Biological Activity of Celery Extract Using Different Extraction Methods

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Abstract. Celery is a medicinal plant that is widely used as an ingredient in traditional medicine in Indonesia. Scientifically, celery extract has the ability as an antioxidant, antibacterial, anti-breast cancer, and inhibiting ACE enzyme activity as antihypertensive. The use of maceration and ultrasound-assisted extraction (UAE) methods will give different results on the amount and biological activity of the extract. This study aims to determine the biological activity of celery extract using maceration and ultrasound-assisted extraction method. TEAC, FRAP, NO, and DPPH scavenging assays were used to determine in vitro antioxidant activities. Folin-Ciocalteu reagent and aluminium chloride colorimetric assay were used to determine total phenolic and flavonoid contents, respectively. The sulforhodamine B and angiotensin-converting enzyme (ACE) inhibition assays were used to determine anti-breast cancer and antihypertensive activities, respectively. The celery extract from both extraction methods had antioxidants activity, inhibited the growth of MDA-MB-231 breast cancer cell line, and showed as antihypertensive by ACE inhibition. The maceration gave better activities than UAE with significant results in antioxidants, especially in a water solvent. The use of solvents had more effect on antioxidant activity, especially the FRAP and DPPH free radical scavenging method, as well as the TPC and TFC values of the extract compared to the extraction technique. It was found that ethanol solvent was better than water. The maceration provided better activities than ultrasound-assisted extraction in anti-breast cancer. However, there was no significant difference in antihypertensives. The UAE extraction method produced higher yields but had lower antioxidant and anticancer activity than a maceration.

Keywords: biological activity · celery · extraction · maceration · ultrasonic

1 Introduction

Celery (*Apium graveolens*) is an annual or biennial herb native to Eurasia and grown in coastal areas. Celery is widely cultivated in temperate climates as a popular garden

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plant and vegetable [1]. Celery is an important medicinal plant throughout the world. This plant is used in the pharmaceutical, food, and ornamental plant industries, causing a fairly high commercial value [2]. All parts of the celery plant such as seeds, leaves, stems, and roots can be used as medicinal plants [2, 3].

The content of its chemical compounds causes the benefits of celery as a medicinal plant. The most secondary metabolite in celery are phenolic compounds, including apigenin as a marker compound [4]. These phenolic compounds are responsible for their several pharmacological activities. Celery has been reported to have pharmacological activities that include antioxidant [5], antibacterial [6], anticancer [6], and antihypertensive [8].

The utilization of extraction techniques is increasingly developing. The use of ultrasonic waves in the extraction process is a development of the traditional maceration extraction technique. However, both extraction techniques have their respective advantages and disadvantages. Maceration has the advantage since it is easy to conduct without special skills. It also allows for parallel extraction with a huge sample. However, this technique has disadvantages in producing more waste because it uses relatively large solvents, has low yield, has longer extraction time, and is not environmentally friendly. On the other hand, the extraction technique using ultrasonic waves has advantages in lower costs, environmentally friendly, and faster processes. However, there are still disadvantages when using this technique, including ultrasonic waves that have the potential to damage some chemical compounds, require further filtration steps, and a repeat extraction process is still needed [9].

The application of ultrasound in food technology for processing, preservation, and extraction is the result of the development process of existing conventional techniques. Ultrasound utilizes different physical and chemical techniques than those applied in conventional extraction, processing, or preservation techniques. Ultrasound offers advantages in the form of better productivity, more selectivity, shorter processing time, improved quality, reduced chemical and physical hazards, and more environmentally friendly [10]. Therefore, this study aims to determine the bioactivity of celery extracts using different extraction methods and solvents.

2 Material and Methods

2.1 Materials

The whole part of celery simplicia was obtained from the Medicinal Plant and Traditional Medicine Research and Development Center, Tawangmangu, Central Java, Indonesia.

