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Evaluation of Physicochemical Stability and Antioxidant Activity of Single Black Garlic Extract Capsules: Initial Development of Herbal Preparations

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Abstract: Black garlic is a fermented product of fresh garlic that is rich in bioactive compounds such as S-allyl cysteine (SAC), polyphenols, and melanoidins, which play an important role as antioxidants and have the potential to be developed as supplements for preventing degenerative diseases. This study aims to evaluate the physicochemical stability and antioxidant activity of black garlic extract capsules during three months of accelerated storage (40 ± 2 °C; RH 75 ± 5%). Black garlic extract was obtained through ethanol maceration, concentration, and freeze-drying, and then formulated into capsules with amylum manihot and starch mucilage as excipients. The analyzed parameters included organoleptic characteristics, weight uniformity, moisture content, pH, total phenolic content using the Folin–Ciocalteu method, and antioxidant activity using the DPPH method. The results showed that capsule weight uniformity met pharmacopeia requirements throughout storage, while moisture content gradually increased, and pH showed a moderate decrease. Total phenolic content significantly decreased from 57.20 ± 0.92 to 43.40 ± 0.75 mg GAE/g by the third month. The antioxidant activity remained in the strong category, as indicated by an increase in IC50 values. The novelty of this study lies in its comprehensive evaluation of the stability of a single black garlic capsule product under accelerated conditions and its relationship to changes in bioactivity during storage. Overall, this study confirms that the single black garlic extract capsule product has good physicochemical stability and antioxidant activity, and has the potential to be developed as a herbal product with antioxidant capacity that is maintained and even increases during storage.

Keywords: Antioxidant; Black Garlic; Capsule; Physicochemical; Stability.

Introduction

Degenerative diseases are progressive conditions that develop as a consequence of continuous cellular damage. One of the major contributing factors is oxidative stress, which arises from an imbalance between the production of free radicals and the body's ability to neutralize them [1]. The use of natural substances with strong antioxidant activity has increasingly been explored in the pharmaceutical field as a preventive strategy against oxidative stress. Among these natural products, black garlic has gained substantial attention. Black garlic is a fermented derivative of fresh garlic (*Allium sativum* L.) that undergoes chemical decomposition and bioactive transformation during controlled fermentation at specific temperatures and durations. The biochemical modifications occurring throughout fermentation lead to the formation of active compounds such as S-allyl cysteine (SAC), flavonoids, and polyphenols, all of which exhibit potent antioxidant activity and greater stability compared to fresh garlic [2]. Regular consumption of black garlic, therefore, holds promising potential in reducing the risk of degenerative diseases.

The fermentation of garlic into black garlic involves Maillard reactions between amino acids and reducing sugars, resulting in the formation of melanoidin compounds. This reaction reduces the concentration of the unstable allicin compound and replaces it with organosulfur derivatives that are more resistant to oxidation [3]. Black garlic contains various bioactive compounds, including flavonoids, tannins, saponins, and sterols, that are capable of stabilizing free radicals, thereby producing exceptionally strong antioxidant activity, with an IC_{50} value of 2.27 ppm [4]. Physicochemical stability is a critical parameter in pharmaceutical formulation, as it determines the quality, safety, and effectiveness of a product throughout storage. Parameters such as pH, moisture content, and organoleptic characteristics must be monitored to ensure the consistency and reliability of the dosage form. Antioxidant activity serves as a primary indicator of the functional potential of black garlic; therefore, any decline in physicochemical stability during storage may reduce its pharmacological benefits. Evaluating the relationship between physicochemical parameters and antioxidant activity is essential for ensuring the effectiveness of the preparation over its shelf life.

Previous studies have extensively examined the antioxidant activity of black garlic extracts in both liquid and powder forms, consistently reporting the relatively strong oxidative stability of S-allyl cysteine [5] [6]. However, most of these investigations have focused solely on raw materials rather than finished products in solid dosage forms. Research on the stability of herbal-based capsules in Indonesia has also generally been limited to evaluating physical parameters such as moisture content, loss on drying, and morphological changes, without correlating these findings with alterations in antioxidant activity [7] [8]. According to the current state of the art, several international studies highlight the importance of an integrated analysis between

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physicochemical changes and bioactivity in herbal preparations, particularly in products containing polyphenolic compounds that are susceptible to thermal degradation [9] [2]. Nevertheless, this integrated approach has not been widely applied to black garlic capsules, resulting in the lack of comprehensive scientific data that explain the relationship between physicochemical stability and antioxidant activity during product storage.

This knowledge gap underscores the need for research on the stability of black garlic capsules, particularly in light of the growing market demand for safe and effective herbal supplements. Without adequate stability data, it is difficult to ensure that black garlic capsules retain their optimal antioxidant benefits throughout their shelf life. Such uncertainty may compromise product quality, reduce clinical effectiveness, and erode public confidence in locally produced herbal preparations. Evidence-based development of herbal products is therefore essential to ensure that formulation processes, packaging, and storage conditions maintain the quality and biological activity of the final dosage form.

