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Quality Testing of Purple Cabbage Extract (*Brassica oleracea* L. var capitata f. rubra) 70% Ethanol Fraction by Percolation Extraction Method

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Abstract. *Multicomponent reactions are considered as the powerful tool in synthetic organic chemistry. They are efficient and one-pot reactions employing more than one starting materials leading to the formation of a final product. A wide range of heterocyclic compounds have been synthesized via multi-component reactions. In a mechanistically interesting MCR between amino triazoles, different carbonyl compounds and aromatic aldehydes, a mixture of isomeric heterocycles are obtained in good yields. Multicomponent reactions (MCRs) of* H_2S *with different carbonyl compounds, amines and hydrazines have been carried out to get a variety of new types of sulphur and nitrogen containing heterocycles of immense medicinal importance. In a microwave assisted three-component reaction of 2,6-diaminopyrimidin-4-one, 4-hydroxycoumarin and aromatic aldehydes in DMF, Chromenopyridopyrimidines are formed in excellent yields .However, addition of acetic acid to the same reaction mixture leads to the formation of a different compound. This article focuses on the recently proposed mechanisms for a series of multicomponent heterocyclization reactions. A wide range of advanced applications of the heterocycles synthesized via MCRs have also been discussed.*

Keywords: *Heterocyclic compounds; Aminotriazoles; Aromatic aldehydes; Hydrazines; Chromenopyridopyrimidines*

1. Introduction

Lack of physical activity, an unhealthy diet, and exposure to vehicle exhaust and cigarette smoke pollution lead to an increase in free radicals. High levels of free radicals in the body can trigger various dangerous diseases, such as cancer. The human body requires antioxidants, such as flavonoids and phenolics, to protect against high levels of free radicals to prevent damage (Ganesha *et al.*, 2020). One plant containing flavonoids is purple cabbage (Brassica oleracea L. var. capitata f. rubra). Purple cabbage has numerous benefits and is rich in protein, carbohydrates, fat, calcium, phosphorus, iron, vitamin A, vitamin B, vitamin C, glycosides, flavonoids, and phenols (Shama *et al.*, 2012; Fang *et al.*, 2019; Kahulinta, 2020). Secondary metabolites found in purple cabbage can be extracted through an extraction process. The percolation extraction method is a cold extraction method that can be used to extract secondary metabolites that are not heat-stable. Extract quality standards must be fulfilled through standardization to ensure pharmacological efficacy, maintain stability and safety, and preserve the consistency of active compounds in the extract. Extract quality standardization is carried out by measuring specific parameters (organoleptic and ethanol-soluble extract content) and non-specific parameters (water content and ash content) (Ministry of Health of the Republic of Indonesia, 2000).

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Purple cabbage plant extract can produce antioxidant activity caused by the content of secondary metabolites of the phenolic compound group (Tensiska & Wijaya, 2007; Fernando *et al.*, 2023). Phenolic compounds are known to reduce the risk of cancer, coronary heart disease, stroke, osteoporosis, inflammation, and neurodegenerative diseases (Pratama *et al.*, 2018). Extraction is a process carried out with the aim of extracting soluble chemical components so that they are separated from insoluble materials with a liquid solvent. The active compounds contained in the extract from the crude drug are divided into soluble and insoluble active compounds. The purpose of determining the presence of active compounds contained in the crude drug is to facilitate the selection of the appropriate solvent and extraction method (Ministry of Health of the Republic of Indonesia, 2000; Putri, 2022). Percolation is the process of extracting crude drugs by flowing an appropriate solvent through the crude drug that has previously been soaked in a percolator. Percolation aims to ensure complete extraction of bioactive compounds from both heat-stable and heat-sensitive plant materials. The principle of percolation extraction is that the extracting solvent flows from top to bottom through the powdered crude drug material. The solvent dissolves the active constituents from the cells it permeates until saturation is reached (Sudarwati & Fernanda, 2019). The percolation extraction method with 70% ethanol solvent aligns with green chemistry principles. The percolation method is performed at room temperature and without heating, indicating low energy consumption and avoiding thermal degradation of active compounds. Furthermore, the use of 70% ethanol is safer because it is a low-toxic and biodegradable solvent.

Phytochemical screening is carried out to identify the groups of secondary metabolites contained in the plants being studied. This method is carried out by observing the color test reaction using a specific reagent according to the secondary metabolite. The advantages of using this method are that it is simpler, faster, and requires simple equipment and reagents, and can detect at low concentrations (Qhoir, 2023). These secondary metabolite compounds are grouped into several groups, namely alkaloids, flavonoids, phenols, saponins, steroids, and triterpenoids (Chatri *et al.*, 2022).

Based on the Decree of the Minister of Health of the Republic of Indonesia No. 22/Menkes/SK/1/2000, traditional medicines distributed in Indonesia must meet quality, safety, and efficacy requirements. Therefore, it is necessary to measure specific and non-specific parameters of the extracts to scientifically determine the content of the herbal medicine as a raw material for medicinal plants, determine its benefits, and ensure its quality and safety (Ministry of Health of the Republic of Indonesia, 2000; Marpaung & Septiyani, 2020).

