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Antioxidant Activities of *Ipomoea carnea* Jacq Extract Serum

Rinandita Febri Susanti*, Anna Fitriawati, Bangkit Riska

Department of Pharmacy, Faculty of Health Sciences, Duta Bangsa University, Surakarta, 57154, Central Java, Indonesia.

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*Coresponding Author :
rinandita1802@gmail.com

Abstract

An antioxidant is a compound that can neutralize free radicals by donating electrons to it. Antioxidant compounds can prevent damage from free radicals in normal cells, proteins and fats. A newly developed form of cosmetic preparation is a serum containing natural antioxidant. This study was an experimental laboratory research. The purpose of this study were to find out the good formulation, to make good quality standards for the physical evaluation of serum preparations and to identifiy of antioxidants activity categories in the *Ipomoea carnea* Jacq. leaves extract serum (*Ipomoea carnea* Jacq). *I. carnea* macerated by ethanol 96%. DPPH ((1,1- Diphenyl-2-Picrylhydrazyl) was a simple and fast method where it can be inhibited by moderate antioxidant on 139,71 ppm. The resulting formula (0) 360 ppm was obtained by having no antioxidant activity, formula I was obtained by the IC_{50} value of 181,15 ppm of weak activity, formula II was obtained by the IC_{50} value of 129,45 ppm moderate antioxidant activity and formula III II was obtained by the IC_{50} value of 122,01 ppm was moderate antioxidant activity. In all four formulations they had moderate antioxidant activity and had a slightly viscoun texture, with green tea aroma, serum pH had met SNI standards and didn’tt show any irritations to the skin.

INTRODUCTION

Excessive exposure to ultraviolet (UV) rays on facial skin can cause skin damage. The sun can produce UV rays such as UVA and UVB. Excessive UVB exposure is the main cause of two different types of skin cancer, namely squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). UVA penetrates into the skin and damages melanocytes and weakens the body's defense system which can develop into the third type of skin cancer, namely melanoma (Jones, 2012). Antioxidant compounds can prevent damage caused by free radicals to normal cells, proteins and fats (Apriani, 2020).

Free radicals are molecules or compounds that can stand alone and contain unpaired electrons, so they are reactive and unstable (Andriani, 2020). Antioxidants are compounds that can neutralize free radicals by donating electrons to them (Andriani, 2020). Antioxidants are compounds that can neutralize and fight free radicals by inhibiting the occurrence of oxidants in body cells thereby reducing oxidation and cell damage (Vifta *et al*., 2020). This is because the side effects are relatively small or even non-existent when compared to drugs derived from synthetic ingredients.

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Many types of plants have been researched and proven to have efficacious effects as medicinal plants and are used as alternative treatments. One of them is *Ipomoea carnea* Jacq (Widyaningrum and Ningrum, 2021). *I. carnea* Jacq has active compounds in the phenolic group which are found mostly in the flowers and there are small amounts of phenolic compounds in the stems. The flavonoid content, especially catechol and quercetin, is distributed in flowers, leaves and stems. Phenolic compounds can be effective as antioxidants and as immunomodulators, while flavonoids have antibacterial, anti-inflammatory, anti-allergic and anti-cancer properties (Widyaningrum and Ningrum, 2021). The research of Abriyani *et al*., 2023 stated that the ethanol extract of *I. carnea* Jacq leaves contains very strong antioxidant levels. In the very strong category, namely <50µg/ml.

Facial care is one of the important activities that many people do, especially by women. Facial care can make a person more confident and keep the skin healthier, more well-groomed and always radiating freshness. One of the steps for facial care is to use skincare products such as serum (Aprilia *et al*., 2022). Serum is a topical preparation that generally has a slightly thick texture with a semi-transparent to transparent color. The advantage of serum is that it provides a more pleasant effect because its viscosity is lower compared to other topical preparations and contains active substances in higher concentrations and spreads more easily on the skin surface (Fatmawati *et al*., 2014) and can provide a faster effect on the skin. and provide a feeling of comfort for the user (Aprilia *et al*., 2022). Based on the description above, the researcher intends to determine the formulation of serum preparations from *I. carnea* leaf extract as an antioxidant using the DPPH method.

MATERIALS AND METHODS

Processing of *Ipomoea carnea* Jacq Ethanol Extract

500 grams of *I. carnea* leaf powder was macerated with 2.5 liters of 96% ethanol for 3 days, stirring every day. After 3 days, it was filtered with flannel cloth, then the filtrate was evaporated. The filtrate was evaporated using a rotary evaporator and then aired until the solvent evaporates and a thick filtrate is obtained. The thick extract was placed in a desiccator for further drying, and the yield of the extract obtained was calculated.

Phytochemical Screening of Simplicia and Extract

Phytochemical screening was carried out on both simplicia and thick extracts to identify the compounds contained in *I. carnea* plants. Tests carried out using the tube test method include preliminary tests to identify chromophore groups, alkaloid tests with dragendrof and Meyer reagents, tests for flavonoids, polyphenols, tannins, saponins, steroids and terpenoids.

