



Antioxidant Potential of Garlic (*Allium sativum*) Ethanol Extract in Gel Formulation Assessed by DPPH Method

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ABSTRACT: Garlic (*Allium sativum*) is known to have antioxidant activity that is beneficial to health. Antioxidant activity is expressed in IC₅₀ value, which is the concentration of the extract that can reduce 50% of free radicals. This study aims to test the antioxidant activity of a gel formulated from garlic extract using the DPPH (2,2-diphenyl-1-pyrrylhydrazine) method. The garlic extract and garlic gel extract were tested to evaluate the % inhibition and IC₅₀ values. Garlic ethanol extract showed an IC₅₀ value of 89,290, and garlic ethanol extract gel showed the % inhibition value of 87,30. The decrease in the IC₅₀ value indicates that the gel formulation provides significant antioxidant activity against DPPH free radicals. In conclusion, the ethanol extract of *Allium sativum* formulated into a gel showed antioxidant activity based on IC₅₀ and percentage of inhibition values obtained from the DPPH assay.

Keywords: *Allium sativum* gel, antioxidant, IC₅₀

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INTRODUCTION

Free radicals are molecules that have one or more unpaired electrons, making them highly reactive. The presence of free radicals in the body can cause cell damage, contributing to various degenerative diseases, including cancer, diabetes, and heart disease (Maharani et al., 2021). To overcome this problem, the body has a natural defense system, but it is often insufficient to fight the amounts of free radicals produced by pollution, stress, and unhealthy diets. Therefore, it is important to consume antioxidants from food sources or supplements to protect the body from oxidative damage (Nurkhasanah et al., 2023). Antioxidants play an important role in health, especially in preventing damage to body cells. They work by neutralizing free radicals and preventing chain reactions that can damage cellular components, such as DNA, lipids, and proteins (Boeing et al., 2012). In addition, antioxidants have benefits for the skin, helping prevent premature ageing and UV-ray-induced damage (Pandel et al., 2013). Various sources of antioxidants can be found in foods such as fruits, vegetables, and spices. One source that has attracted attention is garlic (*Allium sativum*), which is known not only as a kitchen spice but also as an herb with various health benefits.

Garlic plays a role as an antioxidant because it contains curcumin flavonoids and vitamin E. Flavonoids are known to capture free radicals. Flavonoids serve as a basis for isolating compounds with antioxidant potential (Wakhidah, L., & Anggarani, M. A., 2021). Garlic has long been used in traditional medicine across various cultures due to its bioactive compounds, such as allicin, which has potent antioxidant activity (Shang et al., 2019). Previous studies have shown that garlic extract can reduce oxidative stress and increase antioxidant capacity (Verma et al., 2023). However, despite the abundance of research on the health benefits of garlic, there remains a gap in the literature on the effectiveness of garlic extract in gel form for topical application.

Garlic gel preparations are also interesting to study because they can provide direct benefits to the skin. The gel can be used as an effective transport medium for bioactive compounds to deeper skin layers, thereby increasing its potential benefits (Ansong et al., 2023). The purpose of this preliminary study was to compare the antioxidant activity of garlic extract in its native form and when incorporated into a gel base. The results of this study are expected to contribute to the development of more effective and safer herbal-based skin care products.

METHODS

Materials and Equipment

Materials used in the study were analytical grade: DPPH (Sigma-Aldrich), ethanol pa (Smart-lab), methanol pa (Sigma-Aldrich), glycerin (Sigma-Aldrich), Tea (Triethanolamine) (Sigma-Aldrich), carbopol (Sigma-Aldrich), and distilled water obtained from CV Nurra Gemilang. Garlic simplisia (*Allium sativum*) obtained from the Batu Materia Medika Herbal Laboratory. The equipment used included glassware (Pyrex®), analytical balance (Fujitsu®), Ultrasonic-Assisted Extraction (Manufacture Expert®), rotary evaporator (Heidolph®), and spectrophotometer (Shimadzu Single-beam U-1280®).

