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ORIGINAL RESEARCH

Immunocandies: Formulation, Characterization and Antioxidant Activity of Pegagan (Centella asiatica (L.) Urb) Herbal Hard Candy

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ABSTRACT

Pegagan is one of the plants that has antioxidant properties. Everyone very much needs the benefits of antioxidants because they can prevent cell damage due to the bad effects of free radicals. Formulating pegagan in the form of candy is one of the efforts so that the antioxidant effects of pegagan can always be obtained easily because candy is one of the snacks that everyone likes and can be easily obtained anywhere. The formulation of hard candy is made with variations in the concentration of pegagan herb juice (0%, 1%, 5%, 10%, and 15%), tested for physical characterization with organoleptic tests, water content, weight uniformity and stability and antioxidant activity with the DPPH method. The results obtained on hard candy with concentrations of 0%, 1%, 5%, 10%, and 15% have a sweet and slightly bitter taste (typical of pegagan) at a concentration of 5%-15%, have a distinctive aroma of weak sugar, and water content respectively 0.72%, 0.77%, 0.84%, 0.8%, 0.89%, 0.95%, and are stable at a storage temperature of 8°C-15°C with closed packaging, have good weight uniformity, and IC50 values respectively 32552.69 µg/mL, 12992.589 µg/mL, 12077.09 µg/mL 9669.15 µg/mL, 9484.519 µg/mL From the data obtained, it shows that pegagan herb juice can be formulated into candy, hard up to a concentration of 15%, has good physical characteristics, and inactive antioxidant activity.

Keywords: Pegagan, hard candies, characteristics, antioxidants, imuno candies

Introduction

Pegagan may be a stemless herbaceous plant, with brief rhizomes and inching stolons. Pegagan contains glycosides, saponins, asiaticoside, madecacoside, asiatic corrosive and madexatic corrosive (BPOM, 2016). The antioxidant action of the Pegagan herb (Wientarsih et al., 2013) makes this plant wealthy in benefits so that individuals broadly utilize it for treatment, one of which is progressing the resistant framework (Sutardi, 2017). The antioxidant action of the pegagan herb is commonly utilized in pharmaceutical arrangements, to be specific as foaming granules, sunscreen creams (Rahmah et al., 2023), and hand and body salves (Yahya & Nurrosyidah, 2020). Separated from being an antioxidant, pegagan herb is additionally utilized as an antibacterial for Propionibacterium acnes (Sukirawati & Syafitri Khouw, 2023) and Mycobacterium tuberculosis microbes (Ilyas et al., 2016), used moreover as antidyslipidemia, bringing down blood weight (Maulina Rifkiyatul Islami, 2023) and still numerous other benefits. Concurring to an investigation conducted by Yunita and Sari in 2022, the ethyl acetic acid derivation and n-hexane fractions are non-toxic (Yunita et al., 2020).

Cancer prevention agents have an imperative part in well-being since cancer prevention agents can inhibit oxidation responses caused by free radical compounds. The Receptive Oxygen Species (ROS) and free radicals within the body can assault natural molecules such as proteins, lipids, and nucleic acids, resulting in tissue harm (Phaniendra et al., 2015). Oxidative push also plays a part in atherosclerosis, joint pain, diabetes, cancer, untimely maturing, and aggravation. Cancer prevention agents are required to avoid oxidative harm by free radicals (Hossain et al., 2013).

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Department of Pharmacy, Faculty of Pharmacy, Universitas Muslim Nusantara Al-Washliyah, Medan, Indonesia Email: rafitayuniarti@umnaw.ac.id Pegagan which may be a normal antioxidant contains flavonoids that can hinder oxidation responses, the IC50 esteem of ethanol extricate is 78.20 ppm (Yahya & Nurrosyidah, 2020), the antioxidant control of pegagan herb is proportionate to 2,412.9 mg of vitamin C/100g (Saputri et al., 2015). The asiaticoside compound in gotu kola can move forward apprehensive and circulatory disarranges due to the nearness of free radicals within the body (Alaiya et al., 2015).

