



Evaluation of Herbal Honey with Black Cumin and *Curcuma xanthorriza* as an Antioxidant Supplement for Stunting Prevention

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ABSTRACT

Introduction: Honey has been widely used as a nutritional supplement and medicine. Many herbal honey products are developed as nutritional supplements that are combined with SFM to make them more effective, but there are still many shortcomings due to the unpleasant taste produced, unattractive physical appearance, and high susceptibility to oxidation reactions that cause severe damage to the quality of honey, especially in the content of active substances, viscosity, and pH, which can cause the effectiveness of honey to decrease and result in product failure as nutritional supplements to treat stunting problems. We have developed an herbal honey multi-nutrient supplement that combines honey with black cumin oil and *Curcuma xanthorriza* extract called HBCX. Adding *Curcuma xanthorriza* extract and black cumin oil to honey is expected to increase honey's benefits as an antioxidant. This study aims to determine the physicochemical characteristics (pH and viscosity), heavy metal contamination content, polyphenols, and antioxidant activity.

Methods: This experimental laboratory research was conducted at the Pharmacy Lab, Faculty of Pharmacy, UAD. The viscosity and pH of HBCX were observed using an Ostwald viscometer and a pH meter. Total phenol determined by Folin-Ciocalteu reagent and gallic acid standard. Determination of antioxidant activity using the DPPH method. The data are presented in a quantitative descriptive manner and matched with the SNI honey standard.

Results: The results showed that HBCX herbal honey had a higher viscosity than the sampled honey from the market but was still lower than the SNI requirements. HBCX honey has a lower pH than sample honey products from the market but still complies with national standards. HBCX honey is safe from heavy metal contamination and contains polyphenols. The antioxidant activity of HBCX honey is relatively high, with IC₅₀=54.78 ppm.

Conclusion: HBCX has high polyphenol content, is safe from heavy metal contamination, and has sufficient antioxidant activity.

INTRODUCTION

Honey is one of the natural sources of nutrients beneficial to humans (1). Honey contains complex carbohydrates, water, vitamins, minerals, enzymes, organic compounds, free amino acids, and volatile compounds (2). Honey also contains minerals such as Al, Cr, Ni, V, Co, Ca, Mg, K, Na, Zn, Fe, Cu, and Mn. Honey is a source of macro- and micronutrients (3). Honey has been widely used in the food industry, health drinks, herbal medicine, cosmetics, and medicine. In medicine, honey has been used to relieve fatigue and increase immunity, and nutritional supplements to restore fitness in sufferers. Over the past few decades, honey has been considered an effective natural remedy for various disorders. Several studies have shown that honey has antioxidant, anti-microbial, anti-inflammatory, antibacterial, hepatoprotective, and antitumor effects. The benefits of honey are influenced by the composition and content of the honey's active substances. The content in honey also dramatically affects the physicochemical properties of honey and will be related to the expected active substance content (4). Like honey, *Curcuma xanthorrhiza* (CX) and black cumin seed oil (BCSO) contain various active substances, including polyphenols, flavonoids, fiber, fatty acids, protein, polysaccharides, and minerals, and are helpful as nutritional intake, antioxidant and immunomodulator (5), (6), (7).

Stunting is one of the most common chronic nutritional problems in the world. Handling stunting is one of the Indonesian government's national development priorities. Indonesia is one of the loci of stunting in the world, with an incidence rate of 24.4% in 2021; WHO targets the stunting rate in Indonesia to be less than 20% (8),(9). Based on the results of previous studies, stunting has been associated with an increased incidence of infection, lack of immune capacity, increased oxidative stress, premature aging, and increased degenerative diseases (10). Stunting conditions in childhood will have a long-term impact on the nation's quality, so stunting management strategies need to focus on preventing events and inhibiting long-term impacts (11). Recent research has shown a relationship between the gastrointestinal microbiota pattern and the incidence of stunting and its impact on long-term health (12). The gut microbiota is essential for health and primarily implicated in the human body's homeostasis (13). The primary role of gut microbiota residents is to support digestion all through the gastrointestinal tract. Healthy gut microbiota plays an essential role in regulating energy balance. Various factors, including diet, have been associated with a healthy microbiota population in the digestive tract. Consumption of polyphenols and curcumin has been proven by an increase in the population of good microbiota in the digestive tract, thereby increasing the absorption of nutrients, antioxidant capacity, and the body's immune response (13), (14).

