and exploring their potential synergistic interactions.

Keywords: Cantigi, Delphinidin, Metabolic syndrome, Anthocyanins, LC-HRMS

INTRODUCTION

Metabolic syndrome is a cluster of conditions that include obesity, high blood pressure, high blood sugar, and abnormal cholesterol levels. This syndrome increases the risk of cardiovascular disease and type 2 diabetes (Refdanita, 2021). The consumption of anthocyanin-rich foods, such as berries, has been suggested to benefit metabolic syndrome (Jiang *et al.*, 2019; Naseri *et al.*, 2018). Anthocyanins are a type of flavonoid, a group of plant compounds

shown to have antioxidant, anti-inflammatory, and anti-obesity effects. Studies have found that anthocyanin consumption is associated with improved blood sugar control, lower blood pressure, and reduced inflammation. Additionally, anthocyanins may also affect lipid metabolism, with some studies indicating that they can improve cholesterol levels (Roulund *et al.*, 2022). While more research is needed to fully understand the relationship between anthocyanin consumption and metabolic syndrome, the available evidence

suggests that including anthocyanin-rich foods in the diet may benefit this condition.

The development of herbal nutraceuticals has gained attention recently, with many consumers turning to natural remedies for various health benefits. One particular herb that has gained popularity in the nutraceutical industry is Blueberry, a plant species from the *Vaccinium* genus. Blueberry is known for its high content of anthocyanins, which are potent antioxidants that have been linked to a range of health benefits, including anti-inflammatory and anti-cancer properties (Yulyana *et al.*, 2016). Studies have shown that Blueberry extract can improve cognitive function and memory in aging adults, making it a popular ingredient in brain-boosting supplements (Krikorian *et al.*, 2010; Krikorian *et al.*, 2022) and improving metabolite syndrome risk factor (Carvalho *et al.*, 2021). The increasing demand for natural and plant-based supplements has increased the production and marketing of Blueberry-based nutraceutical products, including capsules, tablets, and powders (Gonçalves *et al.*, 2022). As more research is conducted on the potential health benefits of Blueberry, it is expected that the popularity and use of this herb in the nutraceutical industry will continue to grow.

The content of anthocyanins in fruits and vegetables is influenced by several environmental factors, including the growing location, climate, and temperature (de Rosas *et al.*, 2022). Studies have shown that plants grown in regions with higher UV radiation exposure produce higher amounts of anthocyanins. Also, colder temperatures and shorter growing seasons have been linked to increased anthocyanin content in some plant species (Kataoka *et al.*, 2003). However, the relationship between temperature and anthocyanin content can be complex, as some plant species have been found to have optimal temperature ranges for anthocyanin synthesis. In contrast, others may experience a decrease in anthocyanin content at very high or very low temperatures. Furthermore, other environmental factors, such as water availability and soil nutrients, can also affect anthocyanin content (G.H *et al.*, 2006). Overall, the content of anthocyanins in fruits and vegetables is a complex trait influenced by a combination of genetic and environmental factors, making it essential to consider the growing location and environmental conditions when evaluating the potential health benefits of these plant-based compounds.

Understanding the relationship between anthocyanin content and environmental factors is crucial for agricultural production and human health. By studying how environmental conditions affect anthocyanin content in plants, farmers can optimize crop production and improve the quality and nutritional value of fruits and vegetables. Additionally, the potential health benefits of anthocyanins for human consumption have sparked interest in understanding how these compounds are synthesized and regulated in different plant species. Researchers can identify potential targets for breeding crops with higher anthocyanin content by investigating environmental factors that influence anthocyanin content. Furthermore, studies examining the relationship between anthocyanin content and environmental factors can help identify regions or growing conditions that produce fruits and vegetables with the highest levels of these beneficial compounds. The study allows consumers to make informed choices when selecting foods to promote their health.

