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Development of anti-obesity herbal drink from butterfly pea flower (*Clitoria ternatea*) extract and lemon (*Citrus limon*) juice

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Abstract. *Anugrahani AD, Indarto D, Pamungkasari EP, Wijayanti L, Utami F. 2025. Development of anti-obesity herbal drink from butterfly pea flower (*Clitoria ternatea*) extract and lemon (*Citrus limon*) juice. Nusantara Bioscience 17: 129-136*. Obesity is a multifactorial disease with a high global prevalence and higher risk for cardiometabolic diseases. Obesity management comprises diet, physical exercise, and pharmacotherapy. Some obese patients have low compliance with diet and exercise therapy, while long-term use of anti-obesity drugs causes adverse side effects. Herbal drinks have also been used for obesity treatment, but their efficacy remains debatable. Herbal drink products have been developed, including butterfly pea flower extract and lemon juice, which contain flavonoids and anthocyanins that have anti-obesity activity. The purpose of this study was to create a herbal drink for obesity treatment and analyze acceptability, microbiological contamination, and nutritional and phytochemical contents. An organoleptic test assessed the acceptability of the herbal drink. Analysis of macronutrient content used proximate methods. Vitamin C and microbiological tests were performed using spectrophotometric and Total Plate Count (TPC) methods. Delphinidin and hesperidin levels were measured using a High-Performance Liquid Chromatography (HPLC) method. Statistical analysis was performed using Kruskal-Wallis and followed by the post-hoc Mann-Whitney with $p<0.05$ to determine the best formula of the four formulas (F1-F4) in this study. Organoleptic test results showed that formulation F3 of the herbal drink was the most liked and acceptable by the panelists. The herbal drink (F3) contained 0.82% fat, 0.34% protein, 0.099% carbohydrate, 3,527.25 mg/100 g vitamin C, 21.66 ppm delphinidin, and 525.68 ppm hesperidin. In conclusion, herbal drink has the potential to become an alternative anti-obesity treatment. Further research is needed to test the effectiveness of herbal drinks on weight loss and lipid profile improvement in obese patients.

Keywords: Butterfly pea flower, delphinidin, herbal drink, hesperidin, lemon, obesity

INTRODUCTION

Obesity is a multifactorial disease characterized by excessive fat accumulation in the adipose tissues, which increases the risk of cardiometabolic diseases and other non-communicable diseases (Powell-Wiley et al. 2021). Research in Australia states that obese adults had a higher risk of developing type 2 diabetes (OR:12.76, 95% CI: 8.88-18.36), arthritis (OR: 2.25, 95% CI: 1.90-2.68), heart disease (OR: 2.05, 95% CI: 1.54-2.74), asthma (OR: 1.97, 95% CI: 1.49-2.62) and depression (OR:1.96, 95% CI: 1.56-2.48) compared to normal weight adults (Keramat et al. 2021). Globally, adult obesity contributes to 60% of premature deaths (Sultana et al. 2021). Determinants of obesity include high consumption of sweet beverages, lack of exercise, endocrine hormone disruption, decreased energy metabolism, and genetic factors such as MC4R, PPARG, and TCF7L2 gene polymorphisms. In the digitalization era, many people worldwide tend to have several bad habits, such as consuming more fast food than healthy diet food, drinking beverages with higher energy, and having low physical activity (Valeeva et al. 2022).

Current global obesity management focuses on diet and physical exercise, but there is no single diet can be universally applied to everyone with obesity (Roman et al. 2024). A restricted calorie diet, consisting of low carbohydrate and fat, may not be effective in the long term due to poor compliance. A study in a health care center in Guadalajara (Spain), of 209 obese adult patients, showed that 67.5% of patients were not compliant with diet and exercise for weight loss programs. The obstacles experienced by obese patients included 27.8% of patients not having a healthy diet prescription, 17.7% of patients experiencing joint pain, 14.8% of patients being bored with dietary patterns, and 11.5% of patients being lazy (Trujillo-Garrido and Santi-Cano 2022). The second popular diet for obesity is the ketogenic diet, which is characterized by high fat intake (60%) and low carbohydrate intake (10%). The results showed that the effectiveness of the ketogenic diet on weight loss was not better than other types of diets. Not