Formulation of *Ipomoea carnea* Extract Serum Preparations and Physical Evaluation

Serum formulation Proccess

Prepare tools and weigh all materials to be used. First, develop the HPMC with hot distilled water in a hot mortar until a thick mass was formed. Second, add propylene glycol, methyl paraben and propyl paraben to the mortar, stir until homogeneous. Then mix the methyl paraben, propyl paraben and propylene glycol solutions into a mortar, then stir until homogeneous to form a serum mass. The serum base that has been formed is then added to the active substance, namely forest kale leaf extract, into the mortar and then crushed until everything is homogeneous. The final step is to put the serum into the serum container.

Table 1. Base Serum Formulation

Source	Contentration (%)				Function
	F0	F1	F2	F3	
HPMC	-	3	5	7	Gelling Agent
Propilenglikol	-	15	15	15	Humektan
Metyl paraben	-	0,075	0,075	0,075	Preservatif
Propil Paraben	-	0,025	0,025	0,025	Preservatif
Aquadest	-	150	150	150	Solvent

Table 2. Serum Formula Modification

Source	Contentration (g)				Function
	F0	F1	F2	F3	
Extract *I. carnea*	0	2	4	6	
HPMC	1	1	1	1	Gelling Agent
Propilen glikol	7,5	7,5	7,5	7,5	Humektan
Metyl paraben	0,01	0,01	0,01	0,01	Preservatif
Propil Paraben	0,09	0,09	0,09	0,09	Preservatif

Physical Evaluation of Serum Preparations

a. The Organoleptic Test
   Organoleptic testing includes checking the consistency, color and aroma of the serum to determine the physical condition of the serum (Naibaho *et al*., 2013).

b. The Homogeneity Test
   Homogeneity testing is carried out by placing 0.1 gram of the preparation on transparent glass and then leveling it. Serum preparations are

declared homogeneous if there are no coarse grains (Naibaho *et al*., 2013).

c. The Acidity Test
This measurement is carried out using a pH meter. A total of 1 g of the preparation was dissolved in 10 ml of water at room temperature. The electrode that is in contact with the surface of the solution is left for 1 minute (Naibaho *et al*., 2013). In the literature, the pH of facial skin is 4.5-6.5 (Mardhiani *et al*., 2017).

d. The Spreadability Test
This test is carried out by placing 0.5 grams of serum in the middle of a transparent glass. Another transparent glass was placed on top and then a weight of 150 grams was given. After leaving it for 1 minute, the diameter of the spread was recorded (Naibaho *et al*., 2013).

e. The Viscosity Test
Serum viscosity testing uses a Brookfield Viscometer. Place the serum in the container then lower the spindle until it is submerged. The speed and spindle number used are adjusted in such a way that the needle on the tool can take readings on a scale of 0 to 100 (Naibaho *et al*., 2013). The viscosity value for facial serum is 800-3,000 Cp (Septiyanti *et al*., 2019).

f. The Irritation Test
Irritation testing is carried out by placing the preparation openly on human skin. The serum preparation is applied to the inner upper arm with a diameter of 2 cm. After 5 hours, symptoms were observed such as redness, itching and swelling of the skin.

g. The Hedonic Test
Hedonic test on texture, aroma and color parameters. This test was to determine the respondent's level of preference for 3 forest kale leaf extract serum formulas (Suena *et al*., 2020).

Antioxidant Activity of Serum Preparations

1. Measurement of serum absorbance
Make a stock solution for each preparation with a concentration of 5% by taking 2.5g of serum into a measuring flask and adding 50 ml of methanol p.a. then 0.4 mg, 0.8 mg, 1.2 mg and 1.6 mg of each sample were dissolved in 10 ml methanol and filled to the limit mark. Sample solutions were made with respective concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm. 1 ml of each series was pipetted and 1 ml of DPPH solution was added, then covered using aluminum foil. Next, leave it in a dark place during OT, then absorbance is measured using a UV-Vis spectrophotometer with the wavelength obtained and the percentage of inhibition is calculated using the formula:

$$\%\ Inhibition = \frac{control\ abs - sample\ abs}{control\ abs}\ x\ 100\%$$

2. Preparation of DPPH solution
20 mg DPPH was weighed, then put into a 100 ml measuring flask then dissolved with methanol p.a until the mark on the volumetric flask was homogeneous, so that a concentration of 200 ppm was obtained (Permata *et al*., 2023).
3. Making a Vitamin E Comparator
Weighed 5 mg of technical Vitamin E and dissolved it in 100 ml of methanol until a concentration of 50 ppm was obtained. Make a vitamin E concentration series with a concentration of 50 ppm of 0.4; 0.8; 1.2; 1.6 ml and diluted with methanol to a limit of 10 ml in a measuring flask to obtain a concentration of 2, 4, 6, 8 ppm (µg/ml)
4. Preparation of a test solution for forest kale leaf extract
Weigh 2.5 g of *I. carnea* leaf extract, dissolve in 50 ml methanol in a measuring flask (5%). Then make a concentration series of *I. carnea* leaf extract from a 5% ppm solution pipetted at 0.5; 0.6; 0.7; 0.8; 0.9 ml and diluted with methanol to 10 ml in a volumetric flask to obtain a concentration of 25, 30, 35, 40, 45 ppm (µg/ml)
5. Antioxidant Quantitative Test
   a. Determination of maximum absorption wavelength
   Determination of the wavelength was carried out by taking a DPPH stock solution with a concentration of 200 ppm, taking 1 ml dissolved in 10 ml of methanol p.a (20 ppm) then taking 4 ml and putting it in a cuvette and seeing the absorbance in the absorption length range of 400-600 nm used spectrophotometry (Permata *et al*., 2023).
   b. Determination of Operating Time (OT)
   Measurement of the operating time of the DPPH solution with this concentration was then measured for absorbance at the maximum wavelength every 5 minutes for 60 minutes. Observe the time the solution took to produce a stable absorbance which was used as the operating time (Permata *et al*., 2023).
   c. Measurement of absorbance of DPPH solution
   Take 1 ml of the DPPH stock solution and put it in a 10 ml volumetric flask plus methanol p.a until the limit mark after the OT of the

solution was read for absorption using UV-Vis spectrophotometry at the maximum wavelength. This solution was used as a control solution to test the comparison solution and test solution for *I. carnea* leaf extract

d. Measurement of the absorbance of the Vitamin E reference solution
   Pipette 1 ml of the vitamin E concentration series test solution that had been made and add 1 ml of DPPH solution from the stock solution then add methanol p.a to 10 ml, leave for OT, read the absorbance at the maximum wavelength obtained.
e. Measurement of the absorbance of forest kale leaf extract
   Pipette 1 ml of the test solution for the concentration series of *I. carnea* leaf extract that has been made and add 1 ml of DPPH solution from the mother liquor then add methanol p.a to 10 ml, leave for OT, read the absorption at the maximum wavelength obtained.

Data Analysis

The data obtained in this research was analyzed using descriptive methods. Where this descriptive method described an objective situation, with this descriptive method the data obtained could be presented in the form of tables, graphs or percentages for physical evaluation tests of preparations. The percentage differences for each treatment were analyzed using the SPSS method and statistical tests were carried out using One Way ANOVA. If the data on the percentage value of the physical quality of the preparation (viscosity test, spreadability test, pH test) and % antioxidant inhibition met the requirements for normality and homogeneity, it was stated with a p value <0.05.

RESULTS AND DISCUSSION

Drying of simplicia was carried out, so that the active substances contained in simplicia were not damaged, especially compounds that were not resistant to high heat such as flavonoids (Warnis *et al*., 2020). Apart from that, the drying process was also carried out to reduce the water content in order to inhibit the proliferation of bacteria/microbes which damage the quality of simplicial (Yamin *et al*., 2017). The results of the simplicial drying loss from wet raw to dry simplicia which was ready to be processed are presented (Table 1).

After obtaining dry simplicia which was characterized by dry leaves which were dark green in color and had a distinctive smell and break easily when handled, then grind them using a blender, then sift them using a 40 mesh sieve. This was done to get a fine and homogeneous powder and to avoid obstruction of liquid entry extraction into the cell cavity so that the extraction of active substances was not optimal (if it was too coarse), but if it was too fine, extraction was also equally difficult because the filter couldn't penetrate the spaces between cells. The organoleptic resulted of dried simplicia powder were presented in Table 2.

Table 1. The result of raw simplicial lost drying

Fresh Simplicia Weight (g)	Dry Simplicia (g)	Yield (%)
6000	500	8,33

Table 2. Organoleptical Result of Dry Simplicia Powder

Simplicia	Form	Color	Flavor	Odor
Ipomoea carnea	Soft powder	Dark green	Bitter	Dry leaves

Drying loss measurements in this study were carried out using an oven at a temperature of 105°C for 30 minutes to obtain constant drying loss (Table 3).

The results of drying loss measurements on *I. carnea* Jacq leaf simplicia powder showed a drying loss presentation of 5%. The drying shrinkage value did not exceed the specified requirements, namely <10%. So that standardized simplicia could be used as a medicine that contains constant and accountable levels of active compounds. One of the standardizations for the quality of simplicia powder was the water content test, this was done to ensure that the simplicia powder made was truly dry and not damp (Setiadi *et al*., 2022). The water content of dried simplicia was measured by weighing 2 grams each, then placed in a moisture balance at a temperature of 1050C for 3 minutes until the weight was constant (Syafrida *et al*., 2018). The results of testing the water content of simplicia powder were presented in Table 4.