The supplementary feeding program (SFM) for stunted children has been running for a long time (15), but its effectiveness needs to be increased because the SFM program so far has focused on increasing primary nutrition and has not paid attention to the needs of important micronutrients such as antioxidants, immunomodulators, and improvement of the digestive tract microbiota (16), (17), (18). Several polyphenols and active substances from medicinal plants have been shown to improve the digestive tract microbiota and increase the effectiveness of absorption of food extracts (18). Many herbal honey products have been developed as nutritional supplements combined with SFM to make them more effective, but there are still many weaknesses due to the resulting unpleasant taste, unattractive physical appearance, and being very susceptible to oxidation reactions caused by the high content of unsaturated fatty acids. compounds, especially linoleic (19), (20). Oxidation reactions are the main factors that cause severe damage to the quality of honey (2), especially in the content of active substances, viscosity, and pH which can cause the effectiveness of honey to decrease and result in product failure as a nutritional supplement to treat stunting problems.

The addition of *C. xanthorrhiza* extract and black cumin seed oil to honey is expected to increase the benefits of honey as an antioxidant (21), in addition The composition of herbal supplements of *C. xanthorrhiza* and black cumin with honey as a solvent and flavoring can overcome problems that often arise such as unpleasant taste, pungent aroma, and can cause belching which causes children to be less likely to like the preparation (22,23). Therefore, preparations combining honey, *C. xanthorrhiza*, and black cumin with high content of tyroquinone and curcumin accompanied by sweetness will increase consumer interest and are in line with the expectations of researchers (2,24). Herbal honey is a product that combines honey with other herbal ingredients (21). *C. xanthorrhiza* is a medicinal plant native to Indonesia that has been used as an appetite stimulant, anti-inflammatory, antioxidant, and immunomodulator (5), (25). *C. xanthorrhiza*, black cumin oil, and honey have been widely used by the Indonesian people for various health indications (21). Nutritional supplement preparations have been developed from honey (H), black cumin oil (B), and *C. xanthorrhiza* (CX) extract. The addition of *C. xanthorrhiza* extract and black cumin oil

to honey is expected to increase the benefits of honey as an antioxidant and answer the problems that occur in other herbal honey products that have been developed previously. This study aims to determine the viscosity, pH, heavy metal contamination, polyphenol content, and antioxidant activity of HBCX herbal honey.

METHOD

Research Design and Objectives

This research is an experimental laboratory study aimed at determining the viscosity, pH, metal contamination, polyphenol content, curcumin content, and antioxidant activity of herbal honey, black cumin oil, and *C. xanthorrhiza*.

Materials and Instruments

The materials used in this study were herbal honey, black cumin oil, *C. xanthorrhiza*, and two types of comparison honey obtained from the market. Gallic acid (Sigma) is the standard for determining polyphenol levels. 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma), methanol, and ascorbic acid (Sigma) for antioxidant testing. The tools used in this study were a UV-Vis Spectrophotometer, Ostwald viscometer, oven, hot plate, analytical balance, desiccator, 50 mL acid burette, pH meter (pHmeter Crison Instruments, S.A), 500 round bottom flask. mL, 25 and 250mL Erlenmeyer, 50 and 100mL measuring cups, 5mL, 10mL, and 15mL volume pipettes, test tubes, porcelain cups, stopwatch, and sample bottles.

