# Optimization of enzymatic-assisted ultrasonic extraction process of total flavonoids from *Sedum aizoon* L. and its antioxidant activity

Qin Yin[1, 2a*], Christopher G. Batbatan[1b*]

[1]Department of Biology, Central Mindanao University, Maramag, Bukidnon, 8710, Philippines
[2] College of Biological and Food Engineering, Suzhou University, Suzhou, Anhui, 234000, China

**Abstract:** In order to optimize the enzymatic-assisted ultrasonic extraction method of total flavonoids from *Sedum aizoon* L., the effects of solid-liquid ratio, ethanol concentration, extraction temperature, extraction time, and ultrasound power on the extraction rate were investigated by single factor experiment. The optimum technological conditions for enzymatic-assisted ultrasonic extraction of total flavonoids from *Sedum aizoon* L. were as follows: solid-liquid ratio 1:55 (g/mL), ethanol volume fraction 60%, extraction temperature 45°C, extraction time 25 min, and ultrasound power 150 W. Under these conditions, the extraction rate of total flavonoids from *Sedum aizoon* L. could reach 10.77%. The antioxidant activity of flavonoids from *Sedum aizoon* L. was positively correlated with the concentration, and the greater the concentration, the stronger the antioxidant capacity. At a concentration of 0.96 mg/mL, the scavenging rate of the DPPH• radical reached 67.5%. The scavenging rate of the $ABTs^{+}$• radical reached 55.8% at a concentration of 1.92 mg/mL.

## 1. Introduction

*Sedum aizoon* Linnaeus (*Sedum aizoon* L.) is a perennial succulent herb belonging to the genus Sedum in the family Crassulaceae[1,2], also known as Jingtiansanqi, Feicai, Yangxincai, Jiuxincai, etc in China [3]. They have been extensively disseminated throughout the provinces of Jiangsu, Hebei, Fujian, and other regions [4]. *Sedum aizoon* L. was widely used in folk medicine to prevent and treat hyperlipidemia, hypertension, hemorrhage, palpitation [5], and coronary heart disease [6]. The bioactive chemical composition of *Sedum aizoon* L. includes phenols [7], flavonoids [8], polysaccharides [9], alkaloids, and triterpenoids [10]. Among them, flavonoids and phenolic acid components are the main active components in *Sedum aizoon* L. [11] such as gallic acid, quercetin, kaempferol, myricetrin, luteolin, etc. [12].

*Sedum aizoon* L. is a medicinal and edible plant. *Sedum aizoon* L. flavonoids are one of the most important substances in Sedum leaves. Flavonoids are a group of natural polyphenol substances abundant in vegetables, fruits, grains and tea [13]. Flavonoids refer to a series of compounds formed by linking at least one benzene ring containing phenolic hydroxyl groups on both sides of a central three-carbon atom, and its basic core is 2-phenyl chromone (Figure 1).

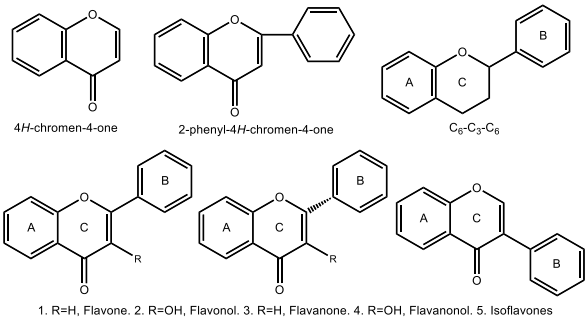


**Figure 1**. Basic structure of flavonoid compounds

Flavonoids are essential in plants, and they are also the main medicinal components of many plants. They have been extensively studied because of their high content, easy availability, and clear pharmacological effects [14]. A large amount of evidence has proved that flavonoids have various physiological activities such as anti-fatigue, anti-oxidation [15], anti-aging, antibacterial [16], anti-inflammatory [17], etc., and these physiological activities are closely related to their own ability to chelate with other substances and their antioxidant properties.

## 2. Materials and methods

### 2.1 Maretials

*Sedum aizoon* L. was specifically sampled from Pei County, Xuzhou City, Jiangsu Province, China. It was

Corresponding author: [a*] s.yin.qin@cmu.edu.ph, [b*] cgbatbatan@cmu.edu.ph

identified and verified by Dr. Anfeng Xiao, who is an expert in botany in Jimei University, China.