MATERIALS AND METHODS

Research Material

The research material used in the study were the fruits of Cantigi (*Vaccinium varingaefolium* Bl. Miq) obtained from Tangkuban Perahu (CTP) and Papandayan (CPP) Mountain in West Java, Indonesia. These two mountains were chosen because they are the largest centers for Cantigi cultivation in Indonesia and have different environmental characteristics. Before processing, the plant was authenticated by the Indonesian Institute of Science: Research Centre for Biology to ensure its authenticity. Overall, using authenticated plant material and carefully considering conservation site factors is essential for producing reliable and informative research results.

Cantigi's Extraction

The modified Tena *et al.* approach provided a basis for the extract's preparation (2022)(Tena & Asuero, 2022). Four kilograms of Cantigi fruits purified by removing impurities were selected. Maceration method preparation of a 70% ethanol extract using a fruit-to-ethanol ratio of 1:10 and supplemented with 1% HCl to obtain pH 3. After going through the process of stirring and filtering, the filtrate was evaporated and concentrated using a rotary vacuum evaporator to obtain a thick extract.

Total Phenolics Content (TPC)

The total phenolic content (TPC) of Cantigi seed extracts was determined using the Folin-reagent Ciocalteu's (FCR) method with a modest modification (Mansouri *et al.*, 2005). The method involved adding methanol, FCR, and 5% CaCO₃ to the seed extract, then measuring the reaction mixture's absorbance at 725 nm using a Hitachi U-2000 spectrophotometer. The TPC was expressed in milligrams of (+)-gallic acid equivalents (GAE) per gram of extract. The FCR method is commonly used to estimate the TPC of plant extracts and is based on the reduction of FCR by phenolic compounds in alkaline conditions.

Total Flavonoids Content (TFC)

Cantigi extracts' total flavonoid content was determined using the method described by Samirana *et al.* (Samirana *et al.*, 2016). The method involved mixing the extract with distilled water and a 5% sodium nitrite solution, then adding 10% aluminum chloride and sodium hydroxide. The mixture was then diluted with distilled water, and the absorbance was measured at 510 nm. The TFC was estimated as mg quercetin equivalents (QE) per gram of extract. Flavonoids are a group of polyphenolic compounds widely distributed in plants and known for their antioxidants and other health-promoting properties.

Anthocyanin Content

The total anthocyanin content of the extracts was calculated using a method previously established by Nile *et al.* (Nile *et al.*, 2015). Each extract was diluted (5:95, v/v) in 1% HCl in methanol to attain an absorbance between 500 and 1.000 at 530 nm. A molar extinction coefficient of 27.900 was applied, and the values were represented as mg cyanidin-3-glucoside equivalents per 100 g fresh weight. All analyses were carried out in triplicate.

Pancreatic Lipase Inhibitory

Lipase inhibitory activity was determined using Lipase Activity Colorimetric Assay Kit (BioVision, Catalog #K722-100). The sample (Cantigi's extract) concentration was 25, 50, 100, 200, and 400 µg/L while the final volume of the reaction mixture was 100 µL (93 µL Assay Buffer, 2 µL OxiRED™ Probe in DMSO, Enzyme Mix 2 µL, 3 µL sample). Changes in absorbance at 570 nm were measured after 60 min using Synergy HTX Multi-Mode Reader (BioTek, Bad Friedrichshall, Germany).

Angiotensin-Converting Enzyme (ACE) Inhibitory Assay

The ACE inhibitory activity of the sample was determined using BioTek Microplate Readers and the spectrophotometric technique. An ACE1 Inhibitor Screening Kit (Catalog # K719-100) by BioVision was utilized. We set sample concentrations for 25, 50, 100, 200, and 400 µg/L. For the ACE activity assay, 25 µL of each (inhibitor control, buffer, and solvent control) was added to 40 µL ACE1 Enzyme Solution. Incubates the cocktail for 15 min at 37 °C (protected from light) and then adds 50 µL of the reaction mix containing ACE1 Assay Buffer 40 µL and 10 µL samples. Re-incubate the mixture and then measure the absorbance at 345 nm. Furthermore, the following formula was used to determine the percentage of ACE activity that has been inhibited:

Inhibition percentage is calculated as $[(\text{Control Activity} - \text{Sample Activity}) / \text{Control Activity}] \times 100$

α-Glucosidase Inhibitory Assay (αGIA)

The αGIA was measured following the protocol from Sigma-Aldrich (α-Glucosidase Activity Assay Kit, Catalog Number MAK123). This assay is based on a kinetic reaction with α-NPG as a substrate. The prepared standard (25, 50, 100, 200, and 400 µg/L) were diluted in phosphate buffer, pH 7.0. Transfer 20 µL of water to two wells of a transparent 96-well plate. Add 200 µL of water into one of these wells and 200 µL of Calibrator to the other wells. Transfer 20 µL of each sample into separate wells of the plate. Transfer 200 µL of the Master Reaction containing Phosphate buffer 200 µL and α-NPG 8 µL into each of the sample wells. Incubate the samples at 37°C, and after 20 min, take the final absorbance at 405 nm.

Chemical Compounds Content

Cantigi's chemicals were examined using a high-resolution mass spectrometer (Q-Exactive, Orbitrap, Thermo Scientific, USA) and an ultra-high performance liquid chromatograph (Vanquish, Thermo Scientific, USA). The technique used for the study was modified slightly from that described by Windarsih *et al.* in 2022 (Windarsih *et al.*, 2022). Analytical column Acclaim Vanquish C-18 (15 mm x 2.1 mm x 2.2 m) was used to evaluate the samples. Three liters of sample injection volume were used. With a flow rate of 0.3 mL/min, formic acid 0.1% in acetonitrile (B) and water (A) were the mobile phases utilized for the analysis. The following analysis was carried out in gradient mode:

Table I. Evaluation of total phenol, flavonoid, and anthocyanin content

Sample	Compounds		
	Phenolics*	Flavonoid**	Anthocyanin***
CPP	267.6±0.38	151.43±0.31	31.28±0.44
CTP	391.09±0.55	160.39±0.58	40.89±0.57

*mg gallic acid eq./100 g extract; **mg quercetine eq./100 g extract; ***mg cyanidin 3-glucoside eq./100 g extract. The highest total phenolics, flavonoid, and anthocyanin contents are found in ethanol extract of CTP.

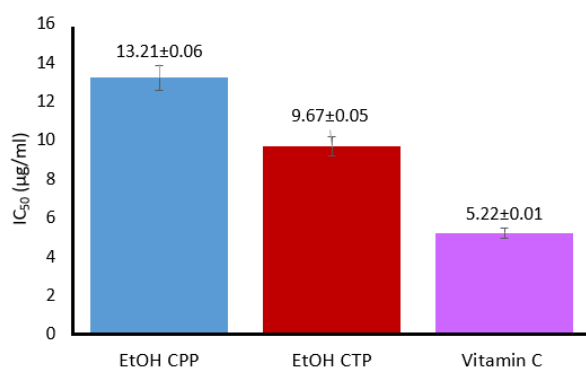
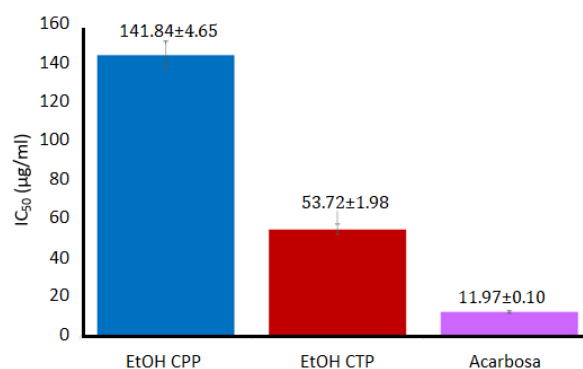


Figure 1. Antioxidant activity of CTP and CPP.