Research Procedure

Viscosity

An Ostwald viscometer was used to analysed the viscosity. Ostwald viscometers should be filled with aqua bides to the limit mark, placed in a beaker, and heated until the temperature reaches 40°C. Use the bulb to suction the liquid through the left tube, and time how long it takes the liquid to flow. The aforementioned procedure was carried out using samples whose viscosity was known in place of the aqua bides. This formula is used to determine viscosity:

$$\eta_{\text{honey}} = (\alpha \times \rho_{\text{(honey)}} \times t_{\text{(honey)}}) / (\eta_{\text{water}} \times t_{\text{water}})$$

where: α = viscosity water at 40°C
 ρ = honey specific gravity
 t_{honey} = time for honey
 η = water viscosity
 t_{water} = time for water

Acidity

A pH meter is used to check the pH of substances. A pH meter made by Crison Instruments, S.A. is the tool that was used. The pH meter should be checked according to the following steps: It is important to check the batteries and electrodes before using the pH meter since they act as a pH indicator for the presence or absence of liquid, which might impact the measurement findings. (ii). Execute calibration. If the condition of the battery and electrodes is good, then calibration is carried out. The pH meter calibration was carried out using a pH = 7 solution; then, the electrode was inserted into the solution and adjusted to pH = 7; then, the electrode was rinsed with distilled water. (iii). After calibration, the pH meter can be used to measure the solution's pH to determine pH. 10 grams of HBCX herbal honey was dissolved in 75 mL distilled water and then poured into a glass cup. The electrode is inserted into the herbal honey solution and waited until the pH value does not change (stable). The stable pH value is read as the pH value of herbal honey. The pH meter electrode is then removed and rinsed with distilled water. The results of the acidity measurement with a pH meter matched the SNI standard for the acidity of the honey. According to the Indonesian National Standard (SNI), the acidity of honey is 3.5 – 5.

Analysis of Metal Contamination in Honey using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

The collected HBCX honey was then analyzed for metal contamination. Weigh 1 gram of forest honey (Apis dorsata) in a 50mL beaker. Add 10mL of 0.1 M HNO₃ and stir. Put the solution into a 100mL volumetric flask, squeeze it to the mark, and homogenize it. Next, analyze with the ICP-AES instrument.

ICP-AES is now widely used as a versatile analytical technique. It is recognized as an environmental measurement technique, similar to atomic absorption spectrometry, and its use will continue to grow in the future. Plasma sources have many characteristic emission lines, making them useful for qualitative and quantitative elemental analysis. ICP-AES produces significantly better quantitative analytical data than other emission sources. The excellence of these results comes from high stability, low noise floor, and lack of interference (26,27).

Analysis of Polyphenol Content

Using the Folin-Ciocalteu reagent and gallic acid as standards, we were able to determine the total phenol concentration. Gallic acid and NaCO₃ solution were combined with 90L of the Folin-Ciocalteu reagent to create the calibration curves. A 10 mg sample was weighed, 10 mL of ethanol was added, and the mixture was then homogenized. The filtrate was utilized for analysis once it had been filtered. Up to 500µL of the filtrate were pipetted, along with 7.5mL of distilled water and 500µL of Folin-Ciocalteu reagent. 8 minutes of homogenization and incubation. 1.5 mL of a 20% sodium carbonate solution was added after incubation. Afterward, the sample was incubated once more for an hour before the quantity of polyphenols in it was determined by measuring the absorbance at a wavelength of 765 nm (28).

Test antioxidant potential

The DPPH technique was used to test the antioxidant activity of HBCX formulations. 10mg of extract was dissolved in 100 mL of methanol PA to create samples of mother liquor with a 100 ppm concentration. Additionally, the material was diluted using methanol as a solvent at different concentrations of 5, 6, 7, 8, and 9 ppm. 5mg of solid DPPH was dissolved in 100 mL of methanol PA to create a DPPH stock solution. Then, a control solution comprising 1mL of 50 ppm DPPH solution and 2mL of PA ascorbic acid was made as a comparison. Test samples required the preparation of 2mL each of sample solution and DPPH solution. After that, they were incubated at 27°C for 30 minutes or until DPPH activity caused a color change. Every sample was manufactured three times. Absorbance values of the incubated HBCX samples were measured at 517 nm with a UV-vis spectrophotometer. The amount of sample needed to scavenge 50% of DPPH free radicals is known as the IC₅₀ value, and it was determined by graphing the percent inhibition against the log of extract sample concentration (29).