2. Methods

150 g of purple cabbage crude drug powder was extracted with a total solvent of 1,500 mL of 70% ethanol (1:10). The powder was placed in a beaker, then 300 mL of 70% ethanol was added to moisten the crude drug, and the mixture was left to stand for 24 hours. The moistened crude drug was gradually transferred into a percolator lined with cotton wool and filter paper. The filter paper was then placed on top of the herbal medicine. The solvent was slowly poured from top to bottom until a 1 cm layer of solvent formed on the surface of the powdered crude drug. It was then left to stand for 24 hours at room temperature with the percolator valve closed. The remaining 70% ethanol solvent was continuously added to the

percolator, and the dripping rate was monitored at 10 drops per minute. The percolation process was continued until the liquid exiting the percolator was clear. The percolate was collected in an Erlenmeyer flask and concentrated using a water bath at 50°C to obtain a thick purple cabbage extract. The quality tests for the purple cabbage extract included:

a. Organoleptic testing. This test examined the appearance, color, and odor of the purple cabbage extract using the five senses.
b. Ethanol soluble extract testing. The thick extract was macerated with 50 mL of 96% ethanol for 24 hours, stirring during the first 6 hours. The solution was then filtered and 4 mL of the filtrate was placed in a tared porcelain dish and heated in an oven at 105 °C for 1 hour until a residue was obtained. Heating and weighing were repeated until a constant weight was obtained. This procedure was performed three times in replication. The formula for the ethanol soluble extract content is:

   Ethanol soluble extract (%) = $\frac{W1-W0}{W} \times 100\ \%$

   W0 = weight of empty cup (g)

   W1 = cup weight + residue after heating (g)

   W = initial extract weight (g)
c. Moisture content test. 1 gram of the thick extract was weighed into a tared crucible that had been preheated at 105 °C for 15 minutes, then left in a desiccator to cool and weighed. The crucible containing the extract was placed in a desiccator to cool, then weighed and the resulting weight recorded. Heating and weighing were repeated until a constant weight was achieved. This procedure was performed three times in replication. The moisture content was calculated as a percentage using the formula:

   Moisture content (%) = $\frac{C-(B-A)}{C} \times 100\%$

   A = weight of empty cup (g)

   B = cup weight + residue after heating (g)

   C = initial extract weight (g)
d. Ash content test. 1 gram of the thick extract was weighed into a silicate crucible that had been preheated in an oven at 105 °C for 15 minutes, left in a desiccator to cool, and then weighed. The crucible containing the extract was heated in a furnace at 500 °C for 4 hours until it turned to black ash. It was then cooled in a desiccator and weighed to a constant weight. Ashing and weighing were repeated until a constant weight was achieved. This procedure was performed three times in replication. The ash content was calculated as a percentage using the formula (Atmaja *et al.*, 2021):

   Ash content (%) = $\frac{W2-W0}{W1-W0} \times 100\%$

   W0 = weight of empty cup (g)

   W1 = cup weight + extract before ashing (g)

   W2 = cup weight + extract after ashing (g)

Phytochemical screening of purple cabbage extract was carried out using the following tests:

a. Flavonoid test. The thick percolated extract was placed in a test tube. The extract was dissolved in 2 mL of 70% ethanol, then 1 mL of 2N HCl and a spatulaful of Mg powder were added. A positive result was indicated by an orange to red solution.
b. Alkaloid test. The thick extract was placed in a test tube. The extract was dissolved in 2 mL of 70% ethanol, then 1 mL of 2 N HCl was added, and the solution was filtered using

filter paper. The filtrate was reacted with 3-5 drops of Dragendroff's reagent. The formation of a white to yellow precipitate indicates the presence of alkaloids (Modified by Sukardiarsyah *et al.*, 2023).

c. Phenolic test. The thick extract was placed in a test tube, then dissolved in 2 mL of 70% ethanol and 3 drops of 1% $FeCl_3$ solution were added. A positive phenolic test was indicated by the formation of a blue to blackish solution.
d. Saponin test. The thick extract was placed in a test tube, then added with 5 mL of 100°C hot water, cooled, and shaken for 10 seconds. The formation of constant foam for 10 minutes indicated the presence of saponins.

3. Results and Discussion

This study aimed to determine the physical quality of purple cabbage extract. The selected purple cabbage was fresh, unrotted, purple in color, firm, and dense. Slicing the purple cabbage accelerated the drying process, while washing with running water removed impurities adhering to the purple cabbage (Handoyo & Pranoto, 2020). Drying reduced the water content in the sample and prevented the growth of mold and other microbes. The drying temperature was 50 °C. Drying was performed at a low temperature because secondary metabolites, such as flavonoids, are destroyed by high heat. Dried purple cabbage samples were characterized by their easy disintegration when squeezed and a brownish-purple color change.