2.2 Sample Preparations and Extractions

The sample was mashed using a blender. Maceration extraction using water solvent (SMA) was carried out based on the methods used by [11] with slight modification. Briefly, as much as 10 g of dry celery powder dissolved in 100 mL of distilled water, then stirred at 150 rpm for one hour at room temperature. The supernatant was then filtered with No. 1 Whatman filter paper and dried with a freeze dryer. Maceration

extraction with 70% ethanol (SME) was carried out based on the methods used by [12] with slight modification. Briefly, as much as 10 g of dry celery powder dissolved in 200 mL of 70% ethanol. The mixture was stirred with a magnetic stirrer for 24 h. The solution was filtered with No. 1 Whatman filter paper and dried using a rotary vacuum evaporator at 40 °C.

Ultrasound-assisted extraction (UAE) method was carried out based on the method used by [13] with slight modification. A total of 10 g of dry celery powder was dissolving in 200 mL of distilled water (SUA) and 70% ethanol (SUE) in an Erlenmeyer flask. The flask was then put in a sonication bath at 25 °C for 30 min in three replications with a fixed transducer frequency of 42 kHz. The solution was filtered with No. 1 Whatman filter paper and dried using a rotary vacuum evaporator at 40 °C for 70% ethanol solvent and a freeze dryer for water solvents.

2.3 Antioxidant Assays

2.3.1 DPPH Scavenging Assay

The antioxidant activity test used the α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical reduction method according to [14]. The samples and DPPH dissolved in methanol. As much as 40 μL of 1 mM DPPH and 100 μL of 2 mg/mL stock sample solutions were pipetted into a 96-well microplate. As much as 60 μL methanol was added to gain a 1 mg/mL sample concentration in 200 μL of the final volume. As a blank, 40 μL of DPPH solution was added with methanol up to 200 μL. All samples of the test solution and blank were incubated at room temperature for 30 min and then measured at a wavelength of 515 nm.

$$\%\,\text{Inhibition} = (\text{A} - \text{B}/\text{A}) \times 100\%$$

A is blank absorption
B is sample absorption

2.3.2 NO Scavenging Assay

The NO scavenging assay was carried out based on the methods used by [15] with slight modification. Briefly, 60 μL of 1 mg/mL extracts (or only extract solvent for the blank) were pipetted into a 96-well plate. After that, 60 μL of 10 mM sodium nitroprusside in phosphate-buffered saline was added to each well. The plate was incubated at room temperature for 150 min. Finally, an equal volume of Griess reagent was added to each well. The absorption of all samples and blanks was measured at 546 nm.

$$\%\,\text{Inhibition} = (\text{A} - \text{B}/\text{A}) \times 100\%$$

A is blank absorption
B is sample absorption

2.3.3 TEAC Assay

Trolox equivalent antioxidant capacity (TEAC) assay was carried out based on the methods used by [16] with slight modification. Briefly, $ABTS^{\bullet+}$ radical cation is generated

by a reaction of 7 mM 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) with 2.45 mM potassium persulfate. The reaction mixture was allowed to stand in the dark for 16 h at room temperature. The $ABTS^{\bullet+}$ solution was diluted with ethanol to give an absorbance of 0.700 ± 0.050 at 734 nm. Ten microliters of diluted sample mixed with 190 μL of diluted $ABTS^{\bullet+}$ solution (final concentration of samples was 1 mg/mL). The mixture was allowed to stand for 6 min at room temperature then the absorbance was immediately recorded at 734 nm. Trolox solution (final concentration 8–40 μM) was used as a reference standard. The results are expressed as μM trolox equivalent/mg dry weight extract.

2.3.4 FRAP Assay

The ferric reducing antioxidant power (FRAP) assay was carried out based on the methods used by [17]. Briefly, the FRAP reagent was prepared from 300 mmol/L, pH 3.6 sodium acetate buffer, 10 mmol/L TPTZ solution in 40 mmol/L HCl and 20 mmol/L $FeCl_3$ solution in proportions of 10: 1: 1 (v/v), respectively. Fifty microliters of 1 mg/mL sample were added to 1.5 mL of the 37 °C warmed FRAP reagent. The absorbance of the reaction mixture was recorded at 593 nm after 4 min incubation. A 100–2000 mmol/L $FeSO_4$ solution was used as the standard curve. The results were expressed as μM Fe (II) equivalent/mg dry weight extract.