It is trusted that the benefits of pegagan as an antioxidant can be felt by everybody so that it can make strides their wellbeing status. But this trust is still troublesome to get since the pegagan plant has not been developed broadly, as it were in certain regions and for certain ranges such as Medan, North Sumatra, the pegagan plant, which is known as pegagan, has not however been developed, but this plant lives wild in rice areas or cultivating ranges. This earthbound pegagan plant flourishes in cool and muggy ranges with adequate sustenance. For the reasons mentioned above, the pegagan plant is made into a item that's prepared for utilization and can be effectively gotten anyplace and is broadly preferred. One item that can be found everywhere and is preferred by numerous individuals is difficult sweet. Difficult sweet has the include of being wonderful to eat since it tastes sweet, contains tall sugar so it is simple to process and gives a source of vitality (Koswara, 2009).

Hard candy contains sucrose or what is frequently called granulated sugar and other sugars such as glucose, fructose, and sugar liquor, in high levels so that it gives a sweet taste and is long-lasting in capacity, so it is secure for well-being since there's no ought to include counterfeit sweeteners and additives which can be perilous. Wellbeing (Koswara, 2009). The generation of hard candy items from pegagan herb juice is anticipated to have an antioxidant movement like other homegrown items. The essential equation for the pegagan home-grown extricate hard candy utilized is the finest equation delivered from an investigation by Hutabarat, et al. in 2022, specifically utilizing sucrose and fructose in a ratio of 1:1, but in this ponder, the fixings utilized were sucrose and glucose syrup since the essential fixings of sweet are for the most part glucose syrup other than sucrose (Koswara, 2009) without altering it with fructose (Hutabarat, MR. et al., 2022).

In an investigation conducted by MR Hutabarat, et al (2022), the fabricate of pegagan home-grown juice sweet had a concentration of 3% and had great characteristics, to be specific organoleptic and water substance that met SNI prerequisites and had a uniform weight (deviation < 5%). In this inquiry, the concentration of pegagn herb juice was utilized to get the ideal characteristics of pegagan herb juice hard candy. This pegagan home-grown juice hard candy has too been socialized to the community in Sidomulyo Town, Biru-Biru District in Community Service exercises supported by the Service of Instruction and Culture, Investigate, Innovation and Higher Instruction in 2022 (Yuniarti et al., 2022)

One of the antioxidant movement test strategies that are widely used is the DPPH (1,1-Diphenyl-2-picrylhydrazyl) method. The points of interest of the DPPH method compared to other strategies are that it is quick, straightforward and as it were requires a little test (Muflihunna & Muhammad Sarif, 2015).

Based on the depiction over, this inquiry was carried out with the point of testing the antioxidant movement of pegagan (*Centella asiatica* L.) homegrown extricate hard candy utilizing the DPPH (Diphenylpicrylhydrazyl) strategy which is communicated by the IC50 esteem.

Materials and Methode

Materials and Tools

The materials used in this study are Pegagan, sucrose, sugar syrup, methanol, diphenyl picrylhydrazyl (DPPH), and the tools are blender (Philips), porcelain cup (Gratec), stirring rod (Iwaki), water bath (Memmert), heater (Cimarec Thermo), thermometer 0-100°C (pyrex), volumetric flask (pyrex), UV-Visible spectrophotometer (Thermo scientific evolution 201).

Method

1. Pegagan Herbal Juice Preparation

The Pegagan herb is washed, drained, and mashed with a blender then filtered to get the juice, then the juice from the pegagan herb is evaporated at a temperature of ± 45 °C until only one third remains. The process of making pegagan herbal juice can be seen in figure 1.

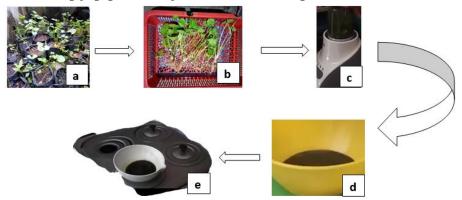


Figure 1. Process of Making Pegagan Herbal Juice

Information:

- a. Pegagan plant (Centella asiatica L. Urb)
- b. Pegagan herbs that have been washed clean and drained
- c. Process of refining pegagan herb
- d. Pegagan herbal juice
- e. The process of evaporating the juice of pegagan herb

2. Hard Candy from Pegagan Herb Juice Preparation

Pegagan herbal juice hard candy is made with several concentration levels of pegagan herbal juice (0%, 1%, 5%, 10%, 15%). This variation was made to obtain a formula that has the best antioxidant activity. The formulation of pegagan herbal juice hard candy (Immuno candies) can be seen in Table 1 below.