Figure 2. The α-glucosidase enzyme IC₅₀ values.

The mobile phase B was initially set at 5% and then maintained at 90% B for 16 min. The original condition (5% B) was maintained for 25 min after the 90% B was held for 4 minutes. The following conditions were applied to the MS parameters: 3.30 kV spray voltage, 320°C capillary temperature, and 30°C auxiliary gas heater temperature. With an auxiliary gas flow rate of 8 arbitrary units (AU) and a sweep gas flow rate of 4 AU, the sheath gas flow rate was set at 32 arbitrary units (AU). Both positive and negative ionization measurement modes were used, and the resolution for full MS was 70,000, and for dd-MS2 was 17,500.

RESULTS AND DISCUSSION

Total Phenol, Flavonoid, and Anthocyanin Content

The Cantigi fruits from both regions have a purple color macroscopically, with slightly different diameters and weights caused by the different sulfur content between the two locations. 70% ethanol aims to extract all polar and highly polar compounds, such as phenols, flavonoids, and their derivatives, even in glycoside form. The addition of HCl aims to adjust the pH to 3 in the hope of binding flavonoid compounds such as anthocyanins, which act as antioxidants. Further, to

date, limited research or literature on the phenolic compounds contained in Cantigi fruits, although several studies have given details on the *Vaccinium* genus's compound makeup (Table I).

Antioxidant activity

Prior studies demonstrated that phenolic compounds such as hydroxycinnamate found in Cantigi significantly affect antioxidant activity (Figure 1).

The 70% ethanol extract of CTP and CPP has antioxidant activity with an IC₅₀ value of 9.67 and 13.21 ppm. Vitamin C was used as the positive control with a value of 5.22 ppm. This indicates that all Cantigi fruit extracts have the potential as potent antioxidants.

The α-glucosidase inhibitor enzyme activity

The α-glucosidase inhibition activity was tested on the 70% ethanol extract of Cantigi fruit and its fractions and compared with the positive control of Acarbose. The research showed that the ethanol extract of CTP has better activity than CPP (Figure 2). The higher total phenolic, flavonoid, and anthocyanin content of CTP compared to CPP correlates with the α-glucosidase inhibition activity.

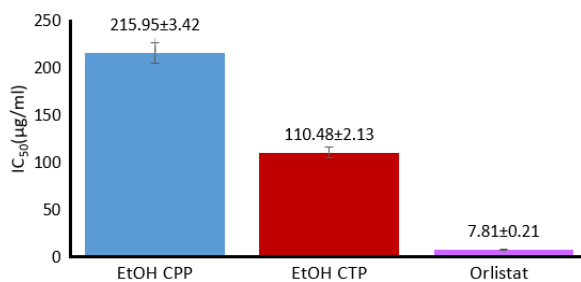


Figure 3. The activity of lipase inhibitors of CTP and CPP.