The whole herb of *Sedum aizoon* L. was selected, rinsed, and dried in an oven at 50°C for 2 d. The moisture content of *Sedum aizoon* L. was controlled to be between 6 - 10%, and after drying, it was finely ground into a powder with a grinder and passed through an 80-mesh sieve. It was sterilized by UV light, put into vacuum bags, and placed in a desiccator.

### 2.2 Extraction and determination of flavonoids from *Sedum aizoon* L.

10 g of dried powder of *Sedum aizoon* L. was accurately weighted, adding 2% cellulase and citric acid buffer solution, and the pH was adjusted to 4.4 - 4.5. The enzymatic digestion was carried out at 50°C for 6 h with 2% cellulase. After enzyme digestion, inactivate the enzyme in a water bath at 75°C for 10 min. After digestion, the enzymes were inactivated in a water bath at 75°C for 10 min. After cooling, draw and filter, scrape the paste off the filter paper into a beaker. The appropriate concentration of ethanol was added and placed in ultrasound for flavonoid extraction. The extraction process should be sealed to prevent evaporation of ethanol, cooled to room temperature and then extracted, leaving the filtrate.

The initial ultrasonic extraction solution of *Sedum aizoon* L. was placed in a test tube, 0.7 mL of 5% sodium nitrite was added, and 0.7 mL of 10% aluminum nitrate was added after 6 min of reaction, and 5 mL of 10% NaOH and 80% anhydrous ethanol was added to 25 mL. The content of flavonoids in the dried powder of *Sedum aizoon* L. was determined by using the standard curve.

According to the rutin standard curve equation to convert the total flavonoid concentration, according to the formula to calculate the total flavonoid yield

Yield of flavonoids: $W\% = (k \times c \times V) / M \times 100\%$

k: Dilution Factor

c: Flavonoid concentration, mg/mL

V: Volume of flavonoid extract solution, mL

M: quality of dried Sedum aizoon L. powder, g

### 2.3 Plotting of rutin standard curve and determination of total flavonoid yield

10 mg of rutin standard was precisely weighed, completely dissolved in 80% ethanol, and transferred to a 50 mL volumetric flask to obtain 0.2 mg/mL rutin standard solution. Add 0, 0.4, 0.8, 1.2, 1.6, 2.0 mL of rutin standard solution into a 10 mL volumetric flask, add 1 mL of ethanol solution and shake well. Subsequently, 0.15 mL of 5% sodium nitrite solution was added, mixed and left for 6 min, then 0.15 mL of 10% aluminum nitrate solution was added, mixed and left for 6 min, then 2 mL of 4% sodium hydroxide solution was added and shaken well. The final volume was fixed with ethanol solution. The absorbance value was measured at 510 nm after 10 min of exposure to light.

### 2.4 Process optimization of enzymatic-assisted ultrasonic extraction of flavonoids from *Sedum aizoon* L.

The effects of solid-liquid ratio (1:45, 1:50, 1:55, 1:60, 1:65), ethanol concentration (40%, 50%, 60%, 70%, 80%), extraction temperature (35°C, 40°C, 45°C, 50°C, 55°C), extraction time (15 min, 20 min, 25 min, 30 min, 35 min) and Ultrasound power (100 Hz, 125 Hz, 150 Hz, 175 Hz, 200 Hz) on the extraction rate of *Sedum aizoon* L. flavonoids were investigated separately.

### 2.5 Determination of scavenging activity of 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH•) by *Sedum aizoon* L. flavonoids

DPPH is a dark purple large prismatic crystal, while DPPH• is a very stable nitrogen-centered radical [18]. It had the maximum absorption at 517 nm and the absorbance was linear with the concentration. If there is a free radical scavenger present, it will scavenge DPPH•, thus reducing the concentration of DPPH• in solution, the color of the solution will become lighter and the absorbance value at 517 nm will decrease. The more the absorbance value decreases, the stronger the ability of the free radical scavenger.

DPPH standard 20.5 mg was weighed accurately, and the solution was prepared in a 250 mL volumetric flask using 95 % ethanol as the solvent and fixed to a concentration of $2 \times 10^{-4}$ mol/L.

