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Forced Degradation Study of Flavonoid in *Portulaca grandiflora* extract using Spectrophotometric UV-Vis

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ABSTRACT

Exploration of the effectiveness from magenta purslane herb extract in traditional medicine is still ongoing. Flavonoid is the major compound that allegedly have pharmacology effect in magenta purslane herb extract. To ensure the stability of the flavonoid content in the magenta purslane herb extract, a stability test was carried out using forced degradation. The aim of this study was to determine the effect of forced degradation by acid hydrolysis, alkaline hydrolysis, oxidation and heat substanes on the quercetin content of magenta purslane (*Portulaca grandiflora*) herb extract using a UV-Vis Spectrophotometer at a wavelength of 424 nm. The results showed in the form of a linear regression equation y=0.0127x + 0.0437 with a value of r^2 = 0.9912 with a quercetin content of 10.59 % weight on weight (w/w). After being subjected to acid hydrolysis degradation, base hydrolysis, oxidation, and heat, a decrease in flavonoid content was observed of 1.65% w/w, 3.86% w/w, and 2.38% w/w, respectively. It was concluded that statistically there was a significant difference with the decrease content in the acid, base and oxidation forced degradation treatments after 2 consecutive hours, whereas no significant reduction was observed under thermal degradation.

Keywords : forced degradation, hydrolisis, oxidation, purslane herb, quercetin, thermal

INTRODUCTION

Indonesia is a country with great potential of medicinal plants, where there are 90,000 plant species and 9.600 among them have been used for medicinal purposes (Kemenkes RI, 2013). Purslane is a wild plant that is also known as an ornamental plant, has been studied and exhibits various pharmacological potentials. Research by Budiawan and Kirana (2022) reported the analgesic properties of magenta purslane (*Portulaca grandiflora*). Studies on the antioxidant activity of magenta purslane have been conducted by Husnawati and Rispriandari (2020). The antioxidant properties of purslane are attributed to phytochemical compounds from the flavonoid group such as quercetin, kaempferol, and apigenin (Zhou et al., 2015). Phenolic and flavonoid compounds that act as antioxidants can be evaluated based on their ability to scavenge free radicals (Sedjati et al., 2017). Flavonoids, carotenoids, polysaccharides, polyphenolic acids, sterols, and reducing substances are some of the chemical compounds contained in purslane (Zhou et al., 2015). Research by Kirana et al. (2023) indicates that flavonoids, alkaloid, saponin, tannin and terpenoid are phytochemical compounds found in magenta purslane (*Portulaca grandiflora*). Flavonoids are the main chemical compounds in *Portulaca grandiflora* (Husein et al., 2021). Flavonoids belong to the phenolic group of compounds, where quercetin is one of the important bioflavonoids found in most plants (Shraim et al., 2021).

Proper storage is essential to maintain stable concentrations of active compounds, including bioactive extracts, as this directly influences the quality of the preparation (Sugiharta and Ningsih, 2021). Inappropriate levels of these compounds may affect the desired therapeutic effect (Fernanda and Maulidia, 2023). Forced degradation, or stress degradation studies, are conducted to assess compound stability by subjecting them to conditions such as acidic, basic, oxidative, and thermal environments (Ramaswamy et al., 2021).

Research on the degradation of magenta purslane (*Portulaca grandiflora*) herb extract remains limited. To date, no studies have specifically addressed the forced degradation of quercetin flavonoids in purslane (*Portulaca grandiflora*). Therefore, this study aims to investigate the forced degradation of magenta purslane herb and to evaluate its effects on flavonoids. Forced degradation of the flavonoid in magenta purslane (*Portulaca grandiflora*) herb extract was evaluated using UV–Vis spectrophotometry. This method was selected due to the presence of chromophore and auxochrome groups, enabling it to absorb radiation in the ultraviolet region when light passes through the solution. As a result, part of the light is absorbed, while the remainder is transmitted (Puspitasari et al., 2023).

METHODS

The instruments used in this study included a 40-mesh sieve (Sieves), grinder, filter paper, micropipette (Socorex), oven (Memmert UN30), a set of glassware, UV–Vis spectrophotometer (Jasco V-730), sonicator (BK-2000), thermostat, analytical balance (US Solid), and rotary evaporator (Biobase).

The materials used in this study included magenta purslane, methanol pro analysis (p.a.) (Merck), ethanol 96% p.a (Merck), technical-grade 96% ethanol, quercetin standard p.a.(Sigma-Aldrich), distilled water (aquadest), 10% $AlCl_3$ p.a. (Merck), 1 M sodium acetate p.a.(Merck), 0.1 N HCl p.a. (Merck), 0.1 N NaOH p.a. (Merck), and 30% H_2O_2 p.a. (Merck).

Extract Preparation

The extract was prepared by maceration of the magenta purslane herb simplicia using 96% ethanol at a ratio of 1:5 for 2 days. The process was repeated twice using the same remaceration procedure. The combined macerates were collected and concentrated using a rotary evaporator, followed by drying in an oven at 60°C for 24 hours to obtain the magenta purslane herb extract.

