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Biochemical Characteristics of the Formulation and Preparation of Telang Flower Kombucha Jelly as an Antioxidant, Food and Pharmaceutical Biotechnology Products

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ABSTRACT

One of the functional food preparations which is soft formulated and made from hydrocolloid ingredients which include agar, gum, carrageenan, pectin and gelatin is jelly candy. In the formulation and preparation of jelly candy, which aims to increase its functional value, in this research, bioactive compounds are added which function to improve the health aspects of consuming it. In this research, jelly candy is made from butterfly pea flower kombucha because it contains organic acid compounds, polyphenols, vitamins, amino acids, so it is good for body health. Kombucha is made from butterfly pea flowers because previous research results have been proven to be antibacterial, antifungal, antioxidant, antifungal, anticholesterol and anticancer. Telang flowers contain secondary metabolite compounds which are divided into alkaloids, flavonoids, saponins, terpenoids, acetogenin which are cytotoxic for the growth of cancer cells and are efficacious as a source of antioxidants. The jelly candy product produced has been tested to meet the SNI 3547.2-2008 quality standard where the water content produced is 16.25%. ash content 0.35%. And pH 4.57. Jelly candy preparations made from the active ingredient telang flower kombucha which are designed as food and pharmaceutical biotechnology products have biochemical characteristics in warding off free radicals which refer to an IC50 value of 62.848 µg/mL.

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Key Messages:

- Research on the formulation and preparation of candy with the active ingredient of telang flower kombucha is the latest innovation from the results of previous research with the active ingredient of soursop leaf kombucha. This research is preliminary research that also needs to be tested in vitro regarding blood sugar inhibition power.

Introduction

One of the favorite food preparations for children and adults in hard, soft, rubber and jelly forms is known as candy. Food preparations in the form of jelly candy are a type of soft candy. Soft candy is designed from a texture containing hydrocolloid excipients in the form of agar, gum, pectin, starch, carrageenan, gelatin, and other excipients (1). The majority of jelly candy in the manufacturing process is given natural or chemical flavor enhancers. The various flavors of jelly candy can still be developed.

This is because food products are a form of preparation that can be innovated. Food products in the form of candy can generally only be used as light snacks aimed at producing energy or soothing the throat. In order to increase its functional value, food preparations that have pharmaceutical value require a bioactive compound.

Bioactive compounds that are used as an increase in functional value have the potential to produce health-based candy consumption (2);(3). One of the bioactive compounds that is highly recommended as an excipient for candy is butterfly pea flower kombucha (Clitoria ternatea L). Butterfly flower kombucha is a fermented tea drink that is probiotic and has many antibacterial capabilities (4) ; (5) ; (6) ; (7) antimicrobial (8) ; antifungal (9) ; (10) ; (11) , anticholesterol (12) ; (13) ; (14) ; (15), antioxidant (16) ; (17) ; (18) and anticancer (19) ; (20) ; (21). Bioactive compound content (22) both primary and secondary metabolites (23) From telang flower kombucha in previous research it has been proven to have potential as a medicinal preparation (24) ; (25) as well as cosmetics (26). Kombucha can ideally be made with black tea leaves or green tea or olong tea (27). However, in this research, butterfly pea flowers were used as raw material for kombucha preparations in the form of food products with pharmaceutical value, namely candy preparations.

Butterfly pea flowers are traditionally and have been widely developed in various herbal medicines. This is due to the content of secondary metabolites which include alkaloids, flavonoids, saponins, terpenoids, polyphenols and acetogenin in butterfly pea flowers which have a cytotoxic effect on the growth of cancer cells. This is in line with the content of secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids, polyphenols and acetogenin, which are secondary metabolite compounds that play an important role in influencing the level of toxicity towards cancer cell growth (28). This research aims to utilize ingredients for making jelly candy from butterfly pea flower kombucha because until now there has been no research that proves making jelly candy from the active ingredient of butterfly pea flower kombucha as a source of antioxidants in warding off free radicals.