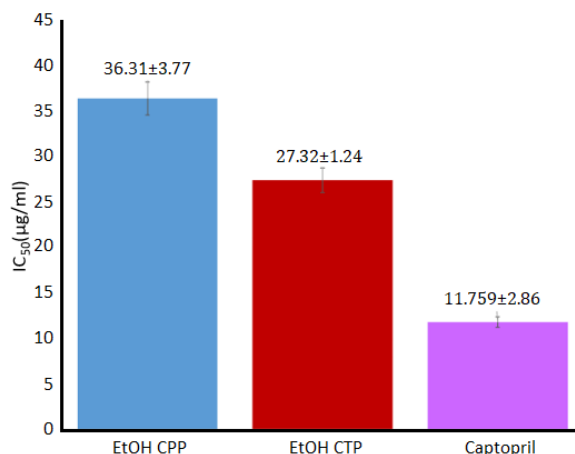


Figure 4. ACE inhibition activity of CTP and CPP

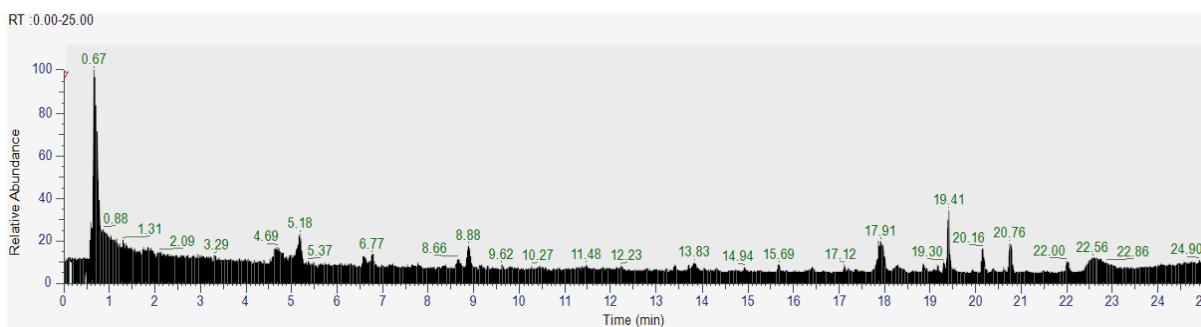


Figure 5. The LC-HRMS profile of CTP

Lipase inhibitor enzyme activity

Foods or drinks rich in flavonoids and pure flavonoids have been proven to lower plasma triglycerides, total cholesterol, and LDL cholesterol or increase HDL cholesterol. Flavonoids can reduce lipid absorption at the gastrointestinal level. However, flavonoids can also modulate the activity of various enzymes involved in lipid metabolism and the expression of transcription factors involved in triglyceride and cholesterol synthesis (Figure 3).

Based on the lipase enzyme inhibitor testing results, the IC₅₀ value of ethanol extract from CTP was 110.48 ppm and better than that of CPP, with a value of 215.95 ppm. This is due to the differences in active compound content, such as phenols, flavonoids, and anthocyanins contained in Cantigi fruit, which affect the lipase enzyme inhibition activity.

Ace inhibitor activity

The anthocyanin content found in Cantigi inhibits ACE activity, similar to delphinidin found in the Hibiscus sabdariffa extract (Figure 4).

The ACE inhibitor activity of 70% ethanol extract from CPP and CTP has an IC₅₀ value of 36.31 and 27.32 ppm, respectively, with captopril as a positive control with a value of 11,759 ppm.

Flavonoid and anthocyanin profiling using HRMS

Total phenolic, flavonoid, and anthocyanin content tests showed that CTP has a higher level than CPP, indicating that CTP could be further developed as an anti-metabolic syndrome agent. The 70% ethanol extract of Cantigi fruit was analyzed for secondary metabolites using LC-HRMS (Figure 5), resulting in several compounds belonging to the phenolic and flavonoid groups.

Table II. The content of flavonoid and anthocyanin compounds in Cantigi ethanol extract is as follows:

No.	Compounds	Molecule formula	MW	Rt	Area	%Area
Phenol						
1.	D-(-)- kuinat acid	C7H12O6	192.06	0.83	106481367	1.38
2.	(-)-Shicimic acid	C7H10O5	174.05	0.83	19052588	0.24
3.	6-methoxyimellein	C11H12O4	208.07	10.66	16060703	0.20
4.	Umbelliferon	C9H6O3	162.03	5.29	479693894	6.23
Flavonoid						
1.	Quersetin	C15H10O7	302.04	10.78	21752753	0.28
2.	Mikuelianin	C21H18O13	478.07	8.83	73708454	0.95
3.	Chlorogenic acid	C16H18O9	354.09	4.69	799817965	10.30
4.	5-kafeoilshikimat acid	C16H16O8	336.08	6.78	245838652	3.19
Anthocyanin						
1.	Delphinidin	C15H11O7	303.05	10.74	13125386.61	0.17
2.	Delphinidin 3-glucosid	C21H21O12	465.10	0.70	8332461.46	0.10
3.	Malvidin 3-glucosid	C23H24O12	492.12	6.89	30769524.83	0.40
4.	Peonidin 3-glucoside	C22H22O11	462.11	7.31	26733584.05	0.34