everyone can adopt a ketogenic diet, such as obese patients with complications of type 1 diabetes, renal failure, and heart disease patients because they have to limit their daily fat intake (Kim 2021). Personalized nutrition is recommended for body weight management of obese people because they are based on the individual specific health condition, gender and age-related needs, food preference, and appropriate motivational support (Roman et al. 2024). Anti-obesity drug therapy is the last alternative if diet and exercise alone do not work or in cases of obesity accompanied by complications of other diseases (Brandfon et al. 2023). Administration of anti-obesity chemical drugs such as phentermine, liraglutide, bupropion, and orlistat can have a weight loss effect. Still, their long-term use can cause side effects that are detrimental to health (Tak and Lee 2021). Therefore, alternative herbal treatments from natural ingredients are needed (Sayed et al. 2023). Anti-obesity herbs that are already popular include green coffee, green tea, ginger, and lemon-garlic. However, their effectiveness is still low (Alfaifi et al. 2020); for example, to provide a weight loss effect of 1.78 kg and a decrease in waist circumference of 2.06 cm, it is necessary to consume 500 mg/day of green tea extract for 12 weeks (Lin et al. 2020).

In our previous study, we made three formulations of butterfly pea flower and lemon combination drink (E1-E3) in the ratio of 75:25%, 80:20%, and 85:15%. The laboratory test results in our study showed that the herbal drink, a combination of butterfly pea flower and lemon, contains flavonoids and anthocyanins that have anti-obesity properties (Indarto and Utami 2023). The most abundant flavonoid in lemon is hesperidin, which can increase leptin sensitivity and inhibit lipogenesis (Xiong 2019). The primary anthocyanin in butterfly pea flowers is delphinidin, which has anti-obesity properties due to its ability to inhibit adipogenesis (Chayaratanasin et al. 2019). Administering a combination of 80:20% and 85:15% butterfly pea flower extract and lemon juice to obese male rats for 21 days reduced body weight, as well as improved lipid profiles (total cholesterol, LDL cholesterol, and triglycerides) and increased HDL cholesterol (Indarto and Utami 2023). However, no data exist on the herbal drink's safety and effectiveness as a human obesity treatment. Therefore, this study aimed to develop an herbal drink for this purpose by analyzing acceptability, microbiological contamination, and nutritional and phytochemical contents.

MATERIALS AND METHODS

Research design

An experimental laboratory was initially conducted to reformulate the herbal drink, followed by a sensory analysis by panelists that evaluated its color, taste, viscosity, and flavor. Collected data were then analyzed statistically. Finally, exploratory research was conducted on the nutritional and phytochemical content of the best formula.

Figure 1. The process of making an herbal drink

Preparation for making an herbal drink

Making an herbal drink refers to the previous study conducted by Indarto and Utami (2023) with a slight modification, locally, this product is called *Litrusia*. Briefly, F1-F4 formulas of herbal drink contained 1% butterfly pea flower extract and were added to lemon juice with an 80:20% ratio (F1 and F2) and 85:15% (F3 and F4. After that, F1 and F3 herbal drinks were added with 3.25% liquid stevia sweetener (Daylistz, CV. Sarsy Sinar Stevia, Subang, West Java, Indonesia), while F2 and F4 herbal drinks were added with 4.87% liquid stevia sweetener. The process of making these herbal drinks has complied with the HACCP (Hazard analysis and critical control points) system procedure, which is a food safety management system to prevent the occurrence of food-borne diseases (Awuchi 2023). The process of making herbal drinks can be seen in Figure 1.

Acceptability analysis of herbal drink

The sensory evaluation of the herbal drink, including color, flavor, texture, and taste, was assessed by 10 expert panelists who worked as lecturers in Nutrition Sciences, Department of Nutrition, State Health Polytechnic Malang, East Java (Djekic et al. 2021). Each panelist was given a glass of mineral water before and after F1-F4 assessments. The acceptability test was adopted from the five hedonic scales: 1: Extremely dislike, 2: Dislike, 3: Somewhat dislike, 4: Like, and 5: Extremely like.