Making jelly candy from the active ingredient of kombucha butterfly pea flower is not focused on performance or appearance alone but refers to preparations that have benefits for human health with the hope that through this research, butterfly pea flower fermented by kombucha will become one part of functional food in helping health. society to always improve. One choice of jelly candy is made from the active ingredient kombucha telang because it contains secondary metabolite compounds such as flavonoids which act as a source of antioxidants . Flavonoids are responsible for warding off free radicals by chelating metal ions and inhibiting the Fenton and Haber-Weis reactions which are sources of oxygen free radical production

Methods

Research Tools and Materials

The tools used in this research include a gas stove, oven, knife, glass jar, blender, sterile cloth, filter, baking dish, watch glass, 10 mL measuring flask, test tube, volumetric pipette, porcelain cup, desiccator, furnace with Thermo Scientific Thermolyne Furnance F48010-33 brand, analytical balance with the ABT-NM Kern brand, pH meter with the Mettler Toledo FiveEasy F30 brand, and UV-Visible Spectrovotometer with the Shinazu UV-1800 brand.

The ingredients used in this research include butterfly pea flower kombucha, where the butterfly pea flower samples obtained were cultivated from Pekuncen village, Ciwedus village, Cilegon city, Banten province. The telang flower sample used came from Pekuncen village because in previous research it had the potential as an active antibacterial substance in the form of telang flower kombucha bath soap.Initial culture of kombucha (SCOBY), plain agar, Food Grade gelatin, granulated sugar, methanol with Merck brand, and DPPH (Brand).

Making Telang Flower Kombucha (Clitoria ternatea L)

The telang flower kombucha produced is based on the results of research conducted by Rezaldi *et* al., (2022) where 500 grams of butterfly pea flowers obtained from cultivation in Pekuncen village, Ciwedus village, Cilegon city, Banten province, were washed in running water (29). Butterfly pea flowers are boiled in 1 liter of water until 250 mL of the solution remains. Filter the boiled water from the butterfly pea flowers and add 80 grams of granulated sugar. Heat until boiling. The boiled telang flower decoction that has matured is stored until it cools down. Transfer the cooled telang flower decoction into a glass jar in sterile conditions. Add the SCOBY solution to the glass jar. Cover the glass jar using a sterile cloth and incubate it for 1 week to 2 weeks in conditions free from light or darkness. Filter and transfer the fermented butterfly pea flower kombucha to a new jar. Telang flower kombucha is left at room temperature which ideally is 23 to 27^{o}C based on research results Rezaldi *et al*., (2022) [61] and the appropriate incubation time refers to research results namely 2 weeks [62].

Making Telang Flower Kombucha Jelly Candy

Put 100 mL of butterfly pea flower kombucha into the pan. Add 30 grams of gelatin and 50 grams of sucrose. Heat the mixture until it reaches 80^0C. Transfer the jelly candy mixture into a baking dish and let it cool until it solidifies. Cut the butterfly pea flower kombucha jelly into 2x2 cm sizes. Bake the candy for 2 hours at 60^oC. Testing the water content, ash content and pH of telang flower kombucha jelly candy (1). The making of telang flower kombucha jelly candy refers to the results of previous research (33).

Organoleptic Testing

Testing of jelly candy preparations made from the active ingredient telang flower kombucha refers to the results of research conducted by Sirait *et al*., (2023) based on modification [30].The parameters used in organoleptic testing of telang flower kombucha jelly preparations include taste, texture and color. Organoleptically testing the preparation of telang flower kombucha jelly candy, there were two test samples in the form of a preparation of telang flower kombucha jelly candy and a preparation of jelly candy without butterfly pea flower kombucha. This organoleptic testing involved 20 individual panelists. The rating scale in this organoleptic test consists of 1 to 7. A score scale of 1 indicates that the panelists really do not like a jelly candy preparation, whether the active ingredient is telang flower kombucha or not. Scale 2 indicates that the panelists do not like a jelly candy preparation made from the active ingredient of butterfly pea flower kombucha or not. A scale of 3 indicates that the panelists somewhat dislike a jelly candy preparation made from the active ingredient of butterfly pea flower kombucha or not. A scale of 4 indicates that the panelists somewhat liked a jelly candy preparation made from the active ingredient of butterfly pea flower kombucha or not. A scale of 5 indicates whether the panelists like a jelly candy preparation made from the active ingredient of butterfly pea flower kombucha or not. A scale of 6 indicates that the panelists really like a jelly candy preparation made from the active ingredient of butterfly pea flower kombucha or not. A scale of 7 indicates that the panelists really like or most like a jelly candy preparation made from the active ingredient of telang flower kombucha or not. The panelists used in this research were untrained panelists with the hope of being able to provide an assessment of this functional food preparation product which has pharmaceutical value.

