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Phytochemical Screening and Study of Ethanol Extract of *Capsicum annuum* from Indonesia as Active Substances for Anti-Riot Control Agent

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ABSTRACT

A riot control agent (RCA) is a substance that causes a physiological incapacitating effect when it comes into contact with the eyes, skin (mucosa), or when it is inhaled. The mechanism of action of this substance is by interacting with sensory nerve receptors at the site of contamination, resulting in localized pain and discomfort with associated reflexes. However, there are not many studies that can prove the safety of the active compounds and excipients used as RCA. The research aimed to carry out phytochemical tests and standardize the active ingredient of Capsaicin from chili (*Capsicum annuum*) from various regions in Indonesia as an active ingredient in riot control. The research included the determination of sample, preparation of chili crude drugs, extraction of samples, phytochemical screening, as well as characterization and standardization of extracts. The study was conducted using 10 samples. All extracts samples contain alkaloid compounds. Based on the moisture content, only samples 1 and 3 met the Indonesian Herbal Pharmacopoeia requirements. Based on the parameters of total ash content, all samples met the requirements of the Indonesian Herbal Pharmacopoeia. However, in terms of acid insoluble ash content, only samples 4 and 7 met the requirements of the Indonesian Herbal Pharmacopoeia. In determination of capsaicin content, it was found that sample No. 6 had the highest capsaicin content, namely 5.092 ± 0.028% (w/w). The results that have been carried out will be continued through further studies for sensitivity tests and toxicity tests of the active ingredients.

1. Introduction

Riot control agents are compounds that are strong irritants to sensory receptors, generally have low toxicity, the effect of which is influenced by the dose and time of exposure. Collectively, these compounds have been referred to as 'paralyzing agents' or as lacrimators, and in common parlance are often referred to as 'tear gas. The benefits of riot control agents have been widely used in both civil and military situations. Generally, as a means of self-defense and disperse large crowds of people. Physiologically, riot control agents can be classified according to their types: lacrimators, which primarily cause eye irritation and lacrimation; emetic agents, which also induce vomiting; and the sternutatory, which mainly causes uncontrolled sneezing and coughing. Riot suppression agents have also been referred to as irritants, that is, agents that are short term incapacitating or incapacitating.

The ingredients in RCA are believed to be short-term irritants, not causing death or causing permanent damage, especially when administered at relatively low levels in open spaces. However, at high levels in confined spaces, it can be deadly. Until now the use of anti-riot materials still causes pros and cons. Initially, the RCA that was widely used was Chloroacetophenone (CN) and Chlorodihydrophenarsazine. However, for reasons of safety and less irritating effects, these compounds have been replaced by 'safer' compounds such as o-chlorobenzylidene malononitrile (CS) and the natural ingredient capsicum oleoresin (OC), Piperine. However, there are not many studies that can prove the safety of the active compounds and excipients used in this RCA, one of which is the active ingredient OC. Likewise with the dose concentration of OC exposure which is still within safe limits. Therefore, this research will be carried out on standardization and characterization of Capsaicin as an active ingredient in riot suppression.

2. Methods

2.1 Sample Preparation

Capsicum annuum (CA) collection is carried out by collecting from regions in Indonesia which include Bukit Tinggi, Jayapura, Pontianak, NTT (3 varieties), Bandung, and Tapin. Crude drugs drying is done after proper cleaning, done by drying in direct sunlight until dry. In order to determine the water content of the crude drugs, it is done by calculating the water content of the dried crude drugs.

Preparation of crude drugs powder is done by grinding the dried CA. The step taken is to grind it by making powder with a mixer and grinder followed by sieving with a 60-mesh sieve repeatedly to get the whole fine powder. Furthermore, the refined powder is stored in a certain place to keep the powder having a certain moisture content, to avoid mold and other impurities.