Table 4 showed that the average water content of simplicia powder was 6.67% ± 0.76. This showed that the water content was <10% as per the requirements regarding the water content of simplicia powder from Materia Medika Indonesia Edition IV. Research by Puspitasary *et al*., (2019) stated that simplicia which was dried using a drying cabinet or oven had a lower water content than if it was dried simply by airing it.

After the drying process, simplicia powder was extracted using 96% ethanol solvent. The 96% ethanol solvent had semipolar properties, this provided an advantage during the active substance withdrawal process, both polar and nonpolar compounds would be completely absorbed (Widyaningrum *et al*., 2021).

Table 3. The result of powder simplicial lost drying

Empty Cup (g)	Powder (g)	Cup and Powder before Heating (g)	Cup and Powder after Heating (g)	Lost drying (%)	Standart (%)
44,80	2	46,80	1,90	5	<10

Table 4. Results of simplicial powder water content

Replication	Powder initial weight (g)	Final Powder Weight (g)	Water Content (%)	Standart (%)
1	2	1,87	6,5	
2	2	1,85	7,5	
3	2	1,88	6,0	<10%
	Average of Water Content ± SD		**6,67 ± 0,76**	

Table 5. Phytochemical screening test of forest kale leaf extract

Assay	Reagen	Color Result	Result
Flavonoid	Zn powder + HCL ➔ red color intensive showed flavonoid (Widyaningrum dan Ningrum, 2021).	Red color intensive	+
Saponin	Shaked as long as 10 second + HCl_{aq} ➔ foam undisappear showed there was a saponin (Widyaningrum dan Ningrum, 2021).	Stable foam formation	+
Polifenol	$FeCl_3$ 10% Dark blue or black green showed there was tannin or polyphenols (Widyaningrum dan Ningrum, 2021).	Greenish black or blackish	+
Alkaloid	Mayer reactor was made white precipitate if its positive alkaloids (Widyaningrum dan Ningrum, 2021).	White to yellowish precipitate	+
	Dragendroff reactor would be positive if its formed orange precipitate (Widyaningrum dan Ningrum, 2021).	Brick red precipitate	+

*Note : (+) Contain substances and (-) No substainces contain

Table 6. Organoleptical result of *I. carnea* leaves extract serum

Formulation	Form	Consistency	Color	Smell
F0	Semi solid	A little bit liquid	Light green	Green tea
F1	semi solid	A little bit liquid	Light green	Green tea
F2	Semi solid	A little bit thick	Light green	Green tea
F3	Semi solid	A little bit thick	Light green	Green tea

Table 7. Anova And Post Hoct Result

Component	ANOVA	*Post Hoc* (LSD)
Significancy value	0,002<0,05	Continued by *post hoc*
Formula 0 vs Formula 1	(significantly	0,004<0,05 (significantly different)
Formula 0 vs Formula 2	different)	0,000<0,05(significantly different)
Formula 0 vs Formula 3		0,008<0,05 (significantly different)
Formula 1 vs Formula 2		0,052>0,05 (no significantly different)
Formula 1 vs Formula 3		0,613>0,05 (no significantly different)
Formula 2 vs Formula 3		0,023<0,05(significantly different)

Tabel 8. ANOVA and post hoc result

Component	ANOVA	*Post Hoc* (LSD)
Significancy value	0,019<0,05	Continued by *post hoc*
Formula 0 vs Formula 1	(significantly	0,233>0,05 (no significantly different)
Formula 0 vs Formula 2	different)	0,160>0,05(no significantly different)
Formula 0 vs Formula 3		0,003<0,05 (significantly different)
Formula 1 vs Formula 2		0,803>0,05 (no significantly different)
Formula 1 vs Formula 3		0,022<0,05(significantly different)
Formula 2 vs Formula 3		0,033<0,05(significantly different)

Apart from that, the use of semipolar solvents was expected to provide maximum yield results (Widyaningrum et al., 2019). Ethanol solvent was a universal solvent that was more selective than water, microbes and fungi were difficult to grow in it was not toxic, was neutral, could mix with water in all proportions and was easily evaporated (Widyaningrum and Ningrum, 2021).

The process of extracting the active compounds from *I. carnea* Jacq leaves used the maceration method for 3 days, with at least 1 stirring every day. This was done to even out the surface of the powder so that every part of the powder was exposed to the filter solution so that the active compounds could be extracted optimally. Apart from that, it was also to prevent saturation of the filter fluid during the extraction process (Warnis *et al*., 2020). The advantage of using the maceration method for the extraction process was that the method was simple, easy, cheap, did not require special equipment and was suitable for active substances that could not withstand heating (Yamin *et al*., 2017). The following was the percentage yield of *I. carnea* Jacq leaf extract can be seen in Table 5.