Preparation of Extract *Allium sativum*

The extraction process used in this study was UAE (Ultrasonic Assisted Extraction) maceration, in which 150 grams of garlic simplicia was placed in an Erlenmeyer flask, and 1500 mL of ethanol solvent was added, resulting in a material-to-solvent ratio of 1:10 (w/v). It was then placed in an ultrasonic bath operating at 53 kHz at room temperature

for 15 minutes. After filtering the extract, a rotary evaporator was used to remove the solvent, producing a thick extract, which was then oven-dried for 48 hours at 40°C (Wulandari et al., 2023). The yield of the concentrated garlic extract (*Allium sativum*) that had been evaporated from the solvent was weighed, calculated based on the sample weight relative to the initial dry weight of the sample (dry weight of the raw material). Then, the yield was calculated using the following equation (Ansong et al., 2023).

Formulation and Manufacturing of Extract *Allium sativum* Gel

Garlic extract is formulated in gel form with the following formula (Yulita et al., 2019):

Table.1 Gel Preparation Formulation

Ingredients	Function	Concentration
Garlic extract	Active ingredients	1%
Carbopol 940	Gelling agent	1%
TEA	Emulsifying agent	6%
Glycerin	Humectant	1%
Aquadest	Solvent	Ad 100%

The preparation of the gel starts with preparing the tools and ingredients, then weighing them. Carbopol is developed by adding distilled water at $\pm 70^{\circ}\text{C}$ for 10 minutes, then stirring vigorously until a thick gel base forms (mixture 1). Add TEA and glycerin to mixture 1 and stir until homogeneous; then add distilled water and stir until a homogeneous gel mass forms. Weigh the concentrated garlic extract and stir until dissolved (mixture 2). Add mixture 2 to mixture 1, stirring until homogeneous. Place in a gel pot and label. The gel is prepared in 3 replicates to assess the repeatability of gel production and to evaluate the gel preparation (Thomas et al., 2023).

Physical Test of *Allium sativum* Gel

This study used several techniques to evaluate the physical quality of gel preparations, including organoleptic testing, homogeneity testing, spreadability testing, pH (acidity) testing, and viscosity testing. The physical properties of a preparation are one of the requirements for acceptability by consumers (Isnawati & Trianggah Fauziah, 2023)

Organoleptic Testing

Organoleptic testing was conducted by directly observing the formula using the five senses, including color, aroma, and shape/consistency. The gel was generally clear with a semi-solid consistency (Suryani et al., 2017).

Homogeneity Testing

Homogeneity was tested by applying the gel to glass or other transparent materials. An appropriate ointment must be homogeneous and free of clumping particles. This test assesses the preparation's composition, which should be homogeneous and free of clumps (Ardiansyah et al., 2023).

Spreadability Test

The spreadability test was conducted by placing a specified amount of the substance on a calibrated glass. Then, the top is covered with the same glass, and the load is increased using weights of 50g and 100g. Then, a time range of 1-2 minutes is given, and the spread diameter is measured at each load addition when the preparation stops spreading (Fatmawati et al., 2020).

pH Test

The pH test was conducted using a pH meter, which was calibrated using pH 4 and pH 7 buffer solutions. The electrode was rinsed with distilled water and dried with tissue. The pH measurement was performed by taking 1 g of the sample using a medicine pot, then dipping the electrode into the container. The number displayed by the pH meter was the pH of the gel.

Viscosity Test

Viscosity measurements were performed on the gel preparation using a Brookfield viscometer (NDJ 8S) by placing the preparation in a 100 ml wide-mouth glass and inserting the appropriate spindle until it was submerged. The rotor is turned on until a stable reading is obtained, as indicated by the pointer. The standard viscosity of a good preparation is 500–10,000 mPas (Farhan et al., 2023).

Antioxidant Test

The antioxidant activity of garlic is measured using the DPPH method. The absorbance of 0.1 mM DPPH was measured at wavelengths between 400 and 600 nm using a spectrophotometer. The wavelength obtained was 517 nm. A sample of 10 mg was added to 100 mL of methanol solvent in a measuring flask. The concentrations used were 60 ppm, 70 ppm and 80 ppm. Each concentration was pipetted into 3 ml and added with 3 ml of DPPH solution. Absorbance was measured at 517 nm.