Preparation of Standard Solutions

A stock standard solution of quercetin was prepared by dissolving an appropriate amount of quercetin standard in methanol p.a to obtain a concentration of 1000 µg/mL. Working standard solutions were prepared by diluting the stock solution to obtain concentrations of 20, 25, 30, 40, and 50 µg/mL.

Determination of Maximum Wavelength (λmax)

The maximum absorption wavelength (λmax) of quercetin was determined using a 30 µg/mL working standard solution. Absorbance was measured over a wavelength range of 400–800 nm using a UV–Vis spectrophotometer, and the wavelength corresponding to the highest absorbance was selected as λmax.

Measurement of Flavonoid Absorbance

Standard and sample solutions were prepared by mixing 0.5 mL of the test solution with 1.5 mL ethanol p.a, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate, and distilled water to a final volume of 5 mL. The mixture was homogenized and incubated at room

temperature for 30 minutes prior to absorbance measurement at λmax. A blank solution was prepared using 0.5 mL methanol p.a in place of the test solution, followed by the same reagent addition and treatment (Mubarokah and Kusumaningtyas, 2023)

Determination of Quercetin Content in the Extract

A total of 50 mg of magenta purslane (*Portulaca grandiflora*) herb extract was dissolved in 20 mL methanol p.a, sonicated for 30 minutes, and filtered. The filtrate was transferred into a 25 mL volumetric flask and diluted to volume with methanol p.a.

An aliquot of 0.5 mL of the extract solution was mixed with 1.5 mL ethanol p.a, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate, and 2.8 mL distilled water. The mixture was homogenized and incubated for 30 minutes at room temperature. Absorbance was measured at λmax in triplicate.

Forced Degradation Studies

Forced degradation studies were conducted under acidic, basic, oxidative, and thermal conditions, following the method described by Ramaswamy *et al*. (2021). For all conditions, 50 mg of magenta purslane extract was dissolved in 20 mL methanol p.a, sonicated for 30 minutes, filtered, and transferred into a 25 mL volumetric flask.

- **Acidic degradation:** 0.25 mL of 0.1 N HCl was added, and the solution was diluted to volume with methanol p.a.
- **Basic degradation:** 0.25 mL of 0.1 N NaOH was added, and the solution was diluted to volume with methanol p.a.
- **Oxidative degradation:** 0.25 mL of 30% H_2O_2 was added, and the solution was diluted to volume with methanol p.a.
- **Thermal degradation:** the solution was diluted to volume with methanol p.a and heated at 60°C.

All degradation processes were carried out for 2 hours. Following degradation, 0.5 mL of each treated solution was reacted with 1.5 mL ethanol p.a, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate, and 2.8 mL distilled water. The mixtures were homogenized and incubated for 30 minutes at room temperature. Absorbance was measured at λmax in triplicate.

Data Analysis

The paired t-test and Wilcoxon signed-rank test were used to evaluate the statistical significance of the decrease in compound levels, depending on data normality.

RESULTS

Quercetin was used as a reference standard because it is a flavonoid compound capable of reacting with $AlCl_3$ to form a complex (Bangun et al., 2021). The maximum wavelength (λmax) was determined using a 30 ppm solution, and the λmax obtained was 424 nm, with the absorption spectrum shown in Figure 1.

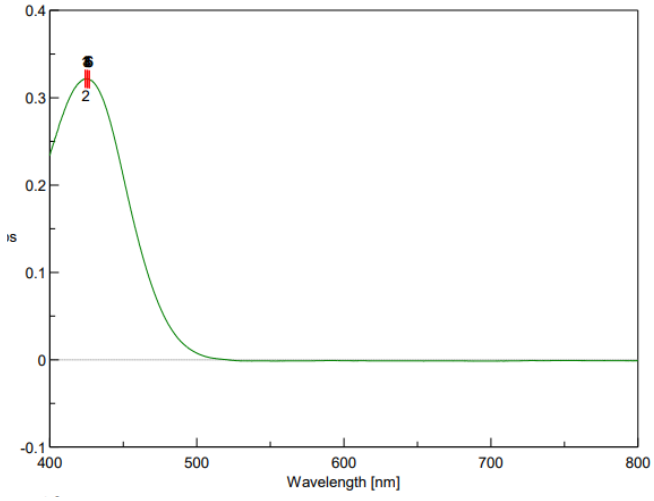

Figure 1. Absorption spectrum of quersetin un the range of 400-800 nm

The quercetin content was calculated by constructing a calibration curve using standard solutions at concentrations of 20, 25, 30, 35, 40, and 50 µg/mL, with absorbance measured at 424 nm. Based on the calibration curve, the linear regression equation obtained was $y = 0.0127x + 0.0437$, with a correlation coefficient (r) of 0.9912, as shown in Figure 2.