2.2 Extraction

The extraction process was started by weighing the dried CA powder. The next step is extraction using a continuous method using a Soxhlet apparatus with 70% ethanol solvent. The extraction process is carried out until it reaches 48-72 hours. Furthermore, the results of the extraction were concentrated using a rotary-evaporator until the extract was obtained. In order to obtain a thick extract followed by evaporation with a water bath, the final result of the extract to calculate the yield of the thick extract is carried out by weighing it by comparing the dried chili used with the thick extract.

2.3 Phytochemical Screening

Phytochemical analysis of all the solvent extracts was performed for the detection of active secondary metabolites or different constituents such as tannins, alkaloids, flavonoids, terpenoids, steroids, carbohydrates, proteins, and saponins. The dried extracts extracted by Soxhlet apparatus were reconstituted in methanol, and each extract was subjected to standard phytochemical analysis.

2.4 Extracts Characterization

The preliminary characterization of ethanol extracts of C. annuum, was carried by the determination of Total ash, determination of acid insoluble ash, and moisture content determination. The percentage of ash with reference to the extract with the formula:

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$$\%Total\ Ash = \frac{((Ashed\ weight) - (Crucible\ weight))}{(Crucible + Sample\ Weight) - (Crucible\ Weight)} \times 100$$

2.5 Determination of Capsaicin

Capsaicin analysis was performed by HPLC with silica gel C-18 stationary phase and isocratic elution using 65% methanol. The detector used is a UV detector with a wavelength of 290 nm. Observation for 15 minutes with an injection volume of 20 µL.

2.6 Calibration Curve

A number of capsaicin comparators were weighed and then dissolved in Pro HPLC methanol. Then a series of concentrations of 50, 100, 250, 500, 750, 1000 ppm was made. After that, a number of comparison solutions were taken and then injected into the HPLC tool. Each concentration was measured three times.

3) Sample Measurement

A number of samples were weighed and then dissolved in *ProHPLC* methanol. Then was filtered using a 0.22 µm filter membrane. After that, a number of samples were injected into the HPLC. Calculation of levels was carried out using a capsaicin calibration curve.

3. Results

3.1 Sample Naming

The chili samples used in the study were 10 (ten) samples, then dried directly with the help of sunlight. The dried simplicia was refined with the help of a blender, then extracted with an alcohol solvent. The results of the extraction were then carried out by examining the yield of chilies (Capsicum annum) from Belu NTT as many as 3 (three) varieties, chilies (Capsicum annum) from Tapin West Kalimantan, chilies (Capsicum annum) from Bandung, West Java, chilies (Capsicum annum) from Pontianak West Kalimantan as many as 2 (two) varieties, Chili (Capsicum annum) from Jayapura Papua and Chili (Capsicum annum) from Bukit Tinggi, West Sumatra, with details in the table

Table 1. Names and Samples Information

Sample	Species	Origin	Extraction Method	Extraction Solvent	Rendement (%)
1	*Capsicum annum*	Varietas 1, NTT	Continuous Extraction	Ethanol 70%	28,945
2	*Capsicum annum*	Varietas 2, NTT	Continuous Extraction	Ethanol 70%	36,129
3	*Capsicum annum*	Varietas 3, NTT	Continuous Extraction	Ethanol 70%	36,480
4	*Capsicum annum*	Tapin, Kalbar	Continuous Extraction	Ethanol 70%	52,938
5	*Capsicum annum*	Bandung	Continuous Extraction	Ethanol 70%	30,340
6	*Capsicum annum*	Pontianak, Kalbar	Continuous Extraction	Ethanol 70%	26,528
7	*Capsicum annum*	Pontianak, Kalbar	Continuous Extraction	Ethanol 70%	35,310
8	*Capsicum annum*	Pontianak, Kalbar	Continuous Extraction	Ethanol 70%	38,660
9	*Capsicum annum*	Jayapura	Reflux	Ethanol 95%	16,710
10	*Capsicum annum*	Bukit Tinggi	Continuous Extraction	Ethanol 95%	35,090

3.2 Phytochemical Screening

The thick chili extract was then screened for phytochemicals which included alkaloids, tannins, quinones, saponins, flavonoids and steroids/triterpenoids, with the results of the phytochemical screening as shown in table 5.2.