Data Analysis

Performing antioxidant activity tests using the DPPH method expressed in IC_{50} values, then testing the Independent Sample T-test analysis using SPSS 26 by comparing the antioxidant activity of garlic extract and garlic gel.

RESULT AND DISCUSSION

Garlic was extracted to obtain its chemical components. The extraction yielded 21.54 grams of thick extract, with a yield of 6.96%. The yield obtained from the garlic extract is above average, indicating that the extract is of good quality, according to Kurniawan's 2021 study, which reported yields of garlic using ultrasonic extraction techniques to be between 2.83% and 12.35% (Yuniarto et al., 2021). Meanwhile, the maceration method described by Damayanti et al. (2024) yielded 13.37%. However, extraction yield alone does not reflect extract quality, as extraction parameters influence phytochemical composition and antioxidant activity and must be evaluated independently.

Quality Evaluation of *Allium sativum* Gel

Organoleptic observations in Table 2, conducted after preparing the garlic extract gel formulation using the above formula, indicate that the gel has a semi-solid form with an elastic consistency, characteristic of gels in general. The yellow color and distinctive odor of the extract are due to the ethanol it contains. The gel produced is clear and yellow, as shown in Figure 1. This is due to the concentration of garlic extract; the higher the concentration, the darker the color and the stronger the distinctive aroma of ethanol garlic extract. The results of the homogeneity test on the gel preparations in Table 2 and Figure 2 show that none of the preparations exhibited coarse particles upon visual inspection, indicating they are homogeneous (Pramiastuti et al., 2021). A homogeneous gel indicates that the active substance is the same at the time of collection and use (Novia Inda Kharisma et al., 2020).



Figure 1. *Allium sativum* gel front view (a) and side view (b). The figure presents the organoleptic characteristics of the *Allium sativum* gel from front (left) and side (right) perspectives. The formulation exhibits a yellowish-orange color with a visually homogeneous matrix, showing no evidence of phase separation or sedimentation. The side view further confirms uniform consistency throughout the gel, indicating acceptable physical stability of the formulation.

The results of the spreadability test in Table 2 indicate that the garlic formula's spreadability is consistent with the literature. Spreadability testing is an important requirement in gel preparations. Good gel spreadability is said to be between 4-7 cm. The greater the spreadability value, the easier it is to apply and spread the gel on the skin. In addition, it can also increase comfort when used and can provide maximum effect. Based on the results of this study, a spreadability of 4-5 cm was observed at loads of 50 and 100 grams. This range shows that the gel's spreadability is very consistent and comfortable to use. The obtained spreadability values meet the requirements (Tutik et al., 2021).

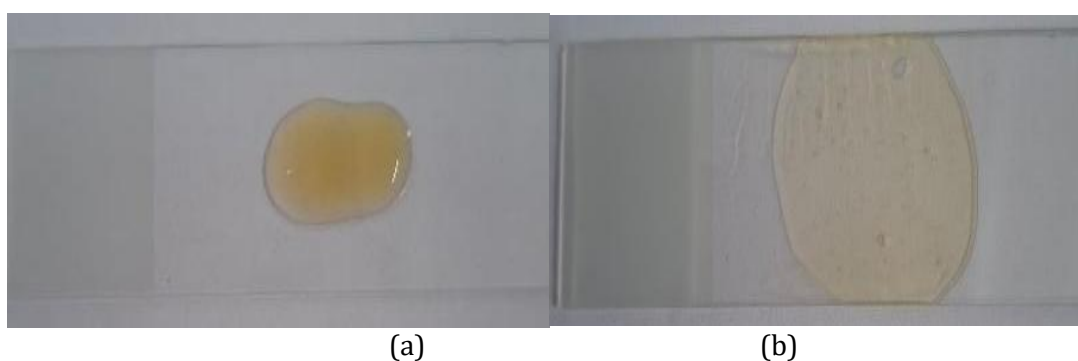


Figure 2. Homogeneity test of *Allium sativum* Gel. (a) A drop of gel placed on a glass side; (b) gel spread with a glass slide. The gel shows uniform color and consistency in both conditions. In (a), the droplet is smooth and free from visible coarse particles or phase separation. In (b), after spreading, the gel forms a thin, even layer without lumps or aggregation, indicating good homogeneity of the formulation.

