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Determination of Capsaicin Levels in *Capsicum annum Linn* Ethanolic Extract using Thin Layer Chromatography Analysis

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ABSTRACT

Active compounds of natural ingredients need to be extensively explored to determine their properties. *Capsicum annum Linn* is widely grown in southern Asia and Southeast Asia. This study aimed to determine the levels of capsaicin in *C. annum L.* ethanolic extract. A total of 300 g *C. annum L.* analyzed in the form of powder and extract solution. This study analysis performed thin layer chromatography (TLC) method to separate the adsorption on the adsorbent thin layer. The ethanol used as a solvent to extract capsaicin. The result showed that the capsaicin levels were found in the powder of 0.36% and extract solution of 1.84%. The linear regression equation was y= 9.4571x + 546