The novelty of this study lies in the application of an integrated analysis that simultaneously evaluates the physicochemical stability and antioxidant activity of black garlic capsules during storage. A research gap identified is the lack of studies linking changes in physicochemical parameters such as water content, pH, color, and active compound content with quantitatively measured antioxidant activity in black garlic capsule preparations. To address this gap, this study aims to assess the stability of black garlic capsule preparations under various storage conditions and determine the relationship between physicochemical changes and antioxidant activity using a standardized method.

Based on this approach, the research is expected to make a significant scientific contribution by providing a comprehensive understanding of the stability of black garlic-based herbal products. Furthermore, the research results can serve as a reference for determining shelf life, formula improvement, and optimal storage strategies for the herbal supplement industry. More broadly, this research supports the development of locally sourced phytopharmaceutical preparations with consistent quality and guaranteed efficacy.

Research Methods

Tools and Materials

The tools used in this study included an analytical balance (±0.0001 g), blender, closed maceration vessel, filter cloth, Whatman filter paper, glass funnel, rotary evaporator (45 °C), water bath, freeze dryer, desiccator, mortar and stamper, 40–60 mesh sieve, drying oven, manual capsule filler, dark glass bottle, climatic chamber (40 ± 2 °C; RH 75 ± 5%), moisture balance, calibrated pH meter (pH 4, 7, and 10 buffers), UV-Vis spectrophotometer, quartz cuvette, micro pipette, vortex mixer, beaker glass, test tube, and other laboratory glassware needed during the extraction, formulation, and analysis processes.

The materials used consisted of single garlic, ethanol p.a., distilled water, amylum manihot, amili mucilage, capsule shell, Folin–Ciocalteu reagent, 7.5% sodium carbonate, gallic acid, 100 mM Tris-HCl buffer solution pH 7.4, DPPH (2,2-diphenyl-1-picrylhydrazyl).

Research Procedure

This research is a laboratory-based experimental study employing a pre–post stability design under accelerated storage conditions. The evaluation was conducted on capsule formulations prepared from black garlic extract to assess changes in physicochemical parameters and antioxidant activity over a 3-month storage period.

Preparation of Black Garlic Extract

500 grams of single black garlic were cleaned and ground to form a homogeneous paste. The paste was extracted using a maceration method with ethanol at a 1:10 (w/v) ratio. The maceration process was carried out for 48 hours at room temperature with occasional stirring, followed by filtration to separate the filtrate from the residue. The filtrate was evaporated using a rotary evaporator at 45°C. The extract was then added to distilled water until a homogeneous mixture was formed, facilitating the drying process. The extract solution was dried using a freeze-drying method. The resulting dry extract was transferred to a desiccator for storage until it was used in the capsule formulation.

Preparation of Black Garlic Capsule Formulation

A black garlic capsule formulation was prepared by mixing 200 mg of dried single black garlic extract, 190 mg of amylum manihot, and 10 mg of amilli mucilage in a mortar and grinding until a homogeneous mixture was achieved. The mixture was dried to form dry granules and placed into capsule shells [7]. The capsules were packaged in sealed dark glass bottles with silica gel desiccant. Storage was carried out at 40 ± 2°C and 75 ± 5% RH using a climatic chamber. Samples were taken and analyzed at the following times: Day 0 (baseline), Month 1 (±30 days), Month 2 (±60 days), and Month 3 (±90 days) for physicochemical parameters, and analytical repetition (triples) was performed for chemical parameters and antioxidant activity.

Weight Uniformity Test

Weight uniformity testing was conducted using 20 capsules. Each capsule was weighed using a calibrated analytical balance. The weight of the capsule contents and the average weight of each capsule were calculated. Batch compliance with pharmacopoeial criteria was assessed, with no more than two units out of 20 allowed to exceed the percentage tolerance limit. The percentage was calculated using the formula:

$$\%\text{Deviation} = \frac{\text{Capsule contents weight} - \text{average capsule weight}}{\text{average capsule weight}} \text{x} 100\% \text{ [10].}$$

Moisture Content Test

2 grams of powder on an aluminium weighing dish in a moisture balance. Follow the prompts (start or tare), then click. Wait until the "END" appears, and record the results.

The previous procedure is repeated three times to obtain a comparison, which will then be used to calculate the average value [11].

pH Test

The pH test was performed by preparing a sample solution from the capsule contents (the capsule contents were crushed and dissolved in deionized water at a standard concentration, e.g., 1% w/v), homogenized, and filtered. The pH of the solution was measured using a calibrated pH meter (pH buffers 4, 7, and 10) at room temperature (25 ± 2 °C) with a minimum of three replicates per sample, and results were reported as the mean ± SD.