Table 1. Formula table for pegagan herbal juice hard candy

			0.0.		<i>-</i>	
Matarial	Amount of ingredients (gram)					
Material	F0	F1	F2	F3	F4	
Pegagan juice	0	1	5	10	15	
Sucrosa	50	50	50	50	50	
Glucose syrup	50	50	50	50	50	
Water	20	20	20	20	20	

Information:

- F0: Pegagan juice hard candy 0% (hard candy blank)
- F1: Pegagan juice hard candy 1%.
- F2: Pegagan juice hard candy 5%.
- F3: Pegagan juice hard candy 10%.
- F4: Pegagan juice hard candy 15%.

Sucrose is added with glucose syrup (1:1), then heated over a water bath at a temperature of 60°C while stirring until it dissolves, then the solution is placed on a heater while continuing to stir until the temperature reaches 150°C, and the temperature is lowered to below 60°C, then the herbal juice of pegagan is added, stir until it thickens and crystal threads form. Pour the solution into a mold and leave it to harden, remove it from the mold, wrap it in plastic and package it. A picture of pegagan herbal juice hard candy (immuno candies) can be seen in the picture 2.



Figure 2. Image of pegagan herbal juice hard candy (imuno candies)

Determination of physical characteristics

1. Organoleptic test

a. Smell test

Five (5) pieces of hard candy are placed on a dry and clean watch glass. Smell the hard candy to find out the smell. Carry out the work by a minimum of three panelists or 1 (one) expert.

b. Taste test

One piece of hard candy is taste tested with the tongue, carried out by a minimum of 3 (three) panelists or 1 (one) exper

2. Water of content

The cup and lid are heated in an oven at a temperature of $100^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for approximately one hour, then cooled in a desiccator and then weighed (the cup and lid) (W₀). Put 5 (five) grams of hard candy into the cup then cover and weigh (W₁). The cup containing hard candy is heated open by placing the cup lid next to the cup in the oven at a temperature of $100^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 3 hours (3 hours after the oven temperature is 100°C). Cover the crucible while it is still in the oven, transfer it immediately to a desiccator and cool, then weigh. Warm-up again and do the same thing until the change in weight between warm-ups has an interval of $\leq 2\text{mg}$ (W₂). Do two repetitions and calculate the water content.

$$water\ of\ content = \frac{w_1 - w_2}{w_1 - w_0}$$

Note:

 W_0 = is the weight of the empty cup and lid.

 W_1 = is the weight of the cup lid and sample before drying

 W_2 = is the weight of the cup lid and sample after drying.

3. Stability Test

The steadiness test was carried out by setting hard candy in a holder and putting away it for 2 weeks at cool temperature (8° C - 15° C) and room temperature (15° C - 30° C) with the bundling open and closed.

4. Testing The Antioxidant Activity of Pegagan Herbal Juice Hard Candy

a. Preparation of DPPH Standard stock solution

10 mg of DPPH was weighed and put into a 50 ml volumetric flask then dissolved with methanol and filled with methanol to the limit mark until a DPPH solution was obtained with a concentration of 200 ppm (Molyneux & Associates, 2004).

b. Preparation of Blank Solution

Pipette 1 (one) ml of DPPH standard arrangement with a concentration of 200 ppm at that point put it into a 5 ml measuring jar and include methanol to the restrain check (concentration 40 ppm). The arrangement is put away in a put secured from light (Molyneux, 2004).

c. Determination of the Maximum Wavelength of DPPH

1 ml of DPPH solution with a concentration of 200 ppm was pipetted, then put into a 5 ml measuring flask and added with methanol to the limit mark (40 ppm concentration), then the absorption of the solution was measured at a wavelength of 400-800 nm using a UV-Visible spectrophotometer to obtain the length maximum DPPH wave (Molyneux & Associates, 2004)

d. Determination of Operating Time (Working Time)

1 ml of DPPH solution with a concentration of 200 ppm was pipetted into a 5 ml measuring flask, and added with methanol to the limit mark (40 ppm concentration). Then the absorbance of the solution was measured every minute at the maximum wavelength for 30 minutes until a stable absorbance was obtained (Molyneux & Associates, 2004).

e. Preparation of sample solution

- 1. Pegagan herb juice: Fresh pegagan herb is blended and squeezed using a planel cloth. Take 2.5 g of pegagan herbal juice and add distilled water to 250 ml (concentration 10,000 ppm).
- 2. Pegagan herb juice hard candy: Weigh 2 g of hard candy, dissolve it in a little water in a beaker glass, then put it in a 100 ml volumetric flask and dilute it with water to the limit mark (concentration 20,000 ppm).