Microbiological contamination, anthocyanin content, macro and micronutrient levels in the herbal drink

A microbial contamination-free test was conducted using a total plate count method. Determination of anthocyanin content of herbal drink was performed using a differential pH method, pH 1 and pH 4.5 buffer solutions were added to two test tubes containing herbal drink samples. The measurements were taken using a UV-Vis spectrophotometer with a 510 nm wavelength and analyzed by comparing the absorbance values at both pHs. Fat, protein, mineral, and water concentrations in the herbal drink were tested using Soxhlet, Kjeldahl, dry ash, and thermogravimetric methods. Carbohydrate concentration was assessed by subtracting the total percentage (100%) from water, ash, fat, and protein percentages. Calculating the energy content of an herbal drink using the proximate method involves the composition of macronutrients (4 kcal/g carbohydrate, 4 kcal/g protein, and 9 kcal/g fat. Total energy is the sum of energy contributions from each macronutrient. Finally, vitamin C concentration was determined using a spectrophotometric method.

Analysis of delphinidin and hesperidin levels in an herbal drink

Delphinidin and hesperidin levels were measured using the High-Performance Liquid Chromatography (HPLC Rigol L-3000, Germany. The standard delphinidin and hesperidin compounds were obtained from Sigma Aldrich with CAT 43725 and H5254, respectively. The mobile phase used in HPLC analysis to determine delphinidin and hesperidin levels was formic acid and acetonitrile. The protocol of delphinidin followed the method by Khan et al. (2022) with slight modifications. 1 mg delphinidin was dissolved in 5 mL of methanol solution. Serial dilution ranged from 1.25, 2.5, 5, 10, 25, and 50 ppm. 1.5 mL of herbal drink was centrifuged at 13.200 rpm for 15 minutes, from which the pellets were dissolved with 1.5 mL of methanol. After that, the herbal drink solution was injected into a 5µm C18, 150×4.6 mm Diamonsil column (Cat#99901 Ser#3673520, Dikma Technologies Inc., USA), and the flow rate was set at 1 mL per minute. The protocol of hesperidin analysis followed the method by Büyüktuncel (2017) with slight modifications. 25 mg hesperidin was dissolved with 25 mL Dimethyl sulfoxide (DMSO. Serial dilution ranged from 50, 100, 200, 400, and 800 ppm. 1.5 mL of herbal drink was centrifuged at 13,200 rpm for 15 minutes, and the pellets were dissolved with 1.5 mL of DMSO. After that, the herbal drink solution was injected into a 5µm C18, 150×4.6 mm Diamonsil column, and the flow rate was set at 1 mL per minute.

Statistical analysis

The collected data were analyzed using the SPSS (Statistical Package for the Social Sciences) program version 25. Numerical data were presented as mean ± SD. Because data distribution was not normal, we used the Kruskal-Wallis statistical test to compare four formulations of herbal drinks in terms of color, flavor, texture, taste, and overall (Ruiz-Capillas and Herrero, 2021), followed by the Mann-Whitney post hoc test the Mann-Whitney post hoc test, and a significant value was $p<0.05$.

RESULTS AND DISCUSSION

Organoleptic test results of four herbal drink formulas

Four formulations of herbal drink (F1-F4) were evaluated for their acceptability (color, flavor, viscosity, and taste, Table 1). The four formulations were similar in color, flavor, and viscosity, but the F3 formulation (score=4.50±0.527) had the best taste compared to F4 (score=4.30±0.675), F1 (score=2.50±0.527), and F2 (score=2.60±0.516) formulations.

Referring to Table 1, we find that the taste of F3 was not significantly different from F4 but significantly different from F1 and F2 ($p<0.001$). Panelists liked the taste of formulas F3 and F4, but somewhat like formulas F1 and F2. Overall, the F3 formula was the highest panelists' acceptance compared to the other three formulas, resulting in F3 as the best formula for the herbal drink.

Analyses of microbiological, anthocyanin, macro, and micronutrient levels in the herbal drink

The herbal drink is free from pathogenic bacteria, contains 258.48 ppm anthocyanin, has low levels of fat (0.82%), carbohydrates (0.099%), calories (7.38 kcal), and high levels of vitamin C (3.5%). In addition, it contains 98.90% water and 0.34% protein (Table 2).

The delphinidin and hesperidin contents of the herbal drink

Figures 2 and 3 present the HPLC assay results of delphinidin and hesperidin in the herbal drink. We detected delphinidin at an 8-minute retention time and hesperidin at a 10-minute retention time. The HPLC results also indicated that the herbal drink contains 21.66 ppm delphinidin and 525.68 ppm hesperidin.