Table 2. Phytochemical Screening Results

Sample	Alkaloid	Tannin	Quinone	Saponins	Flavonoid	Steroids/Triterpenoids

1	+	+	-	-	+	+
2	+	+	+	-	+	+
3	+	+	-	-	+	+
4	+	+	-	-	+	+
5	+	+	-	-	+	+
6	+	+	+	-	+	+
7	+	+	-	-	+	+
8	+	+	-	-	+	+
9	+	+	-	-	+	+
10	+	+	-	+	+	+

(+) detected; (-) not detected

Moisture Content and Ash Value

Table 3. Results of Moisture Content and Ash Value of the Samples

Sample	Moisture Content (v/w%)	Total Ash Content (w/w%)	Acid Insoluble Ash Content (b/b%)
1	20,33 ± 0,58	5,93 ± 0,01	1,29 ± 0,70
2	23,33 ± 1,15	8,52 ± 0,62	3,96 ± 1,31
3	14,33 ± 0,58	4,12 ± 0,06	1,60 ± 0,43
4	26,33 ± 0,58	6,31 ± 0,71	0,43 ± 0,28
5	26,67 ± 2,31	8,69 ± 0,07	3,01 ± 0,57
6	27,33 ± 1,15	9,26 ± 1,01	1,79 ± 0,09
7	28,00 ± 2,00	4,79 ± 0,62	0,60 ± 0,35
8	27,33 ± 3,06	5,41 ± 0,19	4,40 ± 0,55
9	27,67 ± 2,52	7,41 ± 0,93	2,70 ± 0,57
10	24,67 ± 3,06	7,80 ± 0,22	2,07 ± 0,46

Capsaicin Content

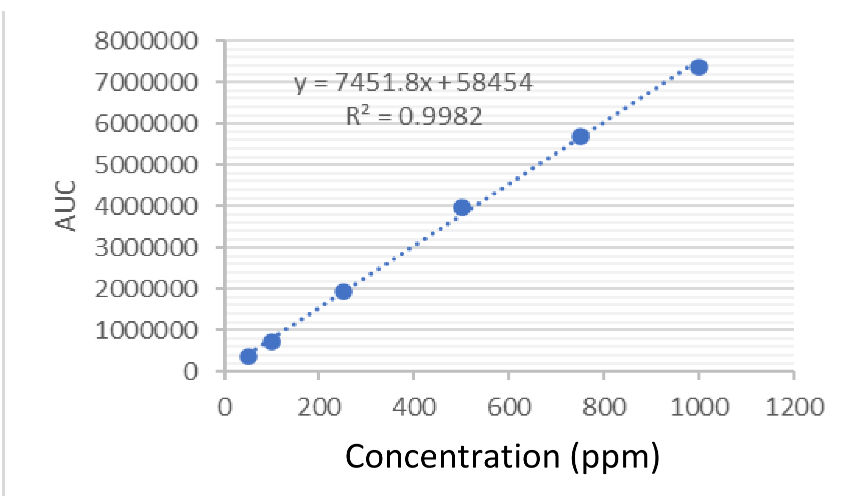

Figure 1. Capsaicin calibration curve

Table 4. Comparison of Capsaicin Levels by Filtration and Non-filtration

Sample	Capsaicin Content (%(w/w))		Significant Value
	Non-filtration	Filtration	
1	2,615	2,606	0,371
1	2,581	2,601	
1	2,557	2,648	

Sig. Value > 0.05 = no significant difference between treatments ($p>0.05$)

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Table 5. Capsaicin Levels Content

Sample	Capsaicin Content (%(w/w))
1	2.618 ± 0,026[a]
2	0,940 ± 0,010[b]
3	2,674 ± 0,019[c]
4	2,124 ± 0,027[d]
5	1,733 ± 0,022[e]
6	5,092 ± 0,028[f]
7	0,954 ± 0,014[b]
8	1,065 ± 0,013[g]
9	1,059 ± 0,012[g]
10	0,297 ± 0,004[h]

a-h = letter differences indicate significant differences between samples ($p < 0.05$).

