

# The Capability of Sedative Effect from Celery (*Apium graveolens* L.) Fraction to Male Mice

*By Arum Setiawan*



## The Capability of Sedative Effect from Celery (*Apium graveolens* L.) Fraction to Male Mice

Shinta Kusuma<sup>1\*</sup>, Arum Setiawan<sup>2</sup>, Salni<sup>2</sup>

<sup>1</sup>Department of Biosciences Program, Faculty of Science, Sriwijaya University, Jalan Padang Selasa 524, Palembang, South Sumatra 30139, Indonesia.

<sup>2</sup>Department of Biology, Faculty of Mathematics & Natural Sciences, Sriwijaya University. Jalan Raya Palembang-Prabumulih km 32, Indralaya, Indonesia.

\*Corresponding author

E-mail address: shintakusuma@gmail.com (Shinta Kusuma).

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### Abstract

*Celery (Apium graveolens L.)* was carried out to determine the ability of the sedation effect of celery fraction compared to celery extracts which have been known to have the ability to effect the previous sedation. This study aims to find out which fraction has the best sedation effect. This study was an experimental study with a Completely Randomized Design (CRD) consisting of 5 treatments and 5 replications. Test animals divided into 5 treatment groups namely negative control group (CMC Na 1%), celery extract group 200mg/kg and 3 treatment groups n-hexane fraction, ethyl acetate, and methanol water fraction with a dose of 200 mg/kg. The sedation effect test was carried out using the Traction Test and Fireplace Test methods. Quantitative data observed were the length of time the mice fell and the length of time the mice went out of the heated tube/glass. The results of the analysis showed that the celery fraction had a better sedation effect than the extract, and the methanol water fraction 200 mg/kg was the most effective fraction in causing sedation effects.

Keywords: Sedation, *Apium graveolens* L. Traction test, Fireplace test.

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### 1. Introduction

Sleep has the same necessity as eating healthy food and exercising to maintain health and stamina. Deep sleep can reduce stress, increase productivity and endurance [1]. If we have good sleep then we will have enough energy to do all our activities the next day. Loss of sleep for a while does not cause significant health problems, but if sleep disturbances occur over a long period of time it can interfere with health such as reduced concentration, alertness and decreased accuracy, slowing reaction time, changes in behavior and frequent appearance of double vision [2][3].

Most people overcome this problem by using chemical drugs that can accelerate the desire to sleep and prolong sleep (sedative - hypnotic). This sedative drug is usually given at night. If these substances are given during the day in lower doses for calming purposes, they are

called sedatives [4]. The use of these chemical drugs has side effects that are not good for health if consumed too often and in the long run such as hypertension, dependence, liver degeneration, coma to death [5].

The use of medicinal plants that can be one of the alternative treatments has been done, even the World Health Organization (WHO) supports the use of medicinal plants in health care because of the enormous potential in them [6]. As an alternative to overcome the side effects of the use of sedative drugs, the use of medicinal plants can be used, one of which is celery (*Apium graveolens* L.) which is a plant of the Apiaceae family. All parts of the celery plant can be used as medicine including leaves, stems, herbs, roots and seeds [7]. Celery has active compounds including alkaloids, flavonoids, and saponins, these active compounds are compounds that have the potential for sedation effects. All parts of celery contain apiin glycosides (flavone glycosides), isoquersetin and umbelliferon and essential oils [8].

There are several studies regarding celery leaves



including celery leaves which have antibacterial power against *Staphylococcus aureus* and *Escherichia coli* [9]. Herba celery as a medicine for sweating sweat, lowering fever, rheumatism, insomnia (insomnia), high blood pressure, gout, and improve the function of disrupted blood, but also can function as anti-inflammatory [10].

In previous studies, it was stated that celery seed extract has a sedative effect [3]. Other studies also stated that the ethanol extract of celery had the effect of sedation in mice and at a dose of 200 mg /kg showed the highest sedation effect.