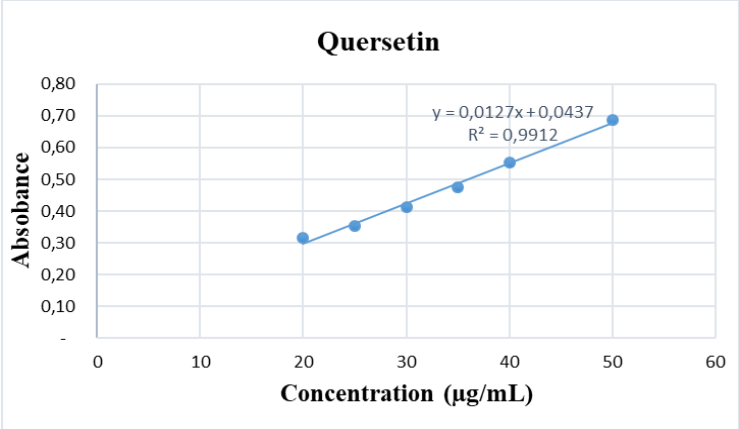

Figure 2. Quersetin calibration curve

Acid hydrolysis was performed using 0.1 N HCl, while base hydrolysis was conducted using 0.1 N NaOH. Oxidative degradation was carried out using 30% H_2O_2, and thermal degradation was performed at 60°C. The flavonoid content, expressed as quercetin, obtained from the forced degradation study is presented in Table 1, which shows statistically significant decreases under several conditions.

Table 1. Forced degradation study

Degradation	Quersetin (%b/b)	
	T0	T2
Acidic	10,59 ± 0,21	8,94 ± 0,05
Basic	10,59 ± 0,21	6,73 ± 0,11
Oxidative	10,59 ± 0,21	8,21 ± 0,25
Thermal	10,59 ± 0,21	10,29 ± 0,20

DISCUSSION

Quercetin is one of the most abundant flavonoid compounds and is widely distributed in plants (Winahyu et al., 2019). Quercetin ($C_{15}H_{12}O_7$) belongs to the flavonol subclass of flavonoids. The determination of quercetin content in magenta purslane herb extract using $AlCl_3$ reagent is based on color complex formation. Measuring absorbance at λmax aims to obtain the maximum absorbance value for each concentration, thereby ensuring optimal analytical results (Winahyu et al., 2019). As shown in Figure 1, absorbance was measured at a wavelength of 424 nm.

The calibration curve exhibited a correlation coefficient (*r*) close to 1, indicating excellent linearity between analyte concentration and absorbance in accordance with the Lambert–Beer law (Mukhriani et al., 2019). This regression equation was subsequently used to quantify quercetin content in the extract.

Forced degradation of quercetin flavonoids in magenta purslane (*Portulaca grandiflora*) herb extract was conducted to evaluate extract stability and ensure quality during storage. This approach also assesses the suitability of the extract for further formulation, particularly its stability under acidic, basic, oxidative, and thermal conditions. Consideration of the chemical structures of both active compounds and excipients is essential to minimize potential interactions, which may arise from reactive functional groups (Alfaridz and Musfiroh, 2020; Patel et al., 2015).

The flavonoid content, quantified as quercetin, obtained from the forced degradation study (Table 1) showed statistically significant decreases under several conditions. Significant differences in quercetin levels were observed over time during acid, base, and oxidative degradation. Acidic conditions (HCl) are known to disrupt the core structure of quercetin, leading to substantial degradation. In contrast, alkaline conditions (NaOH) promote ionization of free hydroxyl groups in flavonoids, accelerating quercetin degradation (Ramaswamy et al., 2021).

Flavonol compounds such as quercetin exhibit bathochromic shifts due to the presence of hydroxyl groups. Oxidative degradation involves electron transfer mechanisms that generate reactive ionic species. Functional groups such as phenols are particularly susceptible to oxidation, forming reactive intermediates that contribute to compound degradation (Ramaswamy et al., 2021). This process may be further accelerated by external factors such as oxygen, light, and trace metals, leading to the formation of highly reactive peroxy radicals that promote quercetin degradation (Patel et al., 2015).

In contrast, thermal degradation did not result in a statistically significant reduction in quercetin content. The flavonoid level decreased slightly from 10.59 ± 0.21% w/w to 10.29 ± 0.20% w/w after 2 hours of heating, which was not statistically significant. Although elevated temperatures can alter physicochemical properties and degrade phenolic compounds (Kapcum and Uriyapongson, 2018), the extent of degradation depends on compound stability and exposure duration. In this study, heating for 2 hours was insufficient to induce significant degradation. Based on the study by Ramaswamy et al. (2021), the quercetin flavonoid content decreased from 97% to 69.44% following heat treatment at 60°C for 30 minutes.

Overall, quercetin flavonoids in magenta purslane (*Portulaca grandiflora*) extract were susceptible to acid, base, and oxidative degradation, while short-term thermal exposure did not produce a significant effect. These findings provide important considerations for the selection of excipients and the optimization of thermal processing conditions in formulations containing magenta purslane herb extract.

CONCLUSION

Based on this study, it can be concluded that the quercetin flavonoid content of magenta purslane (Portulaca grandiflora) herb extract showed significant decreases following 2 hours of forced degradation under acidic, basic, and oxidative conditions, whereas no significant reduction was observed under thermal treatment.

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