2.0 mL of different concentrations of flavonoid solutions were precisely measured in a 10 mL test tube, 2.0 mL of prepared DPPH• ethanol solution was added, shaken well, placed at room temperature for 30 min, and the absorbance value was measured at 517 nm. 2.0 mL of flavonoid solution was mixed with 2.0 mL of 95 % ethanol and the absorbance value Ab was measured at 517 nm, then 2.0 mL of prepared DPPH• ethanol solution was mixed with 2.0 mL of 95 % ethanol and the absorbance value was measured at 517 nm. The antioxidant activity of DPPH• by *Sedum aizoon* L. flavonoids is calculated as equation (1):

$$\text{Antioxidant activity D (\%)} = 100 - \{[(As - Ab) \times 100]/ Ac\} \quad (1)$$

As: Asample, absorbance of 2.0 mL of DPPH• ethanol solution mixed with 2.0 mL of 95 % ethanol.

Ab: Ablank, absorbance of 2.0 mL of Sedum aizoon L. flavonoid solution mixed with 2.0 mL of DPPH• ethanol solution.

Ac: Acontrol, absorbance of 2.0 mL of Sedum aizoon L. flavonoid solution mixed with 2.0 mL of 95 % ethanol.

### 2.6 Determination of scavenging activity of 2,2'-azinobis -(3-ethylbenzothiazoline-6-sulphonic-acid) (ABTs+•) by *Sedum aizoon* L. flavonoids

15 mL 7 mmol/L of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTs) solution and 0.267 mL140 mmol/L of potassium persulfate solution ($K_2S_2O_8$) were mixed well. The reaction was carried out at room temperature and protected from

light for 24 h. Different mass concentrations of the flavonoids solution were added to the above solution, mixed well, and reacted at room temperature and avoid light. The absorbance values were measured at the wavelength of 734 nm for 15 min. The scavenging activity was calculated as equation (2):

$$\text{Antioxidant activity H (\%)} = 100 - \{[(As - Ab) \times 100]/Ac\} \quad (2)$$

As: Asample, absorbance of the solutions with different concentrations of flavonoids added.

Ab: Ablank, absorbance of the sample solution with double distilled water instead of ABTs.

Ac: Acontrol, absorbance of the sample solution with double distilled water instead of flavonoids.

## 3. Results and Discussions

### 3.1 Plotting of rutin standard curve

The results of the standard curve of rutin were plotted with the mass concentration of rutin (μg/mL) as the horizontal coordinate and the corresponding absorbance value $OD_{510}$ as the vertical coordinate, and the results were shown in Figure 2. The regression equation was obtained: y = 11.138 x + 0.0034. The correlation coefficient $R^2$ = 0.9997 (Figure 2).

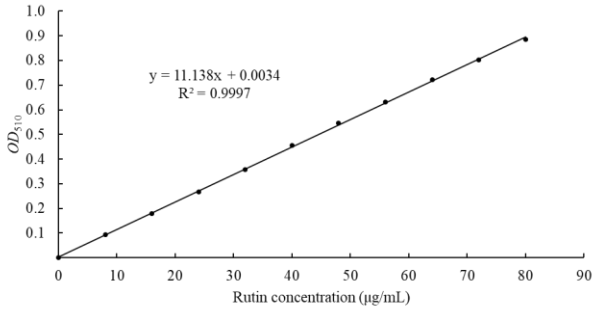


**Figure 2**. Standard curve of rutin

### 3.2 Optimization of enzymatic-assisted ultrasonic extraction of flavonoids from *Sedum aizoon* L.

With the increase of solid-liquid ratio, the extraction amount of total flavonoids from *Sedum aizoon L.* also increased, but when the solid-liquid ratio reached 1:55, the flavonoid content did not increase, but decreased (Figure 3A). This is because the fact that with the increase of solid-liquid ratio, the dissolution of flavonoids in *Sedum aizoon L.* gradually increases, but when the amount of extraction solvent reaches a certain degree, the flavonoids in *Sedum aizoon L.* have been basically dissolved, at this time and then increase the amount of extractant, not only will not increase the dissolution of flavonoids, but may make the amount of dissolution decreased, probably due to too much extraction solvent, which makes the amount of lipid-soluble substances in the extraction solution increased, and the impact on the work of flavonoid extraction increased.