Table 5. The Yield of *I. carnea* Jacq

Powder Weight	Extract Weight	Yield (%b/b)	Standart
500	49,67	9,934 %b/b	> 6,6%

The resulting yield was 9.934% w/w, the use of 96% ethanol solvent could provide optimal filtering results, because it attracted more active compounds compared to nonpolar compounds (Syarifah, 2022). The extract yield that was considered good was not less than 6.6%. Organoleptic observations were carried out with the aim of identifying samples including research on color appearance, aroma and taste. Organoleptic parameters in extracts aimed to provide an initial description of simplicia and its extracts by describing aspects of shape, appearance, aroma and taste (Dirjen POM, 2012). The organoleptic test resulted of *I. carnea* Jacq leaves can be seen in Table 6.

Table 9. Organoleptical Test of *I. carnea* Jacq

Observation	Extract
Form	Thick
Odor	Extract aromatic
Color	Dark green
Flavor	Bitter

The free test was carried out to free the extract from ethanol so that a pure extract was obtained without any contamination (Khaira *et al*., 2022). The following identification of the ethanol free test could be seen in Table 7.

Table 10. Free Ethanol of *I. carnea* Jacq

Identification	Prosedure	Result
Free alcohol test	Extrack + + H_2SO_4 (p) + CH_3COOH ➔ Heated	No ester smell

Based on the ethanol-free test identification table above, it showed that *I. carnea* Jacq leaf extract did not contain an ester odor. The resulting extract was free from ethanol solvent, as indicated by the absence of an ester odor when the extract was reacted with sulfuric acid and acetic acid and then heated.

Determination of water content was carried out to determine the water residue after the thickening or drying process. The water content of the extract was measured by weighing 2 grams each, then placed in a moisture balance at a temperature of 1050C for 3 minutes until the weight was constant (Syafrida *et al*., 2018). The results of determining the water content of *I. carnea* Jacq leaf extract were 4.9%. It can be seen in Table 8.

Table 11. Water Content of *I. carnea* Jacq Extract

Extract Weight (g)	Water Content (%b/b)	Standart (%)
2	4,9	<10

Table 8 showed that the water content in macerated *I. carnea* Jacq leaf extract contained practically no water or the water content met the quality requirements of less than <10% water content. Drying loss measurements in this study were carried out using Moisture Balance to obtain constant drying loss. The results of the drying loss calculations carried out can be seen in Table 9.

Table 12. Drying Shrinkage of *I. carnea* Jacq Extract

Extract Weight (g)	Lost Drying (%b/b)	Standart (%)
2 g	6,5 %	<10

The drying loss value obtained from *I. carnea* Jacq leaf extract was 6.5%. This showed that the amount of water content and compounds lost during the drying process was 6.5%. A good requirement for drying loss was less than 10% because drying loss also represented the evaporated water content (Depkes RI., 2008).

The next step was to ensure that the active compounds extracted were as expected by the researchers, namely by carrying out a phytochemical screening test. Usually phytochemical screening tests used the tube test method, this was done to make observations easier using specific reagents (Widyaningrum *et al*., 2019).

The results of the phytochemical screening test for *I. carnea* Jacq leaf extract can be seen in Table 10.
Based on the results of phytochemical screening using the tube test above, the leaf extract of *I. carnea* was positive for containing secondary metabolites in the form of flavonoids, saponins, polyphenols and alkaloids. Likewise, research conducted by (Hasanah *et al*., 2023) used the same method, only with a different extraction technique, namely soxhletation, *I. carnea* leaf extract contains flavonoids, alkaloids, steroids, terpenoids, saponins, tannins. and polyphenols.
Organoleptic testing was carried out to physically determine the serum preparation produced by observing the visible appearance such as consistency, color, aroma and shape (Naibaho *et al*., 2013). The organoleptic test results of serum preparations could be seen in Table 11.
The results of organoleptic testing showed that the four serum preparations produced were semisolid with a slightly runny consistency (in formulas 0 and 1) and in formulas 3 and 4 they were slightly thicker, light green in color and had a rose scent. This was in accordance with the Based on the results of observations made, both serum formula 0; 1; 2; and 3 all appeared homogeneous, because no coarse particles were found in the serum preparation. This could be said that the serum manufacturing process had been carried out well so that it could be ensured that the uniformity of the serum preparations produced by the four formulas was the same (Astuti *et al*., 2017).
The homogeneity test was related to the even mixing of the active substance with the base used, so that every time it was used or applied for therapy the same results would be obtained. Physically, the serum preparations produced also appear to have the same color, this confirms that the preparations produced were homogeneous (Khaira *et al*., 2022). The preparation made must be homogeneous so that there was no risk of causing irritation to the user and distributed evenly when applied.gel dosage form.
Acidity testing or pH testing was carried out to determine whether the pH of the topical preparation that would be used matches the skin's pH or not. If the preparation was too acidic there was a risk of irritating the skin, whereas if the preparation produced was too alkaline it could cause dry and scaly skin (Purnamasari *et al*., 2023). The acidity test results could be seen in Table 12.
Testing the pH of the preparation is expected to be in accordance with the skin pH range, ideally the normal human skin pH is 4.5 – 6.5. Based on SNI number 16-4399-1996, the pH requirements for topical preparations that can still be tolerated for use on the skin are between pH 4 - 8. The pH of the preparations produced in this study ranges from 6 - 7.75, so it could be said that the pH of the serum preparations produced was adequate according to ideal requirements. One of the requirements that cosmetic products must fulfill when used on the skin is that the acidity level must be within the right range. When the pH is too low it can cause the skin to become dry and sensitive, whereas if the pH is too high it can trigger skin inflammation and the appearance of lots of acne. From the result we can said that formula 3 better than the others, even almost all of the formula were good. It's because formula 3 had the biggest than formula 1 and 2, closer to formula 0.