2.3.5 Total Phenolic and Flavonoid Contents

The measurement of total phenolic content using the Folin-Ciocalteu (FC) reagent refers to [18]. The gallic acid solutions as a standard curve were prepared in distilled water with a concentration series of 6.25, 12.5, 25, 50, and 100 μg/mL. As much as 20 μL of 1 mg/mL sample or gallic acid and 100 μL of 0.2 N FC reagent were added to the 96-well microplate. After 5 min of incubation, as much as 80 μL of 7.5% sodium carbonate was added to the microplate and then incubated in a dark place at room temperature for 2 h. The absorbance was measured at 760 nm using a microplate reader. TPC values are described as mg gallic acid equivalents (GAE) per g of dried extracts.

The measurement of total flavonoid content using aluminium chloride colorimetric assay refers to [19]. The quercetin solutions as a standard curve were prepared in ethanol with a series of concentrations of 0.5, 1, 2.5, 5, 10, and 25 μg/mL. As much as 50 μL of 1 mg/mL a sample and quercetin for standard curve, 10 μL of 10% aluminium chloride solution, and 150 μL of 96% ethanol were added to the 96-well microplate. As much as 10 μL of 1 M sodium acetate was added to the microplate. The mixture was shaken for a few seconds and incubated at room temperature in a dark room for 40 min. The absorbance was measured at 415 nm using a microplate reader. TFC values are described as mg of quercetin equivalents (QE) per g of dried extracts.

2.3.6 Cytotoxic Activity Assay

The cells used in this study were the MDA-MB-231 Breast cancer cell line (ATCC). The cells were maintained at 37 °C under 5% CO_2 and 100% humidity. The cells cultured in DMEM supplemented with 10% fetal calf serum, 1% streptomycin, and 0.5% fungizone.

When the cells reached confluence, they were detached using 0.2% (w/v) trypsin and transferred to new culture flasks. After sufficient growth for experimentation, the cells were trypsinized and plated in 96-well culture plates at a concentration of 1.5 × 104 cells/well. Each well contained 50 μL of cell and 100 μL of DMEM medium, and the plates were incubated for 24 h at 37 °C under 5% CO_2 to obtain a monolayer culture. After 24 h of incubation, the cells were then treated with the extract 50 μg/mL.

The Sulforhodamine B (SRB) assay was used for cell density determination, based on the measurement of cellular protein content [20]. The method described here has been optimized for the toxicity screening of the compounds to adherent cells in a 96-well plate. After an incubation period, cell monolayers were fixed with 1% glutaraldehyde in PBS, then washed using aquadest three times, and incubate for 20 min. Add 50 μL SRB stained for 20 min; then, the excess dye was removed by washing the cells repeatedly with 1% (v/v) acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution for optical density (OD) determination at 510 nm using a microplate reader.

2.3.7 Antihypertensive Assay

Spectrophotometry-based angiotensin-converting enzyme (ACE) inhibition was used for antihypertensive activity according to [21]. The extracts were prepared in DMSO: borate buffer (at pH 8.2) at 0.5: 9.5, then sonicated for one hour. Hippuryl-L-Histidyl-L-Leucine (HHL) substrate (2.5 mM) was prepared in a borate buffer. As much as 125 μL of extract, 50 μL of HHL, and 25 μL of the ACE enzyme were mixed. The final extract concentration used in this study was 1 mg/mL. After incubation at 37 °C for 30 min, the reaction was arrested by the addition of 200 μL of 1 M HCl, then 400 μL of pyridine was added, followed by 200 μL of Benzene sulfonyl chloride (BSC). The mixture was mixed by inversion and left for 10 min in an ice bath. The absorbance was measured at 409 nm. The blank was prepared by adding 125 μL of the buffer instead of the inhibitor sample, while the control was prepared by adding ACE before the addition of 200 μL of HCl. ACE inhibition was expressed as percent inhibition and calculated using the following equation:

$$\text{ACE inhibition } (\%) = (\text{A blank} - \text{A sample})/(\text{A blank} - \text{A control}) \times 100$$

where: A blank is the absorbance of a blank, A sample is the absorbance of a sample; A control is the absorbance of the control.