Testing of Antioxidant Activity on Formulations and Preparations of Telang Flower Kombucha Jelly (Clitoria ternatea L)

Puree the jelly candy containing the active ingredient kombucha butterfly pea flower using a blender. Put 1 mL of DPPH or 400 µM into a test tube. Add 3 mL of methanol and 0.1 mL of telang flower kombucha jelly candy solution which has been prepared for concentrations of 10 ppm, 50 ppm, 150 ppm, and 200 ppm in methanol. Vortex the mixture next and store in a light-free room for 30 minutes. Measuring absorbance or absorption using a UV-Vis spectrophotometer with a wavelength of 517 nm. Calculate the percentage (%) of inhibitory power for each solution based on the IC_{50} value after producing the absorbance value. (31).

Data Analysis

The data used in this research includes descriptive and statistical data. Organoleptic data on each preparation of telang flower kombucha jelly candy was analyzed descriptively. Meanwhile, the results of antioxidant activity tests on telang flower kombucha jelly candy preparations were analyzed statistically.

Results

Chemical Characteristics of Permanent Preparation of Telang Flower Kombucha Jelly

Proximate analysis used to permanently determine the biochemical characteristics of kombucha jelly preparations includes ash content, water content, and pH as listed in table 1 below.

Table 1. Chemical Characteristics of Permanent Preparation of Telang Flower Kombucha Jelly

Parameter Testing	Results
ash content	0,35%
Water content	16,25%
Ph	4,57

Organoleptic Testing of Telang Flower Kombucha Jelly Candy Preparations

Organoleptic testing of telang flower kombucha jelly candy preparations consisted of taste, texture and aroma as listed in Figure 1.

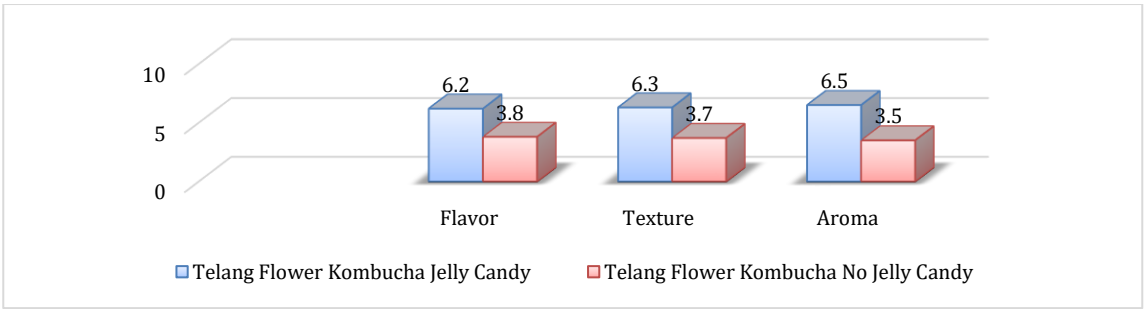

Figure 1. Organoleptic testing of telang flower kombucha jelly candy preparations compared with jelly candy preparations without butterfly pea flower kombucha.

Figure 1 explains that the organoleptic test results such as taste, texture and aroma of the formulation and preparation of jelly candy containing the active ingredient of butterfly pea flower kombucha are higher when compared to the formulation and preparation of jelly candy without the active ingredient of butterfly pea flower kombucha.

Biochemical Characteristics

The biochemical characteristics in this research in the form of the formulation and preparation of jelly candy made from the active ingredient telang flower kombucha can be seen based on its testing in warding off free radicals or which plays an important role as a source of antioxidants. The results of this research are shown in Figure 2 below.

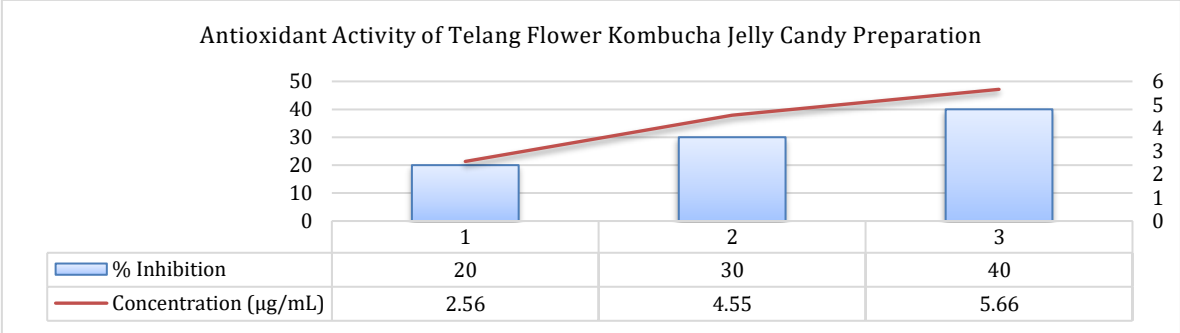

Figure 2. Relationship between the concentration of jelly candy containing the active ingredient kombucha flower based on percentage (%) inhibitory power.