Based on the description above, the researcher wants to know the ability of the sedation effect of celery fraction compared to celery extract which has known the effect of previous sedation effects. This study also aims to determine which fraction has the best sedation effect in the same dose.

## 2. Materials and Methods

### 2.1 Tools and Materials

The tool used in this study is a plastic tub measuring 30 x 25 x 10cm which is equipped with wire netting as a mouse cage, drink bottles, syringe gavage, measuring cups, beaker, analytical balance, Ohaus balance, stop watch, plastic bottles, sieves, and blenders, aluminum foil, gloves, masks, tissue, rotary evaporators, *traction test* devices, hot plates (*fireplace tests*), separating funnels, Erlenmeyer, filter paper, 1000 ml separating flask, water baths, petri dishes, support poles, stirring rod, pipette, stationery, camera.

The materials used were mice (*Mus musculus* L.) Sub-Swiss Webster strain, celery, 96% ethanol, aquadest, phenobarbital, n-hexane, ethyl acetate, methanol, 1% CMC (Carboxy Methyl Cellulose) Na, faucet water, husks and standard feed in the form of pellets.

### 2.2 Animals

The test animals used in this study were male (*Mus musculus* L.) mice of Sub-Swiss Webster strain that were 2-3 months old with a weight of 20-30 grams totaling 24 heads, obtained from the Pharmacology Laboratory of the Department of Pharmacy Faculty of Mathematics and Natural Sciences Bandung Institute of Technology.

### 2.3 Procedures

#### 2.3.1 Collection of Sample

Sample was a simplicia of celery herb (*Apium graveolens* L.) obtained from the Center for Tropical Biopharmaceutical Studies, Bogor Agricultural University.

#### 2.3.2 Extraction

Making celery extract using maceration method using 96% ethanol solvent. Celery powder is put into a well-closed container and protected by light and 96% ethanol is added until it is completely submerged for the first 6 hours

while stirring occasionally, then let stand for 18 hours. The maceration process is carried out for 5 days and 3 repetitions. Maserate is filtered and evaporated in vacuo with a rotary evaporator to obtain a thick extract.

#### 2.3.3 Fractionation

Ethanol 96% extract of celery herbs fractionated with hexane in a separating funnel, whipped enough. After that it is left until 2 layers are formed, namely the hexane layer and the water layer. This treatment was repeated several times until the hexane layer was clear so that the hexane fraction was obtained. The water layer was then fractionated with ethyl acetate and repeated several times as the treatment above so that the water fraction and ethyl acetate fraction were obtained. All hexane, ethyl acetate and water fractions were evaporated in vacuo by rotary evaporator until a thick fraction was obtained. Each extract and fraction added CMC Na 1%. Then dissolved using a small amount of aquadest, then put it in a measuring flask and added with distilled water until it reaches 100 ml, then shake it in one direction until the solution is homogeneous. Each extract and fraction will be tested for the effect of sedation on test animals. The amount of extract taken in 1 gavage is 0.01 ml for every 1 gram of body weight of mice.

### 2.4 Phytochemical Screening

#### 2.4.1 Alkaloid Test

Ethanol extract is added with solution 1% ammonia base and chloroform in the test tube, shaken, then the chloroform layer is pipetted and HCl 2 N is added and then shaken again. The solution obtained was divided into two, each of which was supplemented with Mayer and Dragendorff reagents. Positive results, Mayer reagents cause white deposits and Dragendorff reagents cause turbidity and orange deposits (brick red) [11].

#### 2.4.2 Triterpenoid and Steroid Tests

The extract was dissolved in chloroform, then acetic acid inhydride was added. Next, it drops 3 drops of concentrated sulfuric acid through the tube wall. Positive results for steroid compounds are the emergence of bluish green color while for triterpenoid compounds positive results are indicated by the appearance of brownish or violet red color [12].