MW: Molecular weight; Rt: Retention time

Based on the LC-HRMS analysis, some phenolic, flavonoid, and anthocyanin compounds were detected, which can be seen in detail in **Supplementary Table 1**. Several compounds appear to be dominant in the 70% ethanol extract of Cantigi fruit, including phenolic compounds such as D-(-)-quinic acid, (-)-shikimic acid, 6-methoxymellein, and Umbelliferone. Flavonoid compounds include Myricetin, Quercetin, Chlorogenic acid, and 5-Caffeoylshikimic acid. Anthocyanin compounds include Delphinidin, Delphinidin 3-glucoside, Malvidin 3-glucoside, and Peonidin 3-glucoside. Vaccinium's genus is known for its health advantages, allegedly because of its high phenolic component concentrations or specific, potent polyphenolic chemicals that may interact (additively or synergistically) to improve human health conditions (Seeram *et al.*, 2004). Due to their high levels of phenolic components such as ellagic acid, tannins, ellagitannins, quercetin, gallic acid, anthocyanins, and cyanidins, blackberries rank highly among fruits in terms of antioxidant strength (Hager *et al.*, 2008). The phenol content in CPP was determined to be 267.6 ± 0.38 mg gallic acid eq./100 g extract, while CTP exhibited a higher phenol content of 391.09 ± 0.55 mg gallic acid eq./100 g extract (Table I). For flavonoid content, CPP had a value of 151.43 ± 0.31 mg quercetin eq./100 g extract, whereas CTP showed a slightly higher flavonoid content of 160.39 ± 0.58 mg quercetin eq./100 g extract.

Regarding anthocyanin content, CPP had a value of 31.28 ± 0.44 mg cyanidin 3-glucoside eq./100 g extract, while CTP exhibited a higher anthocyanin content of 40.89 ± 0.57 mg cyanidin 3-glucoside eq./100 g extract. These measurements provide insights into the phytochemical composition of CPP and CTP extracts. Furthermore, the Cantigi ethanol extract contains various phenolic compounds, flavonoids, and anthocyanins (Table II). These compounds are known for their antioxidant properties and potential health benefits. The presence of phenolic compounds such as quinic acid and shikimic acid, flavonoids like quercetin and miquelianin, and anthocyanins, including delphinidin and malvidin, contributes to the overall antimetabolite capacity of the Cantigi extract. The evident that CTP exhibits a higher content of flavonoids, with chlorogenic acid (CGA) being the major flavonoid component (Table II). The esterification of cinnamic acids, such as caffeic, ferulic, and p-coumaric acids with quinic acid produces CGAs, which are phenolic acids with vicinal hydroxyl groups on aromatic residues (Farah *et al.*, 2005).

Antioxidants are crucial in neutralizing harmful free radicals and protecting cells from oxidative damage. Antioxidant activity was assessed using IC₅₀ values, which show the concentration needed to scavenge 50% of the free radicals. CPP exhibited moderate antioxidant activity with an IC₅₀ value of 13.21 ± 0.06 µg/ml, suggesting its ability to scavenge free radicals,

albeit to a lesser extent compared to Vitamin C 15.22 ± 0.0023 $\mu\text{g/ml}$. On the other hand, CTP demonstrated more potent antioxidant activity with a lower IC₅₀ value of 9.67 ± 0.05 $\mu\text{g/ml}$, indicating its potency as an antioxidant compared to CPP. The variations in IC₅₀ values suggest that CTP and CPP possess antioxidant properties, although with differing strengths.