Discussion

Chemical Characteristics

The ash content produced as a chemical characteristic of the permanent telang flower kombucha jelly candy preparation in table 1 in this study has met the SNI 3547.2-2008 quality standard. The ideal maximum ash content in jelly type soft confectionery is around 3%. One of the inorganic or mineral residue substances present in a material is known as ash. High ash content ideally correlates positively with the mineral content of a material according to studies Amelia *et al*., (2021) (32).

The ash content of the formulation and preparation of telang flower kombucha jelly candy in this study came from gelatin as an excipient or additional ingredient. Gelatin contains several mineral components such as iron, lead, zinc, calcium and potassium. The results of the research on the ash content of telang flower kombucha jelly preparations in this study were 0.35%. The results of this research are in line with the results of research conducted by Aini *et al*., (2024) (33) where the preparation of jelly candy made from the active ingredient kombucha soursop leaves produced an ash content of 0.34% and the results of the research regarding the ash content met the SNI 354.72-2008 quality standard (1). The results of previous research conducted by Rezaldi *et al*., (2024) (63).

One component that plays an important role in food products which has the potential to influence the texture, appearance and shelf life is the water content and its levels. The results of observations of the water content in the telang flower kombucha jelly preparation based on those presented in table 1 were 16.25%. These results have proven that jelly candy with the active ingredient kombucha telang flower meets the quality standards of SNI 3547. 2. 2008 with an ideal maximum water content of 20% (1).

The results of this research showed that the water content produced in the telang flower kombucha jelly preparation was 16.25%, which was included in the semi-wet category. The results of this research are also in line with the results of research conducted by Aini *et al*., (2024) where the results of his research have proven that the water content produced by jelly candy preparations made from the active ingredient of soursop leaf kombucha is 16.22%, so it can have a long shelf life (33). to defend it. This statement is supported by Muchtadi & Sugiono (2013) which states that semi-wet food ideally contains a water content of 10 to 40%. This is of course closely related to water activity where the aw range for semi-wet food is around 0.6 to 0.85 (34). The amount of water is one of the water activities that is freely available for microbial growth. Microbes in general, namely bacteria, will reproduce ideally with water activity (aw) exceeding 0.90. This is very positively correlated with the increase in sugar as a food additive.

The purpose of adding sugar to food products is to extend storage life. This is because sugar is hygroscopic which has the potential to reduce the aw of a food product (34). Ideally long-lasting food can occur because the water content decreases to a certain limit. This indicates that increased water content can facilitate the growth of microorganisms which have the potential to shorten the shelf life of food or damage food products (35). The water content of jelly candy can be influenced by several factors including the drying process, added sugar and gelatin. The drying process using an oven aims to reduce the water content quickly. The water content decreases rapidly due to the drying process being longer or longer. Drying using an oven at a temperature of 60^{o}C aims to prevent damage to secondary metabolite compounds, while the water can still evaporate slowly. Increasing sugar in the form of sucrose aims to reduce the water content in a food. This is because sugar can bind water (36). One of the gelling agents that is predominantly used in the food and pharmaceutical industries is gelatin, especially for making jelly candy preparations. Gelatin is used as a chewing agent and functions to increase the elasticity, consistency and stability of a jelly candy preparation (37). The more gelatin used, the more water will bind to the gelatin gel micelles (38).

The result of using telang flower kombucha jelly candy was 4.57. pH is one of the parameters that can influence gel synthesis and the sugar crystallization process (30). The optimal and ideal pH according to Mariana et al., (2023) is 4 to 6 (39) The main ingredient in making jelly candy in this research is butterfly pea flower kombucha where in the fermentation process of butterfly pea flower kombucha the sucrose produced by the two microorganisms is in the form of organic acids which tend to reduce degree of acidity (40).

One of the organoleptic observations in preparing jelly candy with the active ingredient telang flower kombucha is taste. Organoleptic testing can attract consumer interest or attention in selecting food products. The taste is formed due to the combination of food ingredients used in the processing process (30). Telang flower kombucha jelly candy obtained a higher organoleptic value, especially taste, when compared to jelly candy without the active ingredient Telang flower kombucha with an average of 6.2 as shown in Figure 1 above. This is because kombucha produces a fresh sour taste and produces variations to produce a new experience for jelly candy consumers when consuming it.