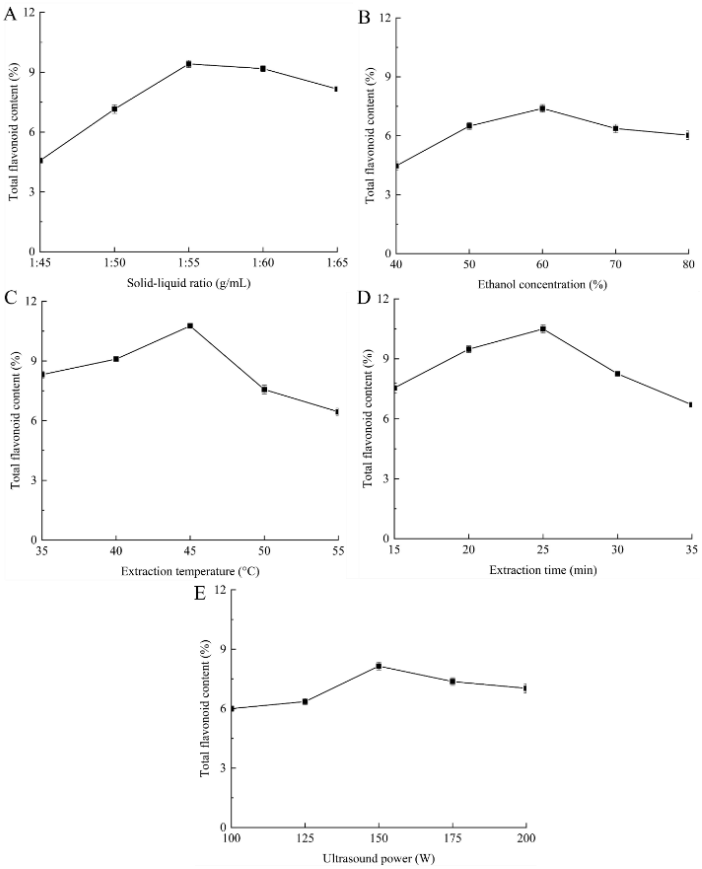


**Figure 3.** Effect of solid-liquid ratio (A), ethanol concentration (B), extraction temperature (C), extraction time (D) and ultrasound power (E) on extraction yield of flavonoids

With the increase of extractant concentration, the flavonoid content in the extract firstly increased, but when the ethanol concentration reached 60% and the extraction of *Sedum aizoon L.* flavonoids reached the peak, the extraction of flavonoids again showed a decreasing trend with the increase of ethanol concentration. It may be due to the fact that flavonoids are generally alcohol soluble, and the polarity of ethanol varies with different concentrations, resulting in different affinities between ethanol and flavonoids. Once the ethanol concentration is too high, the polarity changes, which increases the dissolution of some fat-soluble substances in the extract, thereby affecting the leaching of total flavonoids. After comprehensive consideration, a 60% ethanol solution was selected for subsequent experiments (Figure 3B).

With the increase of the extraction temperature, the extraction amount of flavonoids gradually increased, but when the temperature rose to 45°C, and then increased the extraction temperature, the extraction amount of flavonoids slightly decreased (Figure 3C). It is possible that some flavonoids in *Sedum aizoon L.* were destroyed by oxidation due to the heat of raw materials, and the high temperature led to the loss of solvent volatilization, while the high temperature increased the solubility of other components in *Sedum aizoon L.*, which led to the decrease of flavonoid content. On balance, the extraction temperature of 45°C was chosen as a suitable temperature.

With the increase of time, the extracted amount of flavonoids gradually increased and reached the highest at 25 min, and then the extracted amount of flavonoids showed a decreasing trend with the extension of extraction time. The reason may be that when the time is too short, the flavonoids of *Sedum aizoon L.* are not extracted sufficiently and not completely dissolved, while the time is too long, it may cause some changes in the structure of flavonoids and thus reduce the flavonoid content, so the suitable extraction time of *Sedum aizoon L.* flavonoids is 25 min (Figure 3D).

With the increase of ultrasound power, the yields of the flavonoids of *Sedum aizoon L.* showed a trend of increasing and then decreasing. The maximum yield was obtained when the power was increased to 150 Hz (Figure 3E). This is because ultrasound has a crushing effect on plant cells, and when the ultrasound power is low, the ultrasound has a smaller crushing effect on the cell wall and less flavonoids are leached. With the increase of ultrasonic power, the cavitation effect is enhanced, cell fragmentation is intensified, solvent penetration is increased, and the thermal effect makes the solvent temperature rise, which is conducive to the rapid dissolution of flavonoid substances. However, when the ultrasound power is too high, the molecular structure of flavonoids may be destroyed, the temperature becomes high, and the dissolved impurities will increase, thus reducing the yield of flavonoids.