3.

f. DPPH Absorbance Estimation After Including Test Arrangement

- 1. Measurement of the absorbance of pegagan herbal juice was carried out at concentrations of 100, 150, 200, 250 and 300 μ g/mL, while for pegagan herbal juice hard candy it was carried out at concentrations of 6000, 8000, 10000, 12000 and 14000 μ g/mL.
- 2. Pegagan herb juice: Pipette pegagan herb juice (concentration 500 ppm) each as much as 1; 1.5 ml; 2 ml; 2.5 ml; and 3 ml into a 5 ml volumetric flask. Into each volumetric flask was added 1 ml of DPPH solution with a concentration of 200 ppm then the volume was made up with methanol to the mark line, then left in a dark place. The absorbance was measured at operating time using the maximum wavelength. This was repeated three times.
- 3. Pipette the sample solution (concentration 20,000 ppm) each as much as 1.5 ml; 2 ml; 2.5 ml; 3 ml and 3.5 ml, put into a 5 ml volumetric flask. Into each volumetric flask was added 1 ml of DPPH solution with a concentration of 200 ppm then the volume was made sufficient with methanol to the mark line, then left in a dark place. The absorbance was measured at operating time using the maximum wavelength. This was repeated three times.

g. Determination of Damping Percentage (Data Analysis)

The capacity of antioxidant action was measured through a diminish within the assimilation (absorbance) of the DPPH arrangement (submersion within the purple color of DPPH) due to the expansion of the test arrangement. The retention esteem (absorbance) from the estimation of the DPPH arrangement sometime recently and after including the test arrangement partitioned by the absorbance of the DPPH arrangement estimation sometime recently including the test is calculated as (% weakening) with the taking after equation:

Inhibition (%) =
$$\frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

Information:

A control : DPPH absorbance without sample

A Sample : DPPH absorbance with additional sample

Next, the results of the percent dampening obtained are continued by calculating the linear equation and the sample concentration (ppm) as the X axis (abscissa) and the percent dampening as the Y axis (coordinates).

So a regression line equation is obtained which can then be used to calculate the ability of the sample as an antioxidant by calculating the 50% inhibitor concentration (IC50) value using the following formula:

$$50 = ax + b$$

Information:

50: antioxidant ability to inhibit 50% of free radical activity

a: Slopes

b: Intercept

x: Concentration (Molyneux & Associates, 2004).

h. Determination of Antioxidant IC50 Values

The IC50 esteem may be a number that appears in the test concentration number in $\mu g/ml$ units which gives a DPPH decrease of 50%, competent of inhibiting/reducing the oxidation handle by 50%. An antioxidant movement esteem of 0% implies it has no antioxidant action, whereas an esteem of 100% means total constriction and the test ought to proceed by weakening the test arrangement to see the movement concentration constraint. The calculation comes about entered into a relapse condition with test concentration ($\mu g/ml$) as the X pivot (abscissa) and the percent decrease esteem (antioxidant) as the Y pivot (arrange).

Result and Discussion

1. Results of Making Hard Candy from Pegagan Herb Juice

The pegagan herb juice that will be formulated into hard candy is evaporated first to reduce the amount of water contained in the hard candy so that it does not take a long time to make pegagan herb juice hard candy and to reduce the amount of water content in the candy, because too much water can cause the candy to become soft and easily overgrown with mold or bacteria. The temperature used to evaporate the water in the pegagan herb juice must also be below 60°C so as not to damage the substances contained in the pegagan herb juice, especially flavonoids. Flavonoids are one of the secondary metabolites that are effective as antioxidants.

Making hard candy from pegagan herb juice produces sweetness with a powerless yellow to blackish-yellow color (F0-F4), the higher the concentration of pegagan herb juice utilized, the more seriously the color created. Testing the smell of hard candy with pegagan herb extricate created a normal sugar smell whereas testing the taste, the higher the concentration of pegagan juice utilized, the more bitter the hard candy tasted. Testing the surface of hard candy with pegagan home-grown extricate encompasses a hard candy surface, isn't sticky within the mouth, and is delicate (simple to nibble).