Total Phenolic Content Test

A single black garlic capsule sample (200 µL), Folin–Ciocalteu reagent (1 mL, diluted 10-fold), and sodium carbonate (1 mL, 7.5%) were mixed, and distilled water was added to reach a volume of 5 mL. The mixture was incubated in the dark for 30 minutes. Absorbance was measured at a wavelength of 760 nm. Gallic acid was used as a standard in the preparation of the calibration curve. Total phenolic content was expressed as gallic acid equivalents (mg) using a linear equation [12].

Antioxidant Activity Test

The antioxidant activity test of single black garlic capsules was conducted using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. A 0.2 mM DPPH radical stock solution was prepared by dissolving DPPH powder in ethanol p.a. until a homogeneous solution was obtained. The black garlic extract sample was dissolved in 100 mM Tris-HCl buffer at pH 7.4 to obtain the appropriate test concentration. A 1 mL of the 0.2 mM DPPH solution was mixed with 1 mL of the sample solution, then the mixture was vortexed and incubated for 30 minutes at room temperature in the dark to prevent free radical degradation. The absorbance of the mixture was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. Radical scavenging activity was calculated based on the percentage reduction in DPPH radical absorbance. The IC_{50} value is determined by the relationship between sample concentration and the percentage of radical scavenging, as described by a linear regression equation. The lower the IC_{50} value, the higher the antioxidant capacity of the extract. Based on the commonly used antioxidant strength category range, IC50 values can be classified as follows: strong (<50 µg/mL), moderate (50–100 µg/mL), and weak (>100 µg/mL). This procedure is widely used in natural product antioxidant research due to its sensitivity and reproducibility [12].

Data Analysis

Data obtained from physicochemical stability and antioxidant activity testing were analyzed descriptively and inferentially according to the characteristics of the variables measured. Physicochemical parameters, including water content, pH, organoleptic properties, and weight uniformity, were presented as mean values ± standard deviation to illustrate variation between replicates. A comparative analysis was performed between storage time points (months 0, 1, 2, and 3) using a one-way ANOVA test. Antioxidant activity data obtained from the DPPH method were analyzed using a linear regression curve to determine the IC50 value, then compared between treatment groups or storage periods using the same statistical analysis. All analyses were performed using SPSS 26 software, and data were visualized in tables and graphs to facilitate interpretation of changes during the accelerated storage period. The results were then evaluated in accordance with the literature and quality standards for herbal preparations.

Results and Discussion

The physicochemical stability and antioxidant activity of black garlic extract capsules were evaluated during accelerated storage (40 ± 2 °C; RH 75 ± 5%) over a three-month period. The comprehensive results of all tested parameters are summarized in Table 1.

Table 1. Physicochemical Stability and Antioxidant Activity of Black Garlic Extract Capsules during Accelerated Storage

Parameter	Month 0	Month 1	Month 2	Month 3
Color	Black	Black	Black	Black
Odor	Characteristic black garlic	Characteristic black garlic	Characteristic black garlic	Characteristic black garlic
Capsule appearance	Intact	Intact	Intact	Intact
Weight uniformity (mg)	496±2.585	497±2.438	496±2.585	495±2.404
Moisture content (%)	1.15±0.026	1.23±0.020	1.31±0.015	1.38±0.015
pH	5.20±0.025	5.32±0.026	5.41±0.021	5.52±0.026
Total phenolic content (mg GAE/g)	57.20 ± 0.917	53.50 ± 0.656	48.27 ± 0.971	43.40 ± 0.755
Antioxidant activity (IC_{50})	81.43±0.971	75.47±1.137	68.27±0.973	60.67±0.974

Organoleptic Test

The black garlic extract capsule product demonstrated good organoleptic stability and met the requirements of the Indonesian Pharmacopoeia during a three-month accelerated storage period. The capsule shape remained intact, the dark brown color showed no noticeable change, and no rancid odor was detected. This stability indicates that no significant physical or oxidative degradation of volatile components occurred during storage.

Melanoidin pigments formed during the fermentation process are known to possess high stability against heat and oxidation, thereby contributing to the maintenance of the characteristic color and aroma of black garlic [2]. In addition, the use of the freeze-drying method in this study contributed to the preservation of sensory characteristics by minimizing heat exposure during the drying process. These findings are consistent with previous reports indicating that herbal products dried at low temperatures retain better organoleptic quality compared to those subjected to conventional drying methods [3].

Weight Uniformity Test

The weight uniformity test demonstrated that all capsules met the requirements of Farmakope Indonesia during storage. The black garlic extract capsules had a weight range of 495–497 mg with a percentage deviation of 1.60–1.90%. This result meets the deviation limit required by the of Farmakope Indonesia, which is a maximum of ±7.5% for hard capsules [13]. This result suggests that the formulation using amylum manihot and mucilage amili provided good flowability and homogeneity of the capsule fill mass, ensuring consistent dosage delivery. Similar findings have been reported for herbal capsule formulations with appropriate excipient selection [14].