Data Analysis

The research data were presented descriptively and then interpreted based on the Indonesian national standard (INS) and data from previous studies.

RESULTS

The results of testing the viscosity and acidity of HBCX herbal honey preparations compared to honey products that have been circulating in the community are presented in Tables 1 and 2.

Viscosity

Honey viscosity is one of the quality parameters. The results of the viscosity examination of HBCX herbal honey preparations are presented in Table.

Table 1. The viscosity of HBCX preparations and two honey samples (M1 and M2) circulating in the community

No	Sample product	Viscosity (polse)	Standard (INS)
1	M1 honey	2.53	Minimal 10 poise
2	M2 honey	2.48	
3	HBCX herbal honey	4.92	

Source: Primary Data

The findings of the viscosity analysis revealed that HBCX herbal honey and common honey sold in the market (M1, M2) had viscosities that were less than those required by SNI regulations, or, in other words, did not meet the Indonesian National Standard (INS) on honey viscosity. HBCX herbal honey has a higher viscosity than honey that is bought from a store. Temperature and the amount of water in the honey can both alter its viscosity. Honey's water

content determines how liquid and thick it will be; the higher the water content, the more liquid the honey is. Due to a fermentation process brought on by the honey's diluted state, its flavour becomes more acidic and has an alcoholic aftertaste when consumed.

The Acidity of the HBCX Preparation

The results of measuring the pH of honey samples from the market (M1 and M2) and HBCX herbal honey are presented in the table.

Table 2. Acidity of samples honey (M1, M2 and HBCX)

No	Sample	pH	Standard (INS)
1	M1 honey	4.80	5
2	M2 honey	4.78	
3	HBCX herbal honey	4.5	

Source: Primary Data

The results of the acidity analysis revealed that the acidity value of HBCX herbal honey was within the normal range, however, it was somewhat lower than the sample from the market (4.5 vs 4.78 and 4.80). According to the standard, the acidity level of HBCX honey suggests that bacteria won't be able to grow there, as shown in the table. Honey has a slightly thick texture, which reveals its quality. High acidity levels in honey can be influenced by the water content. Honey will experience a higher level of fermentation due to its high-water content and acidity. Increasing the fermentation process can increase the acidity of the honey's flavour and lessen the nutritional benefits of lowering sugars (glucose). The acidity of the honey is also impacted by storage at humid temperatures.

Heavy Metal Contamination in Herbal Honey

The results of the examination of heavy metal contamination in HBCX preparations are presented in Table

Table 3. Analysis of heavy metal contamination in HBCX

No	Metal Contamination Analysis (mg/kg)	Level in HBCX sample	Standard
1	Timbal (pb)	4.80	0.25
2	Kadmium (Cd)	4.78	0.2
3	Seng (Zn)	4.5	15.2
4	Tembaga (Cu)	0.2563	0.1 - 150

Source: Primary Data

The measurement of heavy metal contamination results indicates that HBCX honey has a lower heavy metal content than the levels set by INS so HBCX is safe for consumption.

The findings of the analysis of polyphenol concentrations

The linear regression equation of the relationship between gallic acid levels/concentrations as a polyphenol standard with absorbance as a standard is presented in Figure 1.

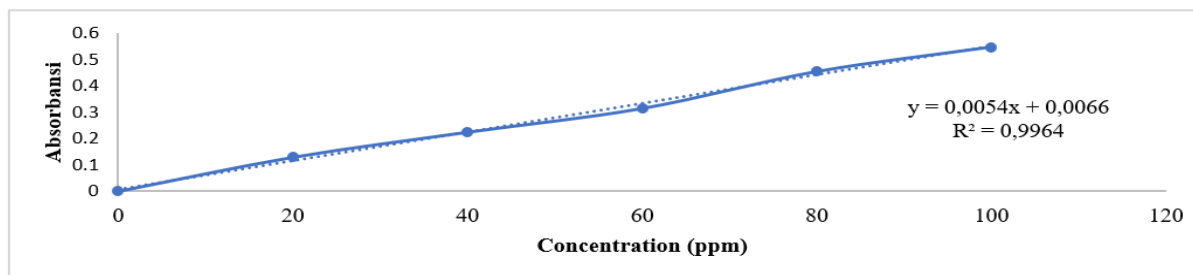


Figure 1. Graph of the linear regression equation of the relationship between gallic acid concentration (ppm) and absorbance

The results of the measurement of polyphenol levels in HBCX are presented in Table 4.