Table 13. Acidity Test of *I. carnea* Leaves Extract Serum

Formulation	pH value $(\overline{X} \pm SD)$	SNI Standart
F0	7,5 ± 0,25	
F1	6,7 ± 0,26	4-8
F2	6,3 ± 0,21	
F3	6,8 ± 0,2	

In the acidity test, statistical testing was carried out using ANOVA to ensure that the four formulas were significantly different or not. Based on the data normality test using the Kolmogorov Smirnov test, it was found that the pH data was normally distributed because the significance value was 0.902 > 0.05. The homogeneity test used the Levine test showed that the pH data was distributed homogeneously, this could be seen from the test results, the significance value was 0.884>0.05 (Dahlan, 2015). The results of ANOVA and post hoc statistical tests could be seen in Table 13.
In the ANOVA test, it could be seen that the four formulas had significant data differences, this could be seen in Table 4.14 where the significance value was 0.002<0.05, which was then continued with a test between variables using a post hoc test (Dahlan, 2015). The test results between variables showed that the pH of formula 1 was not significantly different from formula 2 and formula 3.
The spreadability test is carried out to determine the spread of the preparation when applied to the skin, where the wider the ability to spread the preparation when applied, the more optimal the efficacy of the preparation (Widyaningrum *et al*., 2019). The higher the spreadability of a topical preparation, the easier it was to apply without requiring more pressure to apply. The results of testing the ability to spread serum preparations could be seen in Table 14.
The requirement for good spreadability of serum preparations is if the spread diameter is between 5 - 7 cm (Widyaningrum *et al*., 2019). The results of testing the spreadability of the serum preparations

above (Table 15) showed that the diameter of the four formulas was 4.99 – 6 cm, so it could be said that the spreadability of the preparations was ideal. The serum preparation that was easiest to spread was formula 3 where the diameter obtained was the largest among the others, so it was hoped that it would provide a broad therapeutic effect at the localization of use. After testing the physical properties of each formula, followed by a different test using ANOVA, the following results were obtained.

Table 14. Serum spreadability test result

Formulation	**The average ± Standart of Deviasi**	**Referens** (Widyaningrum et al., 2019)
Formula 0	5,4 cm **±** 0,15	
Formula 1	5,5 cm **±** 0,21	5-7 cm
Formula 2	5,6 cm **±** 0,15	
Formula 3	5,9 cm ± 0,10	

The results of statistical tests show that the four formulas are statistically significantly different using the ANOVA test, but when a post hoc test is carried out between each formula, the data obtained is statistically insignificant. The results of the spreadability of formula 0 are similar to formulas 1 and 2, while formula 1 is also almost the same as formula 2. The spreading ability of the three is almost the same statistically because the significance value is >0.05 (Dahlan, 2015). This is because the basic formula of the base is the same, what is different is the extract content, so it can be said that the extract concentration does not really have an effect on the spreadability of the serum preparation where the concentration ranges between 4% - 8%, if it is more than 10% it will have a significant effect (Andriani, 2020).

The higher the viscosity of a preparation, the greater the viscosity of the preparation. A good serum is a serum with a consistency that is not too thick but also not too liquid (Purnamasari *et al*., 2023). Serum viscosity was measured using *I. carnea* leaf extract using a Brookfield viscometer with spindle number 3 at a speed of 30 rpm. The results of the viscosity test could be seen in Table 16.

Table 15. Viscosity test result of the serum

Formulation	**The average ± Standart of Deviasi**	**Referens** (Septiyan 2019)
F0	868 cPs **±** 2	
F1	957 cPs **±** 1	800 – 3000 cPS
F2	1278 cPs **±** 1	
F3	1547 cPs **±** 1	

The viscosity requirement for gel-based serum preparations is around 800 – 3000cPS (Septiyan, 2019). The serum results in this study met ideal requirements, because it had a viscosity in the range of 800-3000 cPs. Physically, the serum produced was semi-thick, but even so, when applied topically, it spread easily and was light. The ideal viscosity formula was a formula 3, it had the biggest viscosity. The results of the statistical differences between the four formulas are as follows (Table 17).The viscosity test results were in line with the spreadability both statistically and mathematically. This was in accordance with the theory where the inherent ability is in line with the viscosity of a preparation.