Table 1. Organoleptic test results of the herbal drink

Formulas	Organoleptic test results of herbal drink (mean ± SD)				
	Color	Flavor	Viscosity	Taste	Overall
F1	4.30±0.483	4.00±0.000	4.30±0.483	2.50±0.527 [a), b)]	3.80±0.365 [a), b)]
F2	4.30±0.483	4.00±0.000	4.30±0.483	2.60±0.516 [a), b)]	3.83±0.236 [a), b)]
F3	4.40±0.516	4.00±0.483	4.30±0.483	4.50±0.527 [c), d)]	4.26±0.365 [c), d)]
F4	4.10±0.738	3.70±0.483	4.30±0.483	4.30±0.675 [c), d)]	4.14±0.425 [c), d)]
p-value	0.780	0.076	1.000	<0.001*	0.016*

Note: 1: extremely dislike, 2: dislike, 3: somewhat like, 4: like, 5: extremely like. *Significantly different based on Kruskall Wallis test ($p<0.05$. [a)] significantly different from F3, [b)] significantly different from F4, [c)] significantly different from F1, [d)] significantly different from F2 based on Mann Whitney post hoc test

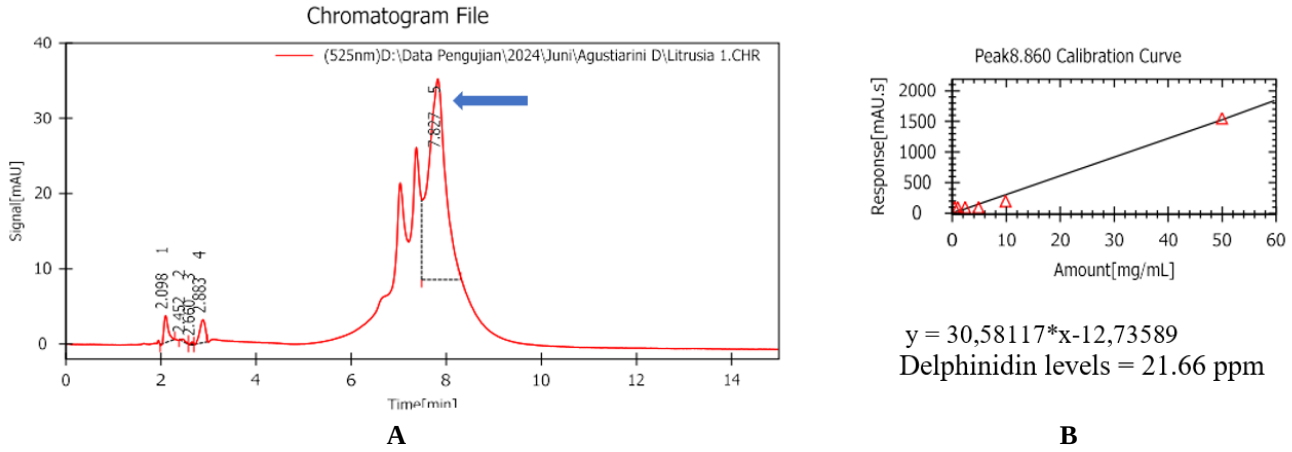

Figure 2. HPLC chromatogram profile: A. Delphinidin content in herbal drink; and B. A standard curve of delphinidin