Table 4. The polyphenol content of the upper, middle, and lower HBCX samples was measured using standards of gallic acid.

No	Sample	Replicas	Absorbance	Intercept	Slope	Concentration
1	Upper part	1	0.189	0.0066	0.0054	33.78
		2	0.194	0.0066	0.0054	34.70
		3	0.187	0.0066	0.0054	33.41
2	Midle part	1	0.214	0.0066	0.0054	38.41
		2	0.225	0.0066	0.0054	40.44
		3	0.227	0.0066	0.0054	40.81
3	Lower part	1	0.198	0.0066	0.0054	35.44
		2	0.214	0.0066	0.0054	38.41
		3	0.214	0.0066	0.0054	38.41

Source: Primary Data

The data reveals that the polyphenol content of HBCX is between 33.70 and 40.81 ppm. The findings of this study suggest that the quantities of polyphenols in HBCX are quite high based on the data. Antioxidants called polyphenols can lessen the production of free radicals in honey. In addition to acting as antioxidants, polyphenols provide other biological advantages and promote health.

HBCX antioxidant activity

Antioxidant activity and the amount of radical-antioxidant complex generated are measured using the DPPH method, which is also used to examine compounds that serve as hydrogen donors or free radical scavengers. The potential antioxidant value of HBCX must be assessed since polyphenols are organic antioxidants. The results of assessing the antioxidant activity of HBCX honey formulations using the DPPH technique are displayed in Figure 2 and Table 5.

Figure 2 shows the association between the standard antioxidant (vitamin C) level and the percentage of DPPH reaction inhibition as a linear regression.

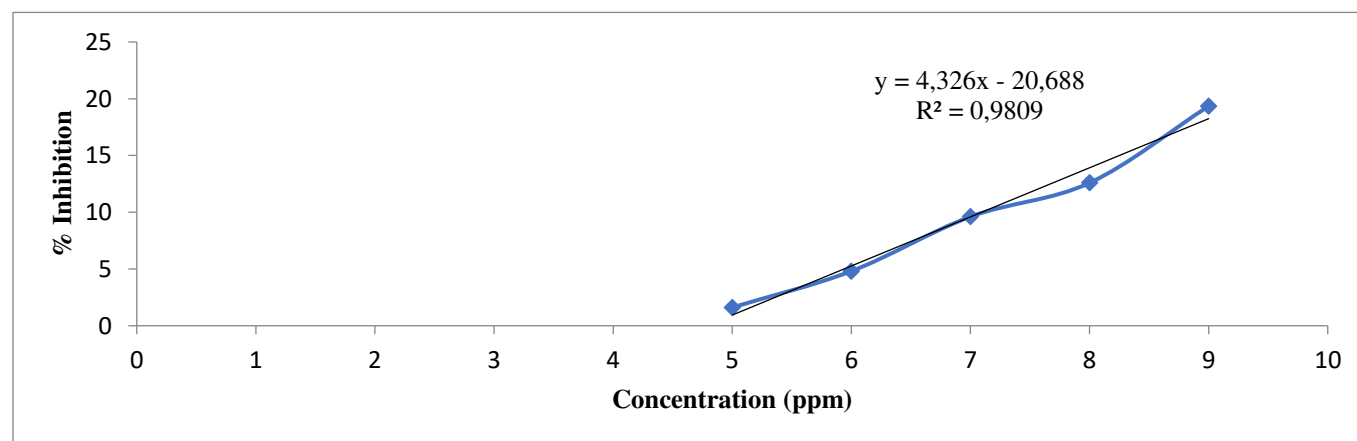


Figure 2. Shows the graph of the linear regression equation for the concentration of HBCX with the free radical binding to the hydroxyl group of DPPH inhibited

The results of the antioxidant activity test of the HBCX samples are shown in Table 5, and the DPPH method was utilized to verify the antioxidant activity of the HBCX preparation.