Observations on garlic extract gel: as shown in Table 2, the pH of the preparations exceeded the skin pH limit due to the relatively high TEA content, in accordance with Adnan's 2022 study, which states that higher TEA concentrations result in higher pH. The pH measurement aims to determine whether the resulting preparation is acceptable for skin pH, as this relates to its safety and comfort when used. If it does not match the skin's pH, the preparation can irritate the skin, leading to discomfort during use. The ideal skin pH ranges from 4.5 to 6.5 (Rashati et al., 2024). Table 2 shows that the viscosity values obtained from the above formula fall within the acceptable viscosity range. This viscosity test aims to determine the viscosity level of a gel preparation. The viscosity of the gel is influenced by the gelling agent used, namely Carbopol 940. Viscosity testing is performed to determine a liquid's resistance to flow. Preparations with excessively high viscosity tend to have low spreadability, making them difficult to apply evenly on the skin surface.

In contrast, excessively low viscosity can cause the preparation to flow easily and adhere poorly. In this study, the viscosity values obtained were within the acceptable range for gel preparations, consistent with the spreadability test results, which showed good spreadability. This indicates that the viscosity of the preparation was balanced, allowing the gel to be easily applied, to adhere sufficiently to the skin, and to be well accepted by users.

Table 2. Physical Test of *Allium sativum* Gel

Parameters	Result	Requirements
Organoleptics	Shape: Elastic and pliable, Color: Clear yellow, Odor: smells like garlic	-
Homogeneity	Homogenous	Homogenous
Spreadability	No load: 3.67 ± 0.153 50g load: 4.90 ± 0.265 100g load: 5.53 ± 0.473	4-7 cm
pH	8.14 ± 0.035	4.5 to 6.5
Viscosity	1076 ± 15.044 mPa.s	500-10.000 mPa.s

Antioxidant Activity

Antioxidant activity testing in this study used the DPPH method. This method was chosen because it offers several advantages, including simplicity, speed, ease, and the need for only a small sample (Romsiah et al., 2020). According to Molyneux (2004), the working principle of the antioxidant test method is a quantitative measurement method that detects DPPH radicals using a compound with antioxidant potential. Free radical scavenging activity is usually expressed as a percentage of DPPH inhibition, stated as the concentration that causes a 50% loss of DPPH activity (IC_{50}). The absorbance value can be determined by UV-Vis spectrophotometry at 517 nm, thereby yielding the free radical scavenging antioxidant activity, expressed as the IC_{50} value.

The inhibition percentage of the Garlic extract was determined, and the regression equation of sample concentration against % Inhibition was calculated in Microsoft Excel 2013. The regression equation of DPPH %Inhibition against concentration was $y=1.6971x-100.97$, while the regression of gel extract equation of DPPH %Inhibition against concentration was $y=1.6106x-90.476$. The regression analysis of DPPH % inhibition versus concentration showed high coefficients of determination ($R^2 = 0.9484$ for the garlic extract and $R^2 = 0.9514$ for the garlic gel extract), indicating a strong linear relationship between

concentration and antioxidant inhibition over the tested concentration range. These values suggest that the regression models fit the experimental data well.