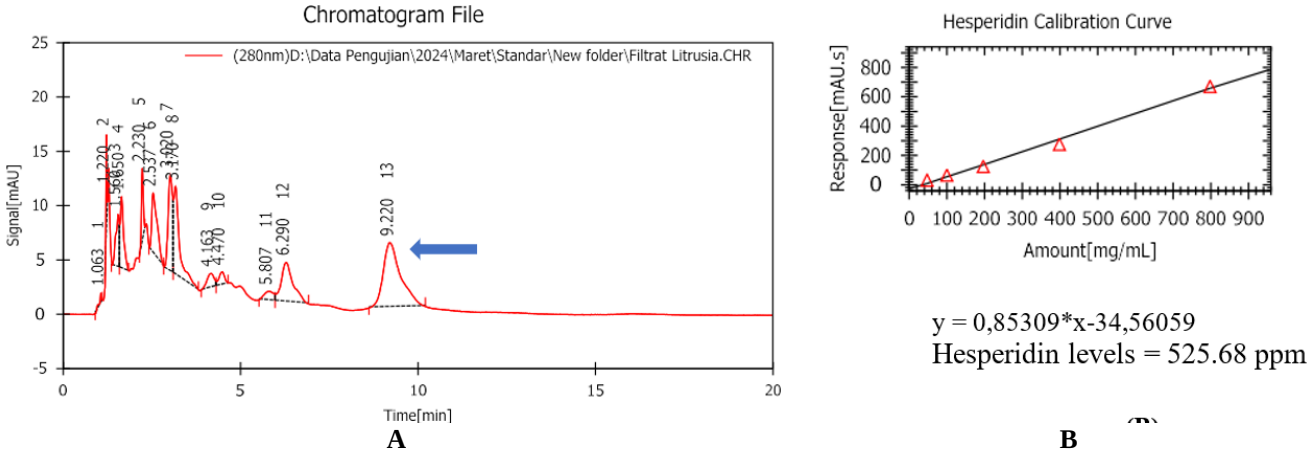

Figure 3. HPLC chromatogram profile: A. Hesperidin content in herbal drink; and B. A standard curve of hesperidin

Table 2. Microbiological, acidity, anthocyanin, macronutrient, and vitamin C contents in the (F3) herbal drink

Types of analysis	Analysis method	Analysis results (wb)
Microbiological	Total Plate Count (TPC)	ND[a)]
Acidity	pH meter	2.83
Anthocyanin	Differential pH	258.48 ppm
Water	Thermogravimetry	98.70 %
Mineral	Dry ash	0.036 %
Fat	Soxhlet	0.82 %
Proteins	Kjeldahl	0.34 %
Carbohydrate	By different	0.099 %
Energy	Total energy calculation [b)]	7.38 kcal
Vitamin C	Spectrophotometric	3.5 % [c)]

Note: [a)] ND: not detected, *ppm: mg/1,000 mL. [b)] Total energy is calculated from energy values of carbohydrate (4 kcal), protein (4 kcal), and fat (9 kcal). [c)] A 100-gram sample of herbal drink contains 3.5 grams of vitamin C

Discussion

In this study, we have demonstrated that the herbal drink with an 85:15% combination of butterfly pea flower extract and lemon juice, added with 3.25% liquid stevia sweetener, is the best formulation compared to the other three formulas based on food acceptability assessment. Our herbal drink formulation is safe from pathogenic bacteria, low energy (7.38 kcal/100 mL), low carbohydrates (0.099%), low fat (0.82%), and high vitamin C (3.5%). Based on Perka BPOM RI No. 1, 2022 on the Supervision of Claims on Labels and Advertisements of Processed Food, the herbal drink can be claimed as low-energy (not more than 20 kcal/100 mL), low-fat (not more than 1.5 g/100 mL), 'No Added Sugar' (sucrose, lactose, and fructose not more than 0.5 g/100 mL. A claim of high vitamin C in the beverage product is more than 2× the Recommended Dietary Allowance (RDA of vitamin C = 90 mg. 100 mL of herbal drink contains 3.5 g (3,525 mg) of vitamin C; hence, it can be claimed as a high vitamin C (Perka BPOM RI 2022). Administering high doses of vitamin C intravenous may result in health risks, but consumption of high vitamin C in foods remains safe, as vitamin C is a water-soluble vitamin that the body will excrete through urine if taken in excess (Doseděl et al. 2021). This herbal drink also contains anthocyanin (258.48 ppm), delphinidin (21.66 ppm), and hesperidin (525.68 ppm). Overall, this formulation of herbal drink may be suitable for an alternative diet treatment for obesity.

Our previous research on three formulations of butterfly pea flower and lemon combination (herbal drink): E1 (75:25%), E2 (80:20%), and E3 (85:15%) contained flavonoids and anthocyanins. Our *in vivo* study indicated that administering an herbal drink with 80:20% and 85:15% for 21 days improved lipid profile and reduced body weight in obese rats (Indarto and Utami 2023). Based on the results of the previous study, re-formulation of the herbal drink with a proportion of 80:20% and 85:15% was carried out by adding liquid stevia sweetener to increase its acceptability. The liquid stevia sweetener is a natural sweetener derived from the leaf extract of the *Stevia rebaudiana* plant, containing zero calories and 50-350 times sweeter than sugar (Samuel et al. 2018). Stevia does not increase postprandial glucose levels and reduces appetite. Therefore, it is recommended for weight loss management, such as for obese patients (Farhat et al. 2019). The FDA conferred 'Generally Recognized as Safe' (GRAS) status for stevia leaf extract in 2018. The European Food Safety Authority (EFSA) sets an acceptable daily intake (ADI) of 4 mg/kg/day for Steviol glycosides. There is no clinical evidence on the side effects of Steviol glycoside sweeteners due to the very small amount required for consumption (Orellana-Paucar 2023). In comparison to other natural sweeteners (high fructose derived from corn extract), high consumption of corn sweeteners (fructose intake >50 mg/day) is involved in several human disorders such as increased insulin resistance, hypertriglyceridemia, hypertension, weight gain, non-alcoholic fatty liver disease (NAFLD), and cardiovascular disease (Khorshidian et al. 2021; Taskinen et al. 2019). In contrast to artificial sweeteners such as sucralose, acesulfame K, aspartame, and saccharin, a large cohort study showed that high artificial sweetener intake was associated with higher mortality, the risks of cardiovascular disease, and cancer (Lizuka 2022).