Further, based on our findings, it can be hypothesized that the antioxidant activity of CTP is primarily attributed to its chlorogenic acid content as the major component (Table II). Several studies previously associated chlorogenic acid with potent antioxidant activity (Liang & Kitts, 2015; Wang *et al.*, 2022; Xu *et al.*, 2012). However, it is essential to note that the anthocyanin content in CTP may also contribute to its antioxidant properties, including delphinidin (Goszcz *et al.*, 2017; Li *et al.*, 2022). Its activity is attributed to its free radical-scavenging and antioxidative properties, which are primarily associated with the hydrogen (electron) donation ability inherent in its flavonoid molecules by donating hydrogen atoms or electrons to neutralize free radicals and oxidative species (Yildiz *et al.*, 2021). Further research is needed to investigate the specific compounds within CTP most responsible for its antioxidant activity. By identifying the key contributors, we can better understand the mechanisms underlying CTP's antioxidant effects and potentially uncover synergistic interactions among its various components.

Anthocyanins, which belong to the flavonoid group, and chlorogenic acid, which belongs to the phenolic acid group, are antioxidants found in blue and red fruits and berries (Meng *et al.*, 2013). Inhibition of the α -glucosidase enzyme is an important strategy for controlling hyperglycemia in diabetes. We compared the IC₅₀ values of three inhibitors, namely CPP, CTP, and Acarbose, for inhibiting the α -glucosidase enzyme. The IC₅₀ values represent the concentration required to inhibit 50% of the enzyme's activity. CPP exhibited a moderate inhibitory effect with an IC₅₀ value of 141.84 ± 4.65 $\mu\text{g/ml}$, while CTP showed stronger inhibition with an IC₅₀ value of 53.72 ± 1.98 $\mu\text{g/ml}$. However, compared with Acarbose, a standard drug, the inhibition α -glucosidase enzyme is 11.97 ± 0.10 $\mu\text{g/ml}$.

Further, Despite CTP demonstrating lower inhibition compared to Acarbose, it is important to note that CTP is still in the form of an extract that contains numerous chemical compounds. While Acarbose shows higher potency as a single

compound, the presence of multiple chemical constituents in CTP may contribute to its overall inhibitory effect on the α -glucosidase enzyme. The complex mixture of compounds in CTP could potentially exhibit synergistic or complementary actions, leading to enhanced inhibitory activity. Additionally, previous studies have demonstrated the potential of anthocyanins to inhibit glucosidase activity (Moein *et al.*, 2022; Promyos *et al.*, 2020). Therefore, further research is warranted to identify and characterize the specific components within CTP that contribute to its inhibitory properties, which may open avenues for developing novel therapeutic agents with improved efficacy and reduced side effects.

Five pathways can be used to categorize anthocyanins' methods to reduce obesity. These strategies include decreasing lipid absorption, boosting energy expenditure, managing food intake, regulating lipid metabolism, and regulating gut flora (Yildiz *et al.*, 2021). Previous studies have demonstrated that anthocyanins exhibit activity against lipid metabolism (Park *et al.*, 2017). Our findings reveal that Cantigi includes three different forms of anthocyanins, namely delphinidin, malvidin, and peonidin (Table II), implying that they may have a role in influencing lipid metabolism. We examine the lipase inhibitory activity of two compounds, CTP and CPP, comparing them to the well-known lipase inhibitor, Orlistat. Lipase inhibition plays a critical role in weight management and the treatment of obesity. The inhibitory activity was assessed by determining the IC₅₀ values, representing the concentration required to inhibit 50% of the lipase enzyme's activity. The results revealed that CPP exhibited an IC₅₀ value of 215.95 ± 3.42 $\mu\text{g/ml}$, indicating a moderate lipase inhibitory effect. Although CPP demonstrated the ability to inhibit lipase activity, it required a higher concentration than the standard lipase inhibitor, Orlistat.