Table 5. Percentage of inhibition of oxidative reactions on DPPH by HBCX honey preparation

No	Concentration	% Inhibition
1	5	1.61
2	6	4.81
3	7	9.61
4	8	12.59
5	9	19.35

Source: Primary Data

The IC₅₀ of the antioxidant activity of HBCX preparations is 54.78 ppm, according to data on the correlation between concentration and percent inhibition. HBCX honey formulations have a moderate level of antioxidant activity based on the IC₅₀ percent inhibition of oxidative reaction inhibition data on DPPH.

DISCUSSION

The physicochemical analysis revealed that the HBCX preparation had a viscosity and pH by the Indonesian national standard (INS) and a relatively high polyphenol content and low heavy metal contamination content, indicating that it was safe for eating. The findings of assessing the antioxidant activity of the HBCX preparation revealed that the IC₅₀ of the HBCX preparation was a moderate antioxidant (54.78 ppm). The HBCX formulation may be used as a dietary supplement and antioxidant in light of the study's findings.

HBCX preparation's polyphenol content and antioxidant activity are critical in enhancing health. Polyphenols play an important role in the prevention and treatment of chronic diseases such as cardiovascular disease (CVD), neurodegenerative disorders, diabetes, and renal failure (2), (30). Polyphenols are a wide collection of bioactive phytochemicals that contain a variety of subclasses, such as flavonoids, stilbenes, phenolic acids, and lignans (31). Flavonoids, an essential part of polyphenols, can be absorbed in the small intestine, and then phase II enzymes will break them down before they are released into the bloodstream. While the small intestine can absorb some dietary flavonoids, the colonic microbiota processes a major portion once they reach the large intestine. Flavonoids, an essential part of polyphenols, can be absorbed in the small intestine, and then phase II enzymes will break them down before they are released into the bloodstream. While the small intestine can absorb some dietary flavonoids, the colonic microbiota processes a major portion once they reach the large intestine (32). According to recent studies, polyphenols and the human gut flora are linked in both directions. For their bioactive qualities, non-extractable polyphenols have not received enough attention; nonetheless, research has shown that they persist in the colon for a considerable amount of time, where gut microorganisms process them to produce active metabolites that are absorbed more quickly (33). Meanwhile, environmental and genetic factors (such as enterotypes, diversity, and content) can affect an individual's gut microbiome's composition (13), (34), and the ability to metabolize specific polyphenols has been linked to distinct phenotypes (18). Polyphenols have been linked to healthy microbiota populations in the gastrointestinal tract and have been demonstrated to improve nutrient absorption in stunted children (35).

Each anatomic site has a different composition of gut microbiota due to variations in temperature, pH, redox potential, oxygen tension, water activity, salinity, and light. The composition of the gut microbiota is also influenced by the role that each regional gut microbiome plays in digestion (36). There are species in the *Lactobacillus*, *Eubacterium*, *Bifidobacterium*, *Clostridium*, and *Bacteroides* that are capable of breaking down carbohydrates. The gut microbiota ferments proteins in the colon using bacterial proteinase and peptidase because of species like *Clostridium*, *Propionibacterium* spp., *Prevotella* spp., *Bifidobacterium* spp., and *Bacteroides*. Research has demonstrated that different species groups in the gut microbiota are activated based on the primary macronutrient consumed. Bacteria that live in the gastrointestinal system and aid in improving the absorption of nutrients in children who are stunted (37), (38). Epidemiological studies have shown that children with stunted growth have an imbalance between the healthy and poor intestinal flora, or dysbiosis (39). Those who are stunted have more harmful gut bacteria, whereas children who are not stunted have more beneficial bacteria. It has been demonstrated that eating a lot of polyphenols increases the population of healthy gut flora. Diet is the major instrument for balancing the gut microbiota (40), (41). Prebiotics, which are mostly dietary elements like indigestible carbohydrates, are being encouraged by this idea. Other food substances, including polyphenols, which are not absorbed by the small intestine

build up in the large intestine where they are exposed to the enzymatic activity of the gut microbiota. Polyphenols may function as prebiotics, according to in vitro research (42), (43).