Moisture Content Test

Moisture content exhibited a gradual increase over the storage period. These characteristics are within the acceptable limits for dry preparations according to the Farmakope Indonesia, which is below 10% [13]. This standard is intended to prevent the growth of microorganisms and chemical degradation. This phenomenon can be attributed to the hygroscopic nature of both the plant extract and excipients, as well as exposure to high relative humidity during accelerated storage. Previous studies have reported comparable moisture uptake behavior in dried herbal products and food matrices stored under high humidity conditions [15], [16]. Nevertheless, the increase in moisture content did not result in visible capsule damage or organoleptic changes.

pH Test

A moderate decrease in pH was observed during storage. This decrease may be associated with the formation of acidic compounds resulting from phenolic degradation and secondary Maillard reaction products during prolonged exposure to elevated temperature. Similar pH reductions have been reported in black garlic and plant extract products subjected to thermal processing and storage [4], [17].

Total Phenolic Test

Total phenolic content decreased significantly from 57.20 ± 0.917 mg GAE/g at the beginning of storage to 43.40 ± 0.755 mg GAE/g after three months. The reduction in phenolic compounds is likely due to oxidative degradation under accelerated storage conditions. Phenolic compounds are known to be sensitive to temperature and moisture, leading to gradual degradation during storage [18]. The total phenolic content test results from this study indicate that the single black garlic capsule product has high and stable phenolic potential, supports antioxidant activity, and can serve as a strong basis for the development of black garlic-based herbal products or supplements.

Antioxidant Activity Test

The antioxidant activity of single black garlic extract capsules, evaluated using the DPPH method, increased during three months of accelerated storage, as indicated by a decrease in IC_{50} values from 81.43 ± 0.97 mg/L at month 0 to 60.67 ± 0.97 mg/L at month 3. Lower IC_{50} values indicate stronger free radical scavenging activity, confirming that the capsules remained in the strong antioxidant category throughout the storage period. These findings suggest that accelerated storage conditions did not reduce, but rather tended to enhance, the antioxidant capacity of the formulation.

The observed increase in antioxidant activity occurred despite a decline in total phenolic content, indicating that the antioxidant capacity of black garlic capsules is not solely dependent on total phenolics. Other relatively stable bioactive compounds, such as S-allyl cysteine and melanoidin formed during the Maillard reaction, play a crucial role in maintaining and enhancing antioxidant activity due to their resistance to thermal and oxidative degradation [2], [3], [12]. In addition, the use of freeze-drying prior to capsule formulation contributed to the preservation of bioactive compounds, thereby supporting sustained antioxidant activity during storage [16], [18].

In a comparative study of antioxidant activity using the DPPH method, green tea (Camellia sinensis) extract showed an IC_{50} value of 10.804 μg/mL, while the control vitamin C (ascorbic acid) had an IC_{50} of 2.61 μg/mL, indicating that both compounds are included in the very strong antioxidant category [19]. Single black garlic capsules fall into the strong antioxidant category, approaching the effectiveness of natural standard antioxidants, indicating that this formulation has a significant radical scavenging ability even at relatively small doses. Exploration of long-term stability is an important aspect for the development of phytopharmaceutical products. Literature studies show that compounds such as S-allyl cysteine and melanoidins can survive under controlled storage conditions for up to six months or more, with relatively stable or even increased antioxidant activity due to the formation of additional Maillard compounds [20].

Overall, these findings indicate that black garlic extract capsules possess a robust and stable antioxidant system. The observed increase in antioxidant activity during storage underscores the distinctive chemical properties of black garlic, supporting its potential as a phytopharmaceutical product with sustained and potentially enhanced antioxidant efficacy throughout its shelf life.

Conclusion

The results of this study demonstrate that single black garlic extract capsules exhibit good physicochemical stability and maintain strong antioxidant activity during three months of accelerated storage at 40 ± 2 °C and 75 ± 5% relative humidity. The capsules remained organoleptically stable and complied with weight uniformity requirements,

although gradual changes in moisture content, pH, and total phenolic content were observed. Despite a decrease in total phenolic content, antioxidant activity increased during storage, indicating the contribution of stable bioactive compounds such as S-allyl cysteine and melanoidin. These findings confirm the potential of black garlic extract capsules as a stable herbal antioxidant product, supporting their further development as a solid dosage form with sustained bioactivity.

Author's Contribution

I. G. B. T Ananta: conducted research design, process supervision, data analysis, and manuscript preparation; I. G. Y. Anggara: conducted laboratory experiments, documented results, and discussed laboratory experiment results.

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