#### 2.4.3 Flavonoid Test

A 2 ml extract was added with a small amount of magnesium powder and 2 ml of HCl 2 N. The positive result was that the solution changed color to orange to red [12].

#### 2.4.4 Tannin Test

Extracts in a test tube were added a few drops of 1% FeCl<sub>3</sub> solution. Color changes to dark blue indicate the presence of tannins [12].

#### 2.4.5 Saponin Test

The extract was put into a test tube, then added 10 mL of hot water, after which it was cooled and shaken vigorously for 10 minutes until a solid foam formed for no less than 10 minutes as high as 1 cm to 10 cm. on the addition of 1



drop of 2 N hydrochloric acid, the foam does not disappear, so there may be saponins [12].

### 2.5 Sedative Activity

Male mice (*Mus musculus* L.) Sub-Swiss Webster strain were adapted for 7 days. Randomization of mice in groups, namely 1 negative control group (CMC Na 1%), 1 group of celery extract dose of 200 mg/kg, and 3 groups of celery fractions namely n-hexane, ethyl acetate, and methanol water each with a dose of 200 mg/kg.

Before treatment the mice are weighed first. After that, the preparation is given orally. Furthermore, mice were put back in the cage and observed and then waited until the mice were no longer active or even asleep. In the 15th, 30th, 60th, 120th minutes after oral administration, the mice were tested for sedative effects using the *Traction Test* and *Fireplace Test* methods.

#### 2.5.1 Traction Test Methods

The front arm/ leg of the mouse is hung on a wire that has been stretched horizontally. Normal mice after hanging on the wire will have time to fall from the old traction test device and will immediately turn around again so that the body position remains balanced (negative). While mice that are affected by the sedative effect will immediately fall from the traction test device and take a long time to condition the body to remain balanced. This shows that mice are under the influence of sedative effects [13][14].

#### 2.5.2 Fireplace Test Methods

The equipment used for this test is a cylindrical/glass tube made of glass. Observations were made by looking at the time needed for the test animal to exit (jump) from a cylindrical glass placed on a hot plate at a temperature of 40-50 °C. Mice are then placed in a cylindrical glass. In the experiment, normal mice will immediately run away and climb the cylindrical glass in a short time. Whereas mice that are affected by sedative effects will take longer to climb or jump from cylindrical tubes [13][14].

### 2.6 Statistical Analysis

Quantitative data collected on the traction test method is the length of time the test animals to reverse the body and the length of time the mice fall from the traction test tool. in the fireplace test method is the length of time the test animal jumps from a cylindrical tube. The data obtained were analyzed statistically using the One Way ANOVA test then continued with post hoc tests assisted with the SPSS 20 programs.

## 3. Results and Discussion

### Phytochemical Screening Results

Celery fraction (*Apium graveolens* L.) contains al-

kaloids, steroids, triterpenoids, flavonoids, tannins and saponins. Each in n-hexane for non-polar, ethyl acetate for semipolar and methanol water for polar compounds.

### Sedation Effect Test Results

Essential oils contained in the celery fraction consist of monoterpenes terpenoid compounds ( $\alpha$ -pinene and  $\beta$ -pinene). This binding of terpenoids and phthalide compounds to the GABA receptor (gamma-aminobutyric acid) causes the chloride channel to open larger, chloride enters the cell resulting in hyperpolarization, inhibition of RAS (Reticular Activating System), also inhibited stimulation and reduced excitation, resulting in sleep [3].

The average value of the time needed by the mouse to fall (*traction test*) and the length of time needed for the fireplace test can be seen in Table 1.