2. Water of Content Test Results

Pegagan herbal juice hard candy has a water content of 0.72% - 0.95% (F0-F4). Water content greatly influences the quality of food because it can affect the color, texture, and taste, water will also affect microbial contamination and shelf life. The resulting water content still meets SNI requirements, namely below 3.5%. The results of the water content test can be seen in Table 2 below

Table 2. Water of Content Test Results for Pegagan Herbal juice Hard Candy

Water level (SNI)	F0	F1	F2	F3	F4
Maximum 3,5%	0,72%	0,77%	0,84%	0,89%	0,95%

3. Stability Test Results

Stability tests were carried out to obtain suitable storage conditions for pegagan herbal juice hard candy. Pegagan herbal juice hard candy has good stability if stored closed (in packaging) at cool temperatures (8-15°C) or room temperature (25-30°C). However, it will melt (unstable) if stored in open packaging at either cool or room temperature. This is because the hard candy from pegagan herb juice is

hygroscopic so it easily absorbs water. This hygroscopic property is because pegagan hard candy uses sucrose which has hygroscopic properties.

4. Results of Antioxidant Activity Tests on Pegagan Herb Juice and Pegagan Herb Juice Hard Candy

Testing the antioxidant activity of pegagan herb juice and pegagan herb hard candy was carried out using the DPPH method at a wavelength of 515 nm because at this wavelength DPPH provides maximum absorbance and provides stable absorbance from the 23rd to the 25th minute.

The results of determining the antioxidant activity of pegagan herb juice and pegagan herb juice hard candy can be seen in the following table 3.

Table 3. IC50 Values and Antioxidant Strength Categories

Sample	IC ₅₀ (μg/mL)	Antioxidant
Pegagan herb juice	179,09	Moderate
F0	32552,69	Inactive
F1	12992,589	Inactive
F2	12077,09	Inactive
F3	9669,15	Inactive
F4	9484,519	Inactive

The table above shows the IC 50 value and antioxidant strength category, where pegagan herb juice has an IC 50 value of 179.09 μ g/mL with a medium antioxidant strength category and pegagan herb juice hard candy with a concentration of 0% (F0), 1% (F1), 5% (F2), 10% (F3) and 15% (F4) shows inactive antioxidant strength, but from the IC50 value shown, it can be seen that the greater the concentration of pegagan herb juice used, the smaller the IC 50 value produced. This shows that if the concentration of pegagan herb extract used is greater, the antioxidant activity will increase. The IC 50 value is inversely proportional to antioxidant activity. The IC 50 value is the ability of a compound to inhibit free radical activity (oxidation) by 50%. The smaller the IC 50 value, the stronger the antioxidant activity.

In this study, pegagan herb juice was used up to 15% and produced inactive antioxidant activity, this is likely because the concentration has not yet reached the right concentration to produce antioxidant activity, because the IC50 value produced decreased with increasing concentration of pegagan herb juice.

In another study conducted by Oktaviani and Rahayuni (2014), it was stated that there was an effect of adding sugar on the antioxidant activity carried out on berry juice. From this study, it was shown that the antioxidant activity of berry juice with the addition of 20g/100ml of sugar decreased by 22.14% from the antioxidant activity of control berry juice (without added sugar). This is in line with what happened in hard candy pegagan herb extract which uses sucrose as the basic ingredient in making hard candy pegagan herb juice, where the antioxidant activity of pegagan herb juice decreased from the moderate category in pegagan herb juice to inactive in hard candy pegagan herb juice.

Conclusion

Pegagan hard candy can be formulated from pegagan herb juice with a concentration of 1% - 15% with good characteristics, namely having a typical sugar color and smell (normal), while for the taste at a concentration of 10% the candy tastes slightly bitter and at a concentration of 15% the candy already tastes bitter. Pegagan herb juice hard candy has a water content of 0.72-0.95% and is stable when stored up to a temperature of 30°C in a closed package. Pegagan herb extract has antioxidant activity in the moderate category and Pegagan herb juice hard candy to a concentration of 15% has inactive antioxidant activity but with increasing concentration of pegagan juice used the IC50 value also increases. This shows that there is an effect of the concentration of pegagan juice used with antioxidant activity (IC50 value)

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