### 3.3 Determination of scavenging activity of 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH•) by *Sedum aizoon* L. flavonoids

The scavenging rate of DPPH radicals increased with the increase of the concentration of Vc and flavonoids from *Sedum aizoon* L. The scavenging rate was faster at lower concentrations, and then gradually stabilized. The scavenging rate of flavonoids on DPPH radicals reached 65.1% when the content of flavonoids and Vc reached 0.24 mg/mL, while the scavenging rate of Vc on DPPH radicals was 80.7%. With the increase of flavonoid concentration, the scavenging effect on DPPH radicals basically tended to be stable (Figure 4A). The flavonoid extract of *Sedum aizoon* L. was able to provide active hydrogen and bind to the lone electron pair of DPPH radicals, reducing the number of free radicals. As the concentration of flavonoid samples increased, the amount of active hydrogen that could be provided increased and the free radical scavenging rate improved.

### 3.4 Determination of scavenging activity of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic-acid) (ABTs+•) by *Sedum aizoon* L. flavonoids

The scavenging ability of the flavonoid from *Sedum aizoon* L. for $ABTs^{+}$• free radicals was not obvious at lower levels, and the free radical scavenging rate of the tested samples showed an increasing trend with increasing flavonoid concentration. The $ABTs^{+}$• scavenging rate of the extracts increased significantly from 0.08 mg/mL to 0.48 mg/mL, while the increasing trend slowed down from 0.48 mg/mL to 1.92 mg/mL, and the $ABTs^{+}$• scavenging rate reached a maximum of 55.8% (Figure 4B). The flavonoid extracts of *Sedum aizoon* L. have a polyphenolic structure, and the active protons present can bind to the lone electron pair of $ABTs^{+}$• radicals, while they can generate a more stable semi-quinone structure themselves, thus achieving the effect of scavenging free radicals.

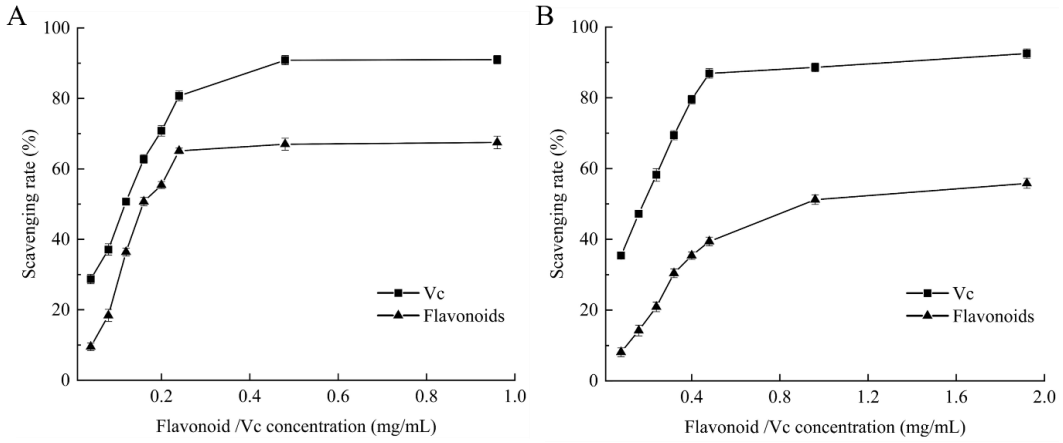


**Figure 4.** Antioxidant ability of flavonoids from *Sedum aizoon* L. evaluated by DPPH• (A) and $ABTs^{+}$• (B)

## 4. Conclusion

Based on the results and discussions presented above, the conclusions are obtained as below:

(1) The optimal technological parameters for the enzymatic-assisted ultrasonic extraction of total flavonoids from *Sedum aizoon* L. were as follows: solid-liquid ratio of 1:55 (g/mL), ethanol volume fraction of 60%, extraction temperature of 45°C, extraction time of 25 minutes, and ultrasound power of 150 W. The total amount of flavonoids extracted from *Sedum aizoon* L. might reach 10.77% under these circumstances.

(2) Flavonoids from *Sedum aizoon* L. had strong in vitro antioxidant activity, and their antioxidant activity was favorably associated with their concentration.

## Acknowledgments

The Scientific Research Platform Open Access of Suzhou University Foundation (2016ykf12) provided funding for this project.

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