Government Regulation of the Republic of Indonesia No. 86/2019 on Food Safety and SNI 01-3719-1995 on the Indonesian National Standard for fruit juice drinks recommend that laboratory tests be conducted before food or beverage products are circulated to ensure that they do not contain contaminants and food additives that can be detrimental to health (BSN 2014; Peraturan Presiden Republik Indonesia 2019). Based on the SNI 01-3719-1995, the quality requirements for fruit juice drinks, among other things, are free from microbial contamination (total plate count maximum 2×102 colonies/mL). The microbial test on herbal drinks using the TPC method showed an ND result, which means that the herbal drink is free from bacterial contamination. Ewansiha (2020) states that drinks with a concentration of 12.5-25% lemon can inhibit the growth of pathogenic bacteria. Pathogenic bacteria cannot grow in an acidic pH environment (pH<4) because acidic pH damages their cell structure and biochemical functions (Lund et al. 2020). Thus, the herbal drink is safe from pathogenic bacterial contamination due to its lemon juice concentration (15%) and acidic pH (pH=2.83).

The anti-obesity activity of butterfly pea flowers has been studied by Hardinsyah et al. (2023) using a fermented beverage of butterfly pea flower kombucha, and then tested by HPLC-ESI-HRMS/MS. They identified 13 essential compounds, including kaempferol, quercetin, dibenzylamine, and pyrrolidinopropiophenone, showing anti-obesity properties in molecular docking studies against pancreatic lipase and amylase. Widowati et al. (2022) examined a *TeMon* drink made from butterfly pea flowers and lemon tea, which contained 2.97 mg flavonoids and 0.28 mg phenols per 100% sample, with 28.66% antioxidant value, higher than butterfly pea flower tea (17.07%) and lemon tea (25.34%).

The herbal drink has an anti-obesity effect due to its anthocyanins, delphinidin, and hesperidin content. The

mechanism of anthocyanins for losing weight is through inhibiting the pancreatic lipase to inhibit lipogenesis, increasing body fat burning through the adenosine monophosphate-activated protein kinase (AMPK) pathway, increasing lipolysis through the Peroxisome Proliferator Activator Receptor (PPAR) pathway, and suppressing the appetite of obese people through the Neuropeptide Y (NPY), Protein Kinase A-α (PKA-α), phosphorylated-Cyclic AMP Response Element Binding protein (p-CREB) pathways (Franco-San Sebastián et al. 2023). Delphinidin can reduce lipid absorption from food through the mechanism of inhibiting pancreatic lipase, and inhibits lipogenesis and adipogenesis through the PPAR-γ and sterol regulatory element-binding transcription factor 1 (SREBP1) (Daveri et al. 2018; Park et al. 2019). Hesperidin is a flavanone glycoside that is anti-obesity by stimulating the growth of intestinal microbes to increase the production of short-chain fatty acid (SCFA), thereby increasing insulin sensitivity and improving glucose homeostasis, suppressing the appetite of obese patients by stimulating the release of cholecystokinin (CCK), an appetite-regulating hormone in enteroendocrine STC-1 cells (Xiong 2019).