On the other hand, CTP displayed a lower IC₅₀ value of 110.48 ± 2.13 $\mu\text{g/ml}$, indicating stronger lipase inhibitory activity compared to CPP. This suggests that CTP is a more potent lipase inhibitor. Furthermore, in another study, the ability of cyanidin and peonidin anthocyanins to stop cholesterol absorption in Caco-2 cells was examined. In light of this, it is clear from earlier studies that cyanidin, one of the anthocyanins present in Cantigi, has hypolipidemic activity, which is explained by its control over lipogenic enzymes (Chen *et al.*, 2018). These findings provide valuable insights into the potential use of CTP and

CPP as lipase inhibitors in weight management strategies or the development of novel therapeutic options for obesity.

By contrasting them with the well-known ACE inhibitor captopril, we examine the activity of angiotensin-converting enzyme (ACE) inhibitors CTP and CPP. The management of hypertension and associated cardiovascular diseases involves using ACE inhibitors significantly. Delphinidin and some natural products high in anthocyanins have been shown to reduce ACE activity (Hidalgo *et al.*, 2012). According to the findings, CPP had an IC50 value of 236.31 g/ml, indicating a moderate ACE inhibitory action. Although CPP was able to reduce ACE activity, it needed a larger dosage than captopril, the well-known ACE inhibitor. Contrarily, CTP showed a substantially lower IC50 value than CPP, at 27.32 g/ml, indicating a more potent ACE inhibitory effect. The result also suggests that CTP is an ACE inhibitor with greater potency. A notable IC50 value for captopril was 11.759 g/ml, demonstrating its potent ACE inhibitory action. The differences in IC50 values indicate that captopril has the most potent ACE inhibitory properties, even though CTP and CPP also have these properties.

Furthermore, phenols, flavonoids, and anthocyanins are known for their potential health benefits, including antioxidant and anti-inflammatory properties (Panche *et al.*, 2016). The higher phenol, flavonoid, and anthocyanin content in CTP suggests its potential for more potent antioxidant and health-promoting effects than CPP. These findings further support the exploration of CTP as a potential source of natural compounds for developing ACE inhibitors, in line with the established ACE inhibitor, captopril. Future studies should investigate the specific phenolic compounds in CPP and CTP extracts and explore their potential bioactivity and contribution to the observed ACE inhibitory effects.

Anthocyanins, such as delphinidin, malvidin, and peonidin, are generally thought to contribute to Cantigi's antimetabolite activity by inhibiting glucosidase, lipase, and ACE enzymes. Anthocyanins have been well-researched for their possible health advantages and are well-known for their various biological functions. The presence of these anthocyanins in Cantigi shows that they are involved in controlling critical metabolic enzymes. Glucosidase inhibitors help control blood sugar levels by delaying the breakdown and absorption of carbohydrates. On the other side, lipase inhibitors can assist in controlling lipid metabolism and

lessen fat absorption. ACE inhibitors are also critical for managing cardiovascular health and blood pressure. The anthocyanin content of Cantigi may enhance its potential as an antimetabolite by blocking these enzymes, providing potential therapeutic uses in controlling disorders linked to lipid metabolism, cardiovascular health, and glucose metabolism. A foundation for creating novel natural compounds for treatments can be laid by further research into the precise mechanisms of action and the synergistic effects of the anthocyanins found in Cantigi.

CONCLUSION

Our research showed that CTP has high antimetabolite activity, especially when it comes to inhibiting α -glucosidase (IC50 = 53.721.98 g/ml), pancreatic lipase (IC50 = 110.482.13 g/ml), and angiotensin-converting enzyme (IC50 = 27.321.24 g/ml). Three anthocyanin compounds, delphinidin, malvidin, and peonidin, were also discovered by our HRMS research, which is thought to be a factor in the antimetabolite activities of Cantigi. These findings confirm the potential of Cantigi as a natural source of bioactive chemicals by offering critical new insights into the precise components responsible for its bioactivity. Future studies should unravel the molecular mechanisms underlying these anthocyanins' effects on the targeted enzymes and investigate potential synergistic interactions between them.

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