Table 1. Average value of mouse time

Treatment	Traction Test (second)	Fireplace Test (second)
P0 (CMC Na 1%)	189.55	18.5
P1 (Extract Celery)	19.5	275.1
P2 (Fraction N-hexane)	18.55	210.8
P3 (Fraction Ethyl Acetate)	13.8	124.35
P4 (Fraction Methanol water)	8.1	384.6

This study has investigated several neuropharmacological activities of celery fraction (*Apium graveolens* L.) as in its extract. The celery fraction also has central nervous system depressant activity as shown by decreased muscle activity of mice in the traction test method and decreased mice reflexes in the fireplace test method shown in Figure 1 and Figure 2. The data presented in this study shows that the three celery fractions have the potential to produce sedation effects. However, the methanol water fraction shows greater potential than other factions. This may be due to the fact that each fraction contains different ingredients.

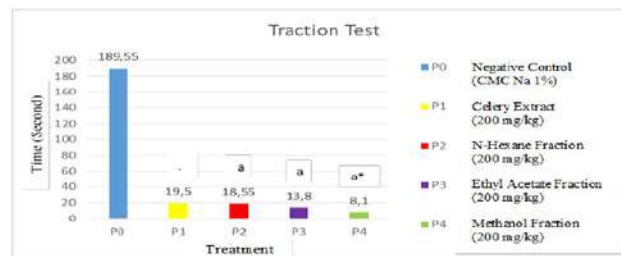


Figure 1. Graph of the length of time the mouse falls in the *Traction Test* method. Data is displayed as Mean  $\pm$  STD of five animals in each group. Value with superscript letters is significant ( $p < 0.05$ ) with each other.

Figure 1. shows the results of the average time for each treatment in the traction test method. The parameter of this method is the length of time it takes for the mouse to fall. The faster the time to fall, the greater the sedation effect, and vice versa. The results of One Way ANOVA analysis are shown in Table 2, showing that there was no significant effect on the significance value of  $p < 0.05$ . So to find out in detail whether there are significant differences between treatment groups the *post hoc* test is carried out.

Table 2. Sedation activity of extract and fraction of Celery (*Apium graveolens* L.) on *Traction Test* in mice.

Group	Treatment	Dose	Traction Test
Control	Aquadest + CMC Na 1%		189.55 ± 34.12
Extract	Celery Extract	200 mg/kg	19.50 ± 2.88
	N-Hexane	200 mg/kg	18.55 ± 8.08
Celery Fraction	Ethyl Acetate	200 mg/kg	13.80 ± 4.11
	Methanol	200 mg/kg	8.10 ± 2.82

Note: All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Duncan's test. \* $P < 0.05$ , significant compared to control.

Saponins bind to the GABA receptor so that GABA receptor activity increases, then causes the chloride channel to open, opening the channel causes chloride to enter the cell, causing hyperpolarization and decreasing excitation. With the subtraction of the part, the resulting effect is a decrease in awareness and arising drowsiness and even sleep [15].

The results of the celery fraction (*Apium graveolens* L.) had a sedation effect as well as the extract both in the *Traction Test* method and *Fireplace Test* method (Table 1). This result is seen from the variation in time that occurred during the treatment. Based on the results of the statistics performed, it shows that all fractions have the ability to produce sedation effects. This is due to the compounds contained in the fraction, where the compound has the ability to cause sedation effects. The active compounds contained therein include alkaloids, flavonoids, and saponins, these active compounds are compounds that have the potential effects of sedation [7]. It's just that the methanol water fraction has a much better effect than the extracts and other fractions at the same dose. Most likely that the polar compounds present in the methanol water fraction have a higher sedation effect compared to other fractions.

Through the *post hoc* test showed that all treatments did not have a significant effect, seen from the same notation in all treatments but very significant compared to the negative control (CMC Na 1%) indicated by the presence of different notations for all treatment groups. From the analysis results it can also be seen that

the methanol water fraction (200 mg/kg) has a better sedation effect than celery extract and other celery fractions with the same dose (Table 3).

Table 3. Results of statistical calculation of the effects of mouse sedation on the *Traction Test* method by further testing *post hoc* test.