Irritation testing is intended to physically determine whether the serum would cause itching when applied to the skin or not. This test is related to detecting the toxicity protection of serum preparations that appear after use (Khaira *et al*., 2022). The irritation test was carried out by applying the serum preparation to the upper arms of 30 panelists. The results of the 5 hour irritation test on the four formulas did not show any signs of irritation in the form of redness and burning itching. This showed that the serum preparation is safe to use.

The hedonic test or preference test was carried out by collecting 30 respondents randomly, then the respondents were asked to assess the physical appearance of the serum preparations made. The assessment of this liking test starts from really like it (5); like (4); somewhat like (3); don't like (2) and really don't like (1). The parameters assessed include aroma, color and texture (Suena *et al*., 2020). The results of the hedonic test were as follows (Table 18).

The results of the liking test show that formula 0 in terms of aroma, texture and color was quite liked by almost all respondents, this was indicated by a mean value of 4 (like). Respondents liked formulas 1 to 3 slightly less because the three serum formulas were green in color and had a distinctive aroma of extract, so they were less liked, while formula 0, because it only contained a base, had a more fragrant smell.

The antioxidant activity test was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The advantages of this method include that it is simple, easy, fast, sensitive and only requires a small sample to evaluate the antioxidant activity of natural product preparations (Hasan *et al*., 2022). If the antioxidants in the extract are reacted with DPPH, they will inhibit DPPH activity, so that the absorption will be seen to decrease (Siswanto *et al*., 2022).

Table 17. ANOVA and post hoc test result of serum

Component	**ANOVA**	***Post Hoc* (LSD)**
Significancy value	0,031<0,05	Continued by *post hoc*
Formula 0 vs Formula 1	(significantly	0,101>0,05 (no significantly different)
Formula 0 vs Formula 2	different)	0,006<0,05(significantly different)
Formula 0 vs Formula 3		0,024<0,05 (significantly different)
Formula 1 vs Formula 2		0,101>0,05 (no significantly different)
Formula 1 vs Formula 3		0,382>0,05(no significantly different)
Formula 2 vs Formula 3		0,382>0,05(no significantly different)

Table 16. Hedonic Test Result

Formulation	**Smell Average Point**	**Texture Average Point**	**Color Average Point**
F0	4	4	4
F1	3	3	3
F2	3	3	3
F3	3	3	3

Table 17. The Result of Serum Inhibition Test

Concreation (ppm)	**Nilai % Inhibition**			
	Formula 0	**Formula 1**	**Formula 2**	**Formula 3**
20	34,62	38,0	35,39	38,71
40	39,38	36,9	39,06	42,31
60	45,05	39,4	40,77	43,96
80	55,68	43,0	43,58	45,12

Table 18. The Linier Equation and IC_{50} value

Treatments	**Equations**	**IC_{50} Value(ppm)**	**IC_{50} Category**
Formula 0 (extract 0%)	Y = 0,34425 + 26,47	360 µg/mL	Very weak weak
Formula 1 (extract 4%)	Y = 0,0875x + 34,95	181,15 µg/mL	weak
Formula 2 (extract 8%)	Y = 0,1314x + 33,13	129,45 µg/mL	moderate
Formula 3 (extract 12%)	Y = 0,1044x + 37,305	122,01 µg/mL	

Table 19. Post Hoc test result by LSD method

Testing Group	**Significance Value**
Vitamin E + Ekstrak *I. carnea* leaves extract	0,054>0,05
Vitamin E + Formula 0	0,000<0,05
Vitamin E + Formula 1	0,000<0,05
Vitamin E + Formula 2	0,000<0,05
Vitamin E + Formula 3	0,000<0,05
I. carnea leaves extract + Formula 0	0,000<0,05
I. carnea leaves extract + Formula 1	0,000<0,05
I. carnea leaves extract + Formula 2	0,000<0,05
I. carnea leaves extract + Formula 3	0,001<0,05
Formula 0 + Formula 1	0,000<0,05
Formula 0 + Formula 2	0,000<0,05
Formula 0 + Formula 3	0,000<0,05
Formula 1 + Formula 2	0,136>0,05
Formula 1 + Formula 3	0,004<0,05
Formula 2 + Formula 3	0,000<0,05

Determination of the maximum wavelength of the DPPH solution was carried out by measuring the absorbance of 200 ppm DPPH stock solution, dissolved using methanol p.a in a 10 ml volumetric flask (20 ppm) which was read in the wave length range of 400-600 nm. Based on the results of these measurements using a UV-Vis Spectrophotometry tool, results were obtained with a maximum wavelength of 515.6 nm, so that these results fall within the range of wavelength values for DPPH which ranges from 515-517 nm.

Operating time (OT) aimed to determine the most stable time for measuring the absorbance of a compound. Determination of OT is necessary to reduce potential errors in measuring antioxidant activity, because several compounds measured in this study form complex compounds with DPPH. OT results can be seen in Table 19.