The extract was prepared by percolation to avoid damage to heat-sensitive compounds. Ethanol 70% was added to the percolator at a rate of approximately 10 drops per minute to ensure optimal and consistent extraction of active compounds from the crude drug. Ethanol 70% has good polarity towards secondary metabolites present in purple cabbage. Polar compounds such as flavonoids will dissolve in polar solvents (ethanol). Ethanol is polar due to the presence of the -OH group, which facilitates the formation of hydrogen bonds between the compound and the solvent (Damayanti *et al.*, 2023). The yield of the purple cabbage extract produced was 37.12%. The yield of purple cabbage extract in this study met the requirements set by the Indonesian Herbal Pharmacopoeia, namely a yield of not less than 10% (Ministry of Health of the Republic of Indonesia, 2017). The yield of purple cabbage extract produced by the percolation method is higher than that obtained by the maceration method. Researcher Humairoh (2023) obtained an extract yield of 13.26%. The higher yield of extract using the percolation extraction method is due to the constant replacement of the solvent, preventing solvent saturation and ensuring complete extraction of the compounds. Solvent saturation can result in incomplete extraction of the phenolic content (Wicaksono *et al.*, 2023). A high yield indicates optimal extraction because it effectively extracts active compounds. However, it is important to be aware that a high yield does not necessarily mean a high number of active compounds; rather, it may involve the extraction of inert (non-efficacious) compounds or the presence of contaminants (e.g., heavy metals or pesticide residues).

The quality of the extract was tested using organoleptic tests. The ethanol soluble extract content, water content, and ash content are listed in Table 1.

Table 1. Quality test of purple cabbage extract

Quality Test	Test Results	Requirements	Interpretation
Organoleptic	Form: Thick extract Color: Blackish purple Odor: Typical purple cabbage	Conforms to specifications	Meets requirements (Merista *et al.*, 2025)
Ethanol soluble extract content	48.8 ± 0.12%	≥ 10–20%	Meets requirements (WHO, 2011)
Ash content	7.37 ± 0.47%	≤ 5% – 15%	Meets requirements (WHO, 2011)
Moisture content	8.69 ± 0.008%	<10%	Farmakope Herbal Indonesia Edisi II (2017)

Organoleptic evaluation included consistency, color, and odor. From the test results, it is known that the purple cabbage extract is thick, blackish purple in color, and has a distinctive purple cabbage odor. The ethanol-soluble extract content of the purple cabbage extract was determined to be 48.8 ± 0.12%. Determination of the ethanol-soluble extract content aims to quantify the amount of active compounds dissolved in ethanol from the extract (Ministry of Health of the Republic of Indonesia, 2000). Ash content testing aims to determine the inorganic mineral content of the extract. Elevated ash content is indicative of a higher mineral load in the material. The ash content in this study was 7.37 ± 0.47%. The ash content is influenced by the type of material, the ashing method, and the temperature and duration of dryin (Erni *et al.*, 2018). A water content test is necessary to determine the amount of water content in the extract. High water content can cause the extract to become susceptible to microbial contamination, including mold and bacteria. From the test results, the water content was 8.69 ± 0.008%. From the quality testing that has been carried out, it can be concluded that purple cabbage extract meets the requirements in Indonesian Herbal Pharmacopoeia and Guidelines for Quality Control Methods for Herbal Materials (WHO).

Phytochemical screening aims to identify secondary metabolites present in the plants under study. Phytochemical screening was performed on flavonoids, alkaloids, phenolics, and saponins. Qualitative tests for secondary metabolites are shown in Table 2.

Table 2. Results of phytochemical screening of purple cabbage extract

Secondary metabolite	**Test Results**	**Interpretation**
Flavonoid	Red	+
Alkaloid	Red-orange precipitate	+
Phenolic	Thick black	+
Saponin	Foam	+

In the flavonoid test, the addition of HCl and Mg powder causes a reduction in the benzopyrone nucleus contained in the flavonoid structure, resulting in a red color indicating a positive flavonoid presence. Purple cabbage extract produces a deep black color, thus positive for phenolic content. This color change is caused by the reaction between the hydroxyl group contained in the phenolic compound and $FeCl_3$. Phenolic form complex compounds with Fe^{3+} ions. In the alkaloid test, Dragendroff's reagent consisting of Bismuth nitrate reacts with potassium iodide to form a bismuth (III) iodide precipitate, which then dissolves in potassium iodide to form a potassium tetraiodobismuthate complex that precipitates as a red-orange potassium alkaloid. The purple cabbage extract is shaken for a while to produce constant foam. This indicates that the extract contains saponins. Saponin compounds contain hydrophilic and hydrophobic compounds, thereby reducing surface tension. When shaken, the hydrophilic group binds with water while the hydrophobic group binds with air. This condition causes saponins to hydrolyze into glucose and other compounds, thus producing a stable foam.

Conclusions

The results of organoleptic testing showed that the extract was thick, blackish purple in color, and had a distinctive purple cabbage odor. The ethanol soluble extract content obtained was 48.8 ± 0.12%, the ash content was 7.37 ± 0.47%, and the water content was 8.69 ± 0.008%. From these results, it can be concluded that the extract meets the quality test requirements in Indonesian Herbal Pharmacopoeia and Guidelines for Quality Control Methods for Herbal Materials (WHO). In phytochemical screening, purple cabbage extract is known to contain flavonoid, alkaloid, phenolic, and saponin.

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Conflicts of Interest

The authors declare no conflict of interest.

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