Organoleptic (Taste) Testing of Telang Flower Kombucha Jelly Candy Preparations

One of the organoleptic observations in candy preparations with the active ingredient telang flower kombucha is texture. Texture is the most important parameter in a candy preparation in determining buyers' interest in the food product they consume. The results of this research have proven that the texture value of jelly candy with the active ingredient telang flower kombucha listed in Figure 1 above has a higher favorability value when compared to jelly candy preparations without the active ingredient telang flower kombucha. This is due to a decrease in sugar content when making jelly candy preparations. This is because the sugar in the jelly candy produced by kombucha itself already contains a certain amount of sugar when it is produced, so that if there is additional sugar content in making jelly candy it can cause violence to the candy preparation which can reduce the interest of consumers as buyers.

The higher the sugar content, the stronger the jelly consistency in forming the texture. Excessive use of sugar can also result in the texture of the jelly candy becoming increasingly hardened due to the crystallization process (41). The telang flower kombucha jelly candy preparation is produced in a chewy form because when drying in the oven the time is reduced so that it does not produce a preparation that is too dry and maintains its elastic quality (42).

One of the organoleptic observations in candy preparations with the active ingredient telang flower kombucha is the aroma. The aroma produced by the telang flower kombucha jelly candy preparation has a higher favorability value when compared to the jelly candy preparation without the active ingredient telang flower kombucha jelly with an average value of 6.5 in figure 1 listed above. This is because kombucha produces a sour aroma that comes from organic acids resulting from fermented

sugar in the form of sucrose. This aroma can cover the fishy aroma that comes from gelatin so that it can increase the panelists' liking for the jelly candy product produced. This is because according to Prihatiningsih et al., (2014), gelatin products generally have an unpleasant fishy aroma or odor due to the process of breaking down urea into ammonia (43).

Biochemical Characteristics of the Formulation and Preparation of Jelly Candy Made from the Active Ingredient of Telang Flower Kombucha Through Antioxidant Testing.

Figure 2 above has been proven that the higher the concentration of telang flower kombucha jelly candy, the greater the ability to ward off DPPH free radicals. The results of this research have also proven that there is a positive correlation based on the two equations y = 0.586x + 12.874 with the resulting regression coefficient or R2 being 0.889. The results of this research are based on the resulting IC50 value of 73.878 µg/mL. The research results obtained prove that jelly candy preparations made from the active ingredient telang flower kombucha are one of the food biotechnology products that have biochemical characteristics through testing for antioxidant activity in the strong category. The theory supported in the results of this research refers to Cruz et al., (2019) (44) where the theory reveals that the concentration to ward off free radicals is 50%, so from the results of this research it has been proven that jelly candy preparations made from the active ingredient kombucha butterfly pea flower are classified as a source of antioxidants in the category strong (45). The results of this research have also been supported by the results of research conducted by Aini et al., (2024) where a preparation of jelly candy made from the active ingredient of soursop leaf kombucha produced activity as a source of antioxidants in the strong category, namely 62,848 µg/mL (33).

The presence of antioxidant activity in the results of this study is because jelly candy with the active ingredient kombucha telang flower contains secondary metabolite compounds in the form of phenolics consisting of quercetin and galamic acid. Phenolic compounds such as quercetin and gallic acid have been widely proven to have the potential to ward off free radicals (46); (47). Another ingredient that has the potential to ward off free radicals is hydroxynamic acid, which is a derivative of phenolic acid, flavonoids, and tannins which are often found in butterfly pea flowers (48). The mechanism of action of phenolic compounds as a source of antioxidants is to stop chain reactions and inhibit the synthesis of free radicals such as superoxide ions, singlet oxygen, hydroxyl radicals and hydrogen peroxide (49). The results of this research have also proven that telang flower kombucha jelly candy has the potential to be a healthy food biotechnology product because it has the potential to be a source of strong antioxidants. The results of this study are also quite recommended for testing other variables towards antidiabetes both in silico (50; 51), in vitro (52;53) and in vivo (54; 55; 56; 57;58; 60).

Conclusion

The research results have concluded that food and pharmaceutical biotechnology products in the form of formulations and preparations of jelly candy with the active ingredient kombucha butterfly pea flower have biochemical characteristics as a source of antioxidants based on an IC50 of 62.848 µg/mL in the strong category.

Conflicts of Interest: This research does not contain any elements of conflict of interest that are unprofessional as academics.

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