2.3.8 Statistical Analysis

Statistical tests were performed by one-way ANOVA ($P < 0.05$) and Duncan's post-hoc test ($P < 0.05$) to determine the significance between treatments.

3 Results

3.1 Sample Extraction

The extraction yields using the maceration method with 70% ethanol and water were 31.10 and 15.00%, respectively. The extraction yields using the UAE method with 70% solvent and water were 35.00 and 17.40%, respectively.

Table 1. Antioxidant Activity of Celery Extracts

No.	Sample Codes	Antioxidant Activity			
		TEAC (μM Trolox equivalent/mg extract)	FRAP (μM Fe (II) equivalent/mg extract)	NO scavenging (%)	DPPH scavenging (%)
1	SMA	$14.06^{c} \pm 0.56$	$105.19^{a} \pm 0.86$	$32.48^{*} \pm 2.02$	$40.23^{b} \pm 2.28$
2	SME	$12.06^{a} \pm 0.55$	$126.79^{d} \pm 2.47$	$34.38^{*} \pm 1.08$	$72.28^{c} \pm 0.73$
3	SUA	$12.40^{ab} \pm 0.64$	$115.43^{b} \pm 1.48$	$31.39^{*} \pm 1.66$	$36.16^{a} \pm 0.15$
4	SUE	$13.02^{abc} \pm 0.94$	$120.12^{c} \pm 2.97$	$31.61^{*} \pm 1.03$	$72.23^{c} \pm 0.24$

* Not significant at the 0.05 level one-way ANOVA. The numbers in the column followed by the same letter are not significantly different at the 0.05 level of Duncan's post-hoc test. Extracts concentration at 1 mg/mL.

3.2 Antioxidant Assays

There were no significant differences in antioxidant activity between treatments in the TEAC method. Maceration treatment with ethanol solvent was not significantly different from ultrasound-assisted using 70% ethanol and water. Similar results were also seen between ultrasound-assisted treatment using 70% ethanol and maceration using water. However, there were significant differences between maceration extraction using water compared with maceration using 70% ethanol and ultrasound-assisted using water as a solvent. In the FRAP method, all treatments showed significant differences in antioxidant activity in terms of the extraction technique and the solvent used. Maceration extraction with 70% ethanol has the highest antioxidant activity, while water has the lowest. In the DPPH scavenging method, there were significant differences in the solvents used in each extraction technique and between extraction techniques, except for 70% ethanol as a solvent between maceration and ultrasound-assisted. Meanwhile, there were no significant differences in all treatments in the NO scavenging method (Table 1).

3.3 Total Phenolic and Flavonoid Contents

Total phenol and flavonoid contents results are present in Table 2. Total phenol and flavonoid contents of celery extract followed the same pattern. There were significant differences in the use of solvents in the same or different extraction techniques. But there were no significant differences when using 70% ethanol as a solvent between maceration and ultrasound assisted.

3.4 Cytotoxic Activity Assay

In anti-breast cancer screening, celery extract from extraction using ultrasound-assisted did not show any activity to inhibit the growth of the cancer cells line. The growth inhibition of the cancer cell line only occurred from the extract obtained by maceration

Table 2. Total Phenolic and Flavonoid Content of Celery Extracts

No.	Sample Codes	TPC (mg GAE/g extract)	TFC (mg QE/g extracts)
1	SMA	$26.52^{b} \pm 0.18$	$1.52^{b} \pm 0.13$
2	SME	$33.54^{c} \pm 0.96$	$2.45^{c} \pm 0.11$
3	SUA	$24.39^{a} \pm 0.42$	$0.90^{a} \pm 0.17$
4	SUE	$33.75^{c} \pm 1.08$	$2.44^{c} \pm 0.03$

The numbers in the column followed by the same letter are not significantly different at the 0.05 level of Duncan's post-hoc test.