Table 20. Operating Time

Time (minutes)	Absorbancy
5	0,554
10	0,560
15	0,570
20	0,578
25	0,580
30	0,586
35	0,589
40	0,595
45	0,605
50	0,605
55	0,605
60	0,614

The OT determination in Appendix 1.11 showed the results that compounds that had antioxidant activity had reacted with DPPH radicals perfectly with stable absorbance values starting at the 45th minute, so absorbance measurements were carried out at the 45th minute. In determining the absorbance, the DPPH control showed a result of 0.540 nm. So this value could be tested with a comparison solution and *I. carnea* leaf extract.

The absorbance measurement results were used to obtain the % inhibition value. The % inhibition value was used to find the IC_{50} value to determine the strength of antioxidant activity in the sample being measured. The parameter used to determine the amount of antioxidant ability was the IC_{50} value. This value was the concentration of antioxidant compounds needed to reduce DPPH radicals by 50%, obtained from a linear regression equation which stated the relationship between the concentration of the extract as the x-axis and the percentage of radical capture as the y-axis, so that the smaller the IC_{50} value, the more active the extract was as a radical scavenger DPPH or as an antioxidant (Table 20 and Table 21).

Table 20. The Result of Vitamin E Inhibition Percentage

Concentration (ppm)	Absorbance	% Inhibition
2	0,389	28,7
4	0,383	29,8
6	0,371	32,11
8	0,345	36,81

Table 21. The Result of *I. carnea* Inhibition Percentage

Concentration (ppm)	Absorbance	% Inhibition
25	0,473	13,43
30	0,456	16,48
35	0,449	17,83
40	0,444	18,68
45	0,436	20,15

From the table above, the ability to capture DPPH free radicals from *I. carnea* leaf extract and Vit E could be measured using the IC_{50} parameter. The IC_{50} value was calculated through regression which compares the sample concentration and the average percentage of each concentration (Table 22).

Table 22. The Linier Equation

Treatment	Equation	IC_{50} value (ppm)	IC_{50} Category
Vitamin E	Y= 1,3339x+25,183	18,65 ppm	Very strong
I. carnea leaves extract	Y= 0,3128x + 6,366	139,71 ppm	modearte

From the table above it could be said that *I. carnea* leaf extract had antioxidants in the medium category, so *I. carnea* leaves could be formulated into an antioxidant serum.

Antioxidant testing was carried out on the four serum preparation formulas, this was done to ensure the ability of the serum preparations as antioxidants. The results of the inhibition values could be seen in Table 23.

For the IC_{50} value in the *I. carnea* leaf serum preparation formula, it could be seen in the linear regression equation between the concentration of the test preparation and the average percentage of DPPH from each concentration (Table 24).

Based on the results of antioxidant activity testing, the results obtained were that the *I. carnea* leaf serum preparation formula contained moderate and weak antioxidant activity, because it had an IC_{50} value of 100-150 mg/L for the weak category and 150-200 mg/L for the medium category. Of the three preparations, it could be seen that the best antioxidant potential was found in formula 3 (12%) with the smallest average IC_{50} value, namely 122.01 µg/mL. This was in accordance with the

statement that the smaller the IC_{50} value indicated the higher the antioxidant activity.

Statistical differences were tested using the ANOVA test, which had previously been tested for requirements first. The requirements for the ANOVA test include the normality test with Kolmogorov Smirnof and the homogeneity test with the Levine test. If the data is normally distributed and homogeneous then continue with the ANOVA test and post hoc test. The prerequisite test results for the normality test and homogeneity test showed that the data wasas normally distributed with a p value of 0.858>0.05 and w declared homogeneous with a p value of 0.063>0.05.

ANOVA test could be carried out to identify significant differences between all treatments in antioxidant activity. The results of the ANOVA test stated that the p value = 0.000 <0.05, which means that it was statistically proven that the six treatments had significant differences in antioxidant activity, because the significance value was 0.000 <0.05. If in the ANOVA test there were significant differences, then it could be continued with a post hoc test between variables/treatments to see the contribution between treatments to the results of the ANOVA test (Dahlan, 2015). The results of the post hoc test could be seen in Table 25.

After testing between treatments using the LSD test, it was found that vitamin E and *I. carnea* extract were not statistically significantly different, because the significance value was 0.054>0.05. Likewise between formula 2 and formula 3 with a significance value of 0.000>0.05 so it was concluded that they were not significantly different. Pharmacologically and from a category perspective, it was appropriate that the two antioxidant activities in formulas 2 and 3 were the same, namely moderate (Syarifah, 2022).

CONCLUSION

Based on the results obtained, several conclusions could be drawn, including the following:

a. *I. carnea* Jacq leaf extract contained active compounds that function as antioxidants, including phenolic compounds, saponins, alkaloids and phenolics.
b. Based on the results of the IC50 value obtained at a concentration of 12% (formula 3), it had antioxidant activity, although it was moderate so it could be added to serum preparations of *I. carnea* Jacq leaf extract
c. The best serum preparation formulation of *I. carnea* Jacq leaf extract met good physical qualities including organoleptic, acidity, spreadability and viscosity was formula 3, its also met for the ideal serum quality.

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