Results in Table 3 indicate that the antioxidant activity test of garlic ethanol extract using the DPPH method showed an increase in the % inhibition to 32.511%. In terms of its potential to scavenge free radicals, garlic ethanol extract showed an IC₅₀ value of 89.290, which is classified as strong. Meanwhile, based on the test results in Table 3, the antioxidant activity of the garlic ethanol extract gel using the DPPH method showed that the % inhibition increased to 36.134%. Regarding its potential to scavenge free radicals, the garlic ethanol extract showed an IC₅₀ of 87.29 mg/L, which is considered strong. When comparing the IC₅₀ of garlic extract with garlic gel extract, it was found that the IC₅₀ of the extract was greater than that of the gel. This is because the extract has a higher concentration than the gel, which has been mixed with other ingredients.

The antioxidant potential of garlic ethanol extract and garlic ethanol gel extract was not significantly different. This is shown in Table 3. Meanwhile, the higher the solution concentration, the higher the % inhibition. This is because the higher the solution concentration, the greater the antioxidant potential of the garlic extract (Amirna et al., 2024). The antioxidant potential in free radical scavenging can be expressed as %Inhibition of DPPH. However, it can also be expressed by the concentration value that causes a 50% loss of DPPH activity (IC₅₀). The IC₅₀ value is the concentration of the test compound that can neutralize 50% of free radicals. The smaller the IC₅₀ value, the greater the antioxidant effectiveness (Amirna et al., 2024). The categories of antioxidant potential include very strong (IC₅₀ ≤ 50), strong (50-100), moderate (IC₅₀ 100-150), weak (IC₅₀ 150-200), and very weak (IC₅₀ ≥ 200) (Molyneux, 2004).

Table 3. Results of Antioxidant Potential Test of *Allium sativum* Gel

Sample	%inhibition	IC ₅₀	Category
Allium sativum Extract	32,511 ± 0,256	89,29	Strong
Allium sativum Gel	36,134 ± 0,243	87,30	Strong

The Independent Samples t-test was conducted to compare the IC₅₀ values between the two groups. Levene's test for equality of variances showed a non-significant result (Sig. = 0.864), indicating that the assumption of equal variances was met. Therefore, the results under the "equal variances assumed" condition were used for interpretation. The t-test revealed a statistically significant difference in IC₅₀ values between the two groups (t = 6.710, df = 4, p = 0.003). The mean difference in IC₅₀ was 1.67, with a 95% confidence interval ranging from 0.98 to 2.36. These results indicate that the IC₅₀ values of the two groups differed significantly, suggesting a meaningful difference in antioxidant activity between the extract and gel formulations.

This study aligns with research (Mokoginta et al., 2020) showing that garlic has potential as an antioxidant, as it contains several compounds that act as powerful antioxidants, including flavonoids. Flavonoid compounds have antioxidant potential because they have two hydroxyl groups. The reaction between the binding of DPPH free radicals and antioxidant compounds causes a color change from deep purple to bright yellow, which is caused by the resonance of the DPPH free radical binding structure. This color change indicates the extract's strong antioxidant potential.

Factors affecting the results of antioxidant tests on garlic extracts include the extraction method, solvent type, active ingredient concentration, and dosage form. The

extraction method determines the extent to which sensitive compounds are preserved; for example, ultrasound-assisted extraction (USE) is superior in preserving aroma and bioactive compounds compared to conventional distillation. The use of polar solvents, such as ethanol, in combination with ammonium sulfate increases allicin extraction efficiency to 94%. In contrast, non-polar solvents (hexane, ethyl acetate) tend to reduce phenolic content and antioxidant activity (Ghali et al., 2025). The concentration of allicin and total phenols also plays an important role; aged garlic extract (AGE) has a higher phenolic content and a lower IC₅₀ than raw extract (Park et al., 2009). The gel formulation can increase allicin stability and improve direct contact with target tissues, thereby increasing the inhibition percentage (36.134% vs 32.511%) and decreasing the IC₅₀ (87.30 vs 89.290).

CONCLUSION

Garlic extract gel exhibited significant antioxidant activity as determined by the DPPH method, with an IC₅₀ of 87.29 mg/L. The percentage of inhibition achieved indicates that the garlic extract-based gel has potential as a topical formulation with effective natural antioxidant content. However, adjustments to the formulation are necessary to ensure that it is safe to use on the skin without irritation.

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