Table 3. Growth Inhibition Activity of Celery Extracts on MDA-MB231 Breast Cancer Cells

No.	Sample Codes	Cancer growth inhibitions (%)
1	SMA	$3.77^{b} \pm 1.06$
2	SME	$9.8^{c} \pm 1.06$
3	SUA	$0^{a} \pm 0.00$
4	SUE	$0^{a} \pm 0.00$

The numbers in the column followed by the same letter are not significantly different at the 0.05 level of Duncan's post-hoc test. Extracts concentration at 50 μg/mL.

Table 4. Antihypertensive Activity of Celery Extracts

No.	Sample Codes	ACE inhibitions (%)
1	SMA	$63.8^{*} \pm 5.71$
2	SME	$70.8^{*} \pm 1.10$
3	SUA	$72.1^{*} \pm 1.10$
4	SUE	$68.9^{*} \pm 4.40$

* Not significant at the 0.05 level one-way ANOVA. Extracts concentration at 1 mg/mL.

extraction. The use of 70% ethanol solvent in the maceration technique showed better anticancer activity than water, although both still showed weak inhibition against the MDA-MB231 breast cancer cell line (Table 3).

3.5 Antihypertensive Screening Assay

In antihypertensive screening, all extracts had the ability to inhibit ACE activity. However, the differences in extraction techniques and solvent types were not statistically significant (Table 4).

4 Discussion

In both extraction methods, 70% ethanol utilized as a solvent gave a higher yield value than the water. It can happen because the compounds in celery are more attracted to 70% ethanol solvent than water. The combination of water and organic solvents can facilitate the binding of soluble compounds in both solvents. So, they attract more compounds than ethanol or water alone [22]. In addition, the use of the UAE method can attract more compounds than conventional maceration methods. The physical and chemical properties of materials treated with ultrasound will change because the propagation and interaction of sound waves will damage plant cell walls. So, it will increase the release of extracted compounds inside plant cells [23].

NO reacts with superoxide radicals (O2-) to produce peroxynitrite (ONOO-). It is a reactive oxidant that reacts with proteins, lipids, and DNA, causing cell and tissue damage. Sodium nitroprusside (SNP) is commonly used as a NO donor both in vivo and in vitro experiments. SNPs are stable in the solid state, but in an aqueous solution produce NO which can interact with oxygen to produce nitrite ions as a stable final product. The first step of this reaction is the release of •NO from the SNP which is unstable in an aqueous solution. •NO can be oxidized to nitrite when reacting with water and oxygen. So that, it was can be calculated by reacting it with Griess reagent. In the presence of antioxidant compounds, the NO released will be scavenged by antioxidants, resulting in the formation of less nitrite [24].

DPPH free radical scavenging method was developed to determine antioxidant activity using the stable free radicals 2, 2-diphenyl-1-picrylhydrazyl. This assay is based on measuring the antioxidant capacity of a compound against free radicals. Odd electrons of nitrogen atoms in the DPPH structure are reduced by accepting hydrogen atoms from antioxidant compounds to form a hydrazine structure. The DPPH reaction is indicated by a purple color when reacted with methanol. Mixing a DPPH solution with a substance that can donate hydrogen atoms will result in a reduced form with the loss of purple color and turning yellow [25]. Our results are consistent with previous studies which reported that *Crataegus meyeri* leaf ethanolic extract had better antioxidant activity than water in DPPH assay [26].

TEAC method used ABTS as a target molecule to evaluate antioxidant activity. ABTS is a target molecule used to evaluate the reactivity of antioxidant samples in the presence of peroxides. ABTS undergoes oxidated with potassium permanganate, potassium persulfate, or 2,2′-azo-bis (2-amidinopropane) to produce the radical cation of the ABTS (ABTS•+) with a blue-greenish color. These radicals can accept electrons and H• from antioxidant compounds present in the sample, causing the reduction of ABTS•+ to ABTS and causing a change in the color of the reaction mixture [26]. FRAP method measures the ability of antioxidants to reduce ferric iron. It is based on the reduction of the complex of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride $[Fe^{III}(TPTZ)]^{3+}$ to an intense blue colored ferrous form $[Fe^{II}(TPTZ)]^{2+}$ at low pH [28]. Our results indicated that solvents with different polarities have significant effects on antioxidant activities [29].