Treatment	N	Subset	
		1	2
Methanol Fraction (200 mg/kg)	5	8.1000 (a)*	
Ethyl Acetate Fraction (200 mg/kg)	5	13.8000 (a)	
N-Hexane Fraction (200 mg/kg)	5	18.5500 (a)	
Celery Extract (200 mg/kg)	5	19.5000 (a)	
Negative Control (CMC Na 1%)	5		189.5500 (b)
Sig.		.311	1.000

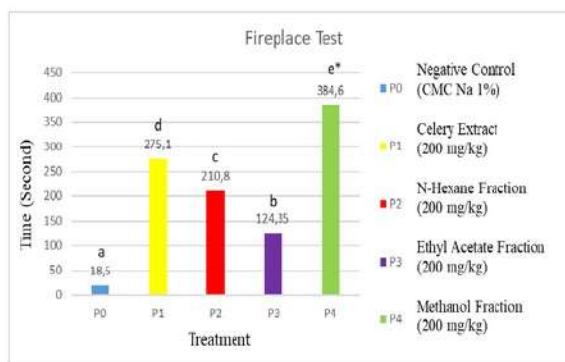


Figure 2. Graph of how long the mouse jumps in the *Fireplace Test* method. Data is displayed as Mean ± SE of five animals in each group. Value with superscript letters is significant ( $p < 0.05$ ) with each other.

Figure 2. shows the results of the average time in each treatment in the *fireplace test* method. The parameter of this method is the length of time it takes for the mouse to jump out of the heated glass tube /container. The longer the jump time of the mouse, the greater the sedation effect, and vice versa. The results of *One Way ANOVA* analysis are shown in Table 4, showing that there was a significant influence between treatment groups, at a significance value of  $p < 0.05$ . So to find out in detail whether there are significant differences between treatment groups the *post hoc* test is carried out.

Through *post hoc* testing shows that all treatments have a significant effect, it can be seen from the absence of the same notation in all treatments. From the results of the analysis it can also be seen that the methanol water fraction (200 mg/kg) has a better sedation effect than celery extract and other celery fractions (Table 5).



Table 4. Sedation activity of extract and fraction of Celery (*Apium graveolens* L.) on *Fireplace Test* in mice.

Group	Treatment	Dose	Fireplace Test
Control	Aquades + CMC Na 1%		18.50 ± 5.14
Extract	Celery Extract	200 mg/kg	275.10 ± 26.14
		200 mg/kg	210.80 ± 10.92
Celery Fraction	Ethyl Acetate	200 mg/kg	124.35 ± 30.53
		200 mg/kg	384.60 ± 31.66

Note: All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Duncan's test. \*P<0.05, significant compared to control.

Table 5. Results of statistical calculation of the effects of mouse sedation on the *Fireplace Test* method by further testing *post hoc* test.

Treatment	N	Subset				
		1	2	3	4	5
Negative Control (CMC Na 1%)	5	18.5000 (a)				
Ethyl Acetate Fraction (200 mg/kg)	5		124.3500 (b)			
N-Hexane Fraction (200 mg/kg)	5			210.8000 (c)		
Celery Extract (200 mg/kg)	5				275.1000 (d)	
Methanol Fraction (200 mg/kg)	5					384.6000 (e)*
Sig		1.000	1.000	1.000	1.000	1.000

Alkaloid will interact with the active side of Ach (Acetylcholinesterase), thus Ach will become inactive and cannot hydrolyze Ach which has been bound to the neurotransmitter receptor. Not hydrolyzed Ach will cause the Ach receptor complex to continue to affect the postmenaptic membrane so that the muscle fibers get stimulated depolarization persists until it finally loses the contraction response and causes paralysis [16].

#### 4. Conclusion

Based on the research that has been done it can be concluded that the celery fraction (*Apium graveolens* L.)

has a better sedation effect than the extract. And also the methanol water fraction with a dose of 200 mg/kg is the best fraction that has the strongest sedation effect compared to the other two fractions.

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