4. Discussion

In this test, a phytochemical screening was carried out to qualitatively detect the presence of secondary metabolites in the chili extract sample. The results showed that all samples showed the presence of alkaloids, tannins, flavonoids, and steroids/triterpenoids. Positive alkaloids in each sample indicate that there is a possibility of the presence of capsaicin compounds because capsaicin is one of the alkaloid group compounds. The presence of a nitrogen atom in the capsaicin structure indicates that the compound is an alkaloid.

In addition to phytochemical screening, samples were also tested according to the requirements in the Indonesian Herbal Pharmacopoeia, namely water content, total ash content and acid insoluble ash content. In Table 2.2 it can be seen that the majority of the sample water content is more than 21% except for samples number 1 and 3. This shows that the majority of the samples still contain a lot of water. The presence of water in the sample greatly affects the condition of the sample. If the water is too high then there will be a possibility of microbes can grow. In addition, the presence of high water will also affect capsaicin levels. Therefore, it is necessary to re-concentrate the chili extract samples so that they meet the requirements of the Indonesian Herbal Pharmacopoeia, namely the water content of the extract is not more than 21%.

The total ash content of the extract was also measured according to the method in the Indonesian Herbal Pharmacopoeia. Table 2.2 shows that all samples meet the requirements, namely the total ash content value is not more than 13.3%. The lowest total ash content was shown by sample number 3 which is approx 4.12%. However, at the acid insoluble ash content of the samples that met the requirements of not more than 1.1%, only samples number 4 and 7. The acid insoluble ash content indicated the inorganic ash content contained in the samples. This shows that the majority of the sample does not meet the requirements.

Indonesian Herbal Pharmacopoeia related to acid insoluble ash content. Therefore, it is necessary to carry out measured cultivation so that the inorganic ash contamination in the sample can be controlled so that during continued production the quality of the starting material can be better guaranteed.

In determining the content of capsaicin as a marker compound in the chili sample, a quantitative measurement was carried out using high performance liquid chromatography (HPLC). Based on system optimization, the mobile phase was obtained using a mixture of methanol - water + 0.01% formic acid (65:35) isocratic with a flow rate of 1 mL/minute and a detection wavelength of 290 nm. The stationary phase used was silica gel C-18. The sample retention time lies in the 10th minute.

Test the effect of filtration on capsaicin levels detected by the tool. In sample no. 1, the capsaicin content was determined in samples that were filtered and not filtered. In Table 2.3 a statistical analysis was carried out and showed that there was no significant difference between the filtered and non-filtrated samples. Therefore, for the next sample, the sample will be filtered before being analyzed by HPLC.

Table 2.4 shows the average capsaicin levels in each sample. The highest capsaicin content was in sample no. 6 and the lowest was in sample no. 2. This difference may occur due to differences in the growing environment which affect the nutrients absorbed by plants. Besides that, Differences in environmental conditions also affect external factors such as temperature, humidity, intervention from other animals and plants.

5. Conclusions

All extract samples have alkaloid compounds. Based on the water content parameter, only samples 1 and 3 met the Indonesian Herbal Pharmacopoeia requirements, namely not more than 21%. Based on the parameters of total ash content, all samples met the requirements of the Indonesian Herbal Pharmacopoeia, namely not more than 13.3%. However, in the acid insoluble ash parameter, only samples 4 and 7 met the requirements of the Indonesian Herbal Pharmacopoeia, namely not more than 1.1%. In determining the capsaicin content, it was found that sample No. 6 had the highest capsaicin content, namely 5.092 ± 0.028% (w/w).

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