The antioxidant activity based on the TEAC value of the 70% ethanol extracts in this study was much higher than in the previous report. The ethanol extracts of celery from Thailand had TEAC values of 0.18 μM trolox equivalent/mg extract [29]. The same thing

happened to the FRAP method. Previous studies reported that the methanol extract of celery only had a value of 2.6 M Fe (II) equivalent/g extract [31]. Although the antioxidant activity through DPPH scavenging in this study had higher activity than in previously reported studies, the antioxidant activity using the NO scavenging method had lower activity. Water extracts of celery at concentrations of 10 mg/mL had DPPH scavenging activity at 25.20% while ethanolic extract of celery had NO scavenging activity of 36.66% at a concentration of 0.8 mg/mL [31, 32]. Differences in antioxidant activity that occur can be influenced by geographical location, growing conditions, extraction method, and solvent system used [33, 34].

Like the measurement of antioxidant activity, total phenolic and flavonoid contents were influenced by the type of solvent and extraction method. From both extraction methods, the highest results of this study was ethanolic extract that had lower levels of total phenols and flavonoids compared to previous studies conducted by [5]. The study reported that ethanol extract of celery had total phenol content of 36.60 mg GAE/g extract, while flavonoid levels was 55 mg QE/g sample. In general, the antioxidant activity of an extract is related to its phenolic compound composition. In addition, the phenolic content of a plant extract is also influenced by the solvent system. These indicated that solvents with different polarities had significant effects on total phenol contents [29]. Therefore, the selection of a suitable solvent is very important for the study of antioxidants, the determination of the phenolic and flavonoid content of a plant extract.

The content of phenolic compounds such as flavonoids, coumarin derivatives, and others contained in certain plant materials is known to ward off oxidative stress in the human body by helping to maintain a balance between oxidants and antioxidants. Oxidative stress is a condition when the content of oxidants and free radicals in the body is more than antioxidants [36]. Antioxidants protect the body from free radicals like Reactive Oxygen Species (ROS). Reactive Oxygen Species (ROS) such as superoxide anions (O_2), hydroxyl (-OH), peroxyl (ROO-), alkoxyl radicals (RO-), and hydrogen peroxide (H_2O_2) will attack proteins, lipids, and or make DNA damage thus causing disease [37].

The maceration technique and the type of solvent affect the extraction process, the type of chemical compound, and the biological activity of the extract [34]. In this study, we use two different types of extraction and solvents. There were significant differences between extraction techniques when using water as a solvent (but not in ethanol) at the antioxidant activity, TPC, and TFC assays. This result is in line with previous studies that reported no significant differences between maceration and UAE in antioxidant activity, TPC, and TFC of *Eruca sativa* ethanol extract [38]. The ethanol extract had better antioxidant activity, TPC, and TFC than the water extract. It was because the ethanol extract has more active compounds dissolved/extracted in the ethanol solvent instead of using water as a solvent [38], so it will affect the biological activity.

Breast cancer is a predominant health problem for women. Epidemiological observations and experimental studies have shown that food composition can influence the development and course of the disease. Consumption of fruit and vegetables has been suggested for cancer prevention, and previous studies suggest a link between celery intake and a reduced risk of cancer [7]. Breast cancer cases are the type of cancer with

the highest number of sufferers in Indonesia, reaching 19.18% of the total population or about 34.30% of the all-female population in Indonesia [40]. The results in this study were in line with previous studies that reported that celery extract had anticancer activity in the MCF-7 breast cancer cell line [41]. Anticancer activity results also showed that the maceration method had better activities than UAE. The results of anticancer activity in this study were in line with previous studies that reported that extracts from maceration had better growth inhibitory activity against HepG2 cell lines compared to the UAE [38].