According to the epidemiological study's findings, children with stunting had higher levels of proinflammatory cytokines and reactive radicals than children without stunting (44). Stunting's oxidative stress and chronic inflammation have a significant role in lowering immunity and accelerating aging-related processes, such as the slowdown of cognitive development (45). The principal mode of action of polyphenols was once believed to be their direct antioxidant properties, as evidenced by the study's findings. However, because these chemicals do not accumulate to concentrations high enough to significantly affect the scavenging of free radicals in most tissues, these effects are no longer thought to be as relevant in vivo (46). Other potential biochemical and molecular mechanisms, however, have been discovered. These include a wide range of effects on intra- and intercellular signalling pathways, such as controlling nuclear transcription factors and fat metabolism, as well as modifying the production of inflammatory mediators like tumor necrosis factor (TNF), interleukin (IL)-1, and IL-6 (47). Additionally, polyphenols are known to enhance cognitive function. A connection between total polyphenol intake and cognitive variables was discovered in a prospective study of adults in their midlife. Clear data indicates that after single dosages, polyphenol-rich products and individual polyphenols regularly increase cerebral blood flow (CBF) or modify brain activity. Long-term intake of diets high in polyphenols may offer protection against the onset of several chronic diseases, including cancer, diabetes, inflammatory disorders, infectious diseases, and neurodegenerative diseases (47),(35),(33). This is highly supported by pre-clinical and clinical research. It has been suggested that increasing consumption of foods high in polyphenols (such as quercetin, epigallocatechin-3-gallate, resveratrol, cyanidin, etc.) can reduce the risk of many chronic oxidative cellular damage conditions (48), Neurodegenerative diseases, bacterial and viral infections, cancers, tissue inflammation, and DNA damage (49), The polyphenol content in HBCX nutritional supplement preparations may one day serve as an alternative nutritional supplement for children who are stunted, according to the most recent research.

Limitation and Implication

This study is limited to evaluating the viscosity, pH, heavy metal contamination, polyphenol content, and antioxidant activity of HBCX herbal honey, so in the future, it is necessary to conduct tests on stunting cases and oxidative stress test animals to determine the efficacy of HBCX formulation as an antioxidant.

The finding that HBCX honey contains high levels of polyphenols and antioxidants can provide an idea that HBCX honey preparations can be used as an alternative solution in therapy to prevent stunting caused by oxidative stress. This will certainly really help the government's efforts to reduce the incidence of stunting in Indonesia, which in recent times has been considered ineffective.

CONCLUSION

HBCX herbal honey has a higher viscosity than sample honey from the market but still falls below INS standards. Compared to sample honey products, HBCX honey has a lower pH. Less heavy metal contamination can be found in HBCX honey. HBCX honey has a high level of polyphenol content and antioxidant activity.

The efficacy of HBCX formulations as an antioxidant and a pharmacological mode of action must be investigated immediately by utilizing stunting and oxidative stress test animals.

AUTHOR'S CONTRIBUTION STATEMENT

All authors contributed equally in conducting research and writing the manuscript. Lukman Hardia designed the research concept, collected data, and prepared the manuscript. Akrom designed the research concept, analysed and interpreted the data, reviewed the manuscript, and was the corresponding author. Titiek Hidayati, collected and conducted literature reviews, and reviewed the manuscript. Nanik Sulistyani searched for literature, designed the study, and criticized the manuscript. All authors agree to publish.

CONFLICTS OF INTEREST

The author declares that all authors have agreed that there is no conflict of interest and the final draft of this paper.

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