The herbal drink contains 258.48 ppm anthocyanins (51.70 mg/200 mL). Administration of 81.16 mg anthocyanins/day from Rosella extract for six weeks in obese men can reduce body weight by -0.77 kg and waist circumference by -3.08 cm (Sari et al. 2018) and administration of *Juçara* pulp powder with 130.7 mg anthocyanins/day for six weeks in obese adults can reduce body weight by -0.58 kg and increase HDL_C levels by 107 mg/dl (Jamar et al. 2020). Delphinidin levels in the herbal drink were 21.66 ppm or 4.34 mg per serving (200 mL). *In vivo* studies have shown that administration of 100 µg delphinidine to rat adipose tissues can reduce lipid accumulation, inhibit lipogenesis and adipogenesis through PPAR-γ, SREBP1, and AMPK without toxicity (Park et al. 2019). In vitro studies have shown that administering 25 µg delphinidine to human adipose tissues has an adipogenesis inhibitory effect similar to the anti-obesity liraglutide drug (Saulite et al. 2019). Herbal drink also contains 525.68 ppm hesperidin. A previous study on 40 amateur cyclists (aged 18-55 years) in Spain, given the 2S-hesperidin intervention (500 mg/day, n=20) and another group taking placebo (microellulose 500 mg/day, n=20) for 8 weeks showed that the hesperidin intervention group had a decrease in body fat percentage (-10.4%, p = 0.035) and a decrease in body weight percentages (-3.7%, p = 0.006) of DXA analysis (Martinez Noguera et al. 2021). The anthocyanin and hesperidin content per serving (200 mL) of the herbal drink was lower than that of the Rosela drink and hesperidin capsules. However, the delphinidin content was higher than that of the delphinidin extract tested in the study by Saulite et al. (2019). The advantage of an herbal drink is that it is made from a brew of butterfly pea flowers and lemon juice, not pure extract, so it contains numerous phytochemical compounds.

The nutritional content of herbal drinks also supports obesity diet therapy because it contains high vitamin C. In obese patients, oxidative stress occurs due to pro-oxidant levels (which come from producing pro-inflammatory cytokines) greater than antioxidant levels in adipose tissues. Therefore, a therapeutic approach using antioxidant foods such as vitamin C to reduce the adverse effects of obesity. Vitamin C administration in obese mice can modulate lipolysis in adipose tissues by activating lipoprotein lipase (Imessaoudene et al. 2022).

The American Heart Association and Diabetes Association recommend low-calorie sweetened beverages to control weight gain and blood sugar because excessive and long-term consumption of sugary drinks leads to obesity and cardiometabolic diseases. The herbal drink is low in carbohydrates (0.099%) and low in calories (7.38 kcal. WHO recommends consuming <10% sugar per day, or equivalent to 50 grams per day for adults with an average calorie requirement of 2,000 kcal, and 25 grams per day is ideal (Chatelan et al. 2024). Stevia contains steviol glycosides, which have a sweet taste 300 times sweeter than granulated sugar and have a non-glycemic effect (Peteliuk et al. 2021). Raghavan et al. (2023) showed that overweight subjects who were treated to replace the commonly consumed granulated sugar with steviol glycosides for 90 days proved to significantly reduce body weight by 2.12 kg and reduce waist circumference by 4.78 cm. The sweetness of 2 g of steviol glycoside contains zero calories; in comparison, 10 g (1 tbsp) of table sugar contains 38 calories. Adopting steviol glycosides as a substitute for table sugar can lower calorie intake by 90 kcal per day, or 2,700 kcal per month, potentially leading to weight loss (Raghavan et al. 2023). Hence, this herbal drink contains 7.38 kcal of energy per 100 mL, 0.82% of fat, 0.34% of protein, 0.099% of carbohydrate, and 3.5% of vitamin C. This herbal drink contains bioactive compounds that have anti-obesity properties, including 258.48 ppm anthocyanins, 21.66 ppm delphinidin, and 525.68 ppm hesperidin. However, herbal drink product has several limitations, as it does not last long at room temperature and change quickly when exposed to sunlight. Secondly, the herbal drink has an acidic pH, so it cannot be given to obese patients who have gastrointestinal disorders.

In conclusion, herbal drinks have low calories, low fats, and high vitamin C. They also contain anthocyanins, delphinidin, and hesperidin, and have the potential as an alternative anti-obesity treatment. The best formula for a herbal drink, determined by an acceptance score of 4.26±0.365, is a combination of 85:15% with 3.25% liquid stevia addition. Liquid stevia is used to enhance the sweetness of the herbal drink without adding extra calories. Further research is needed to improve the durability of herbal drinks by storing them at 4°C and using dark bottles. Moreover, the herbal drink should be consumed after meals and given to obese people without gastrointestinal disorders.

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