Some compounds that act as anticancer in celery include flavonoid compounds, such as apigenin [42]. So far, in vitro and in vivo studies have reported the mechanism of apigenin in inhibiting the development of breast cancer cells. These mechanisms include the induction of apoptosis and cell cycle arrest, inhibition of fatty acid synthase, aromatase, tumor angiogenesis, and anti-invasive metastases [43]. In addition, the anticancer activity of an extract is related to its ability as an antioxidant. In addition to the antioxidant activity itself, antioxidant agents have anticancer abilities through several mechanisms: including antiangiogenic effects, reducing extracellular matrix, inhibition of matrix metalloproteinases, apoptosis-inducing activity, and epigenetic mechanisms [44]. In this study, the extracts were not tested on normal cells because it is still in the screening stage. Therefore, further testing is needed to determine the toxicity of these extracts on normal cells.

Increased blood pressure, especially hypertension (systolic pressure), which poses a significant cardiovascular risk, is an important public health problem. Blood pressure can affect by blood volume, the amount of blood pumped by the heart per minute, and arterial tone balance [44]. Maintaining blood pressure levels involves various elements of the integrated neurohumoral system. It includes the renin-angiotensin-aldosterone system (RAAS), natriuretic peptides, the endothelium, the sympathetic nervous system (SNS), and the immune system. Any disruption to any of the factors involved in blood pressure control can directly or indirectly increase blood pressure [46].

One of the blood pressure regulatory systems, the renin-angiotensin system (RAS), involves two key enzymes, the angiotensin-I converting enzyme (ACE) and renin [47]. The first action in this system is that angiotensinogen is converted to angiotensin-I by the hydrolysis of renin and then broken down by ACE to produce angiotensin-II to control blood pressure [48]. The inhibition of ACE will decrease the formation of angiotensin II and increase the concentration of bradykinin as a vasodilator [49]. Several studies have shown that celery can be antihypertensive by inhibiting ACE activity in vitro [50]. Luteolin and apigenin, the flavonoid compounds in celery, can act as an antihypertensive by inhibiting ACE activity [51].

As with anticancer activity, antihypertensive also has a relationship with antioxidant activity. Under normal conditions, the pro-oxidant activity will be balanced by the presence of anti-oxidative agents. When this condition is disturbed by an uncontrolled environment, various pathological disorders, such as hypertension and another vascular disease can arise [52]. Reactive oxygen species (ROS), which consist of superoxide anions (O2•-) and hydroxyl ions (OH-), play a major role by creating an oxidative stress environment that can trigger hypertension [53]. Therefore, if an extract has antioxidant activity, it indirectly also has the potential to reduce the risk of hypertension.

The celery extract obtained from different extraction techniques and solvents had ACE inhibition activity with no significant difference. These results were different from previous studies that reported that several extracts of native plants from the Extremadura region (Spain) produced from the UAE had higher ACE inhibitory activity than those extracted using agitated maceration [54]. Several traditional medicine industries for standardized herbal medicine and phytopharmaca in Indonesia also use celery as a single ingredient or a mixture with other medicinal plants as an antihypertensive product. However, the claims included in these studies are through the mechanism of increasing nitric oxide, diuretics, and Ca blockers. Therefore, the activity of celery extract as an inhibitor of ACE activity is very promising to proceed to the next stage in pre-clinical and clinical trials. In addition, the application of alternative extraction techniques using ultrasonic waves in this study can increase the yield of extracts with antihypertensive properties that are equivalent to conventional extraction techniques. In the future, these findings are expected to open up opportunities for the application of ultrasonic waves in industrial-scale extraction processes.

5 Conclusion

The water and 70% ethanol extract of celery have antioxidant, anticancer, and antihypertensive activities. The application of ethanol as a solvent provides better biological activities compared to water. The maceration provides better activities than ultrasound-assisted extraction in anti-breast cancer. However, there was no significant difference in antihypertensive activity. The UAE extraction method produces higher yields but has lower antioxidant and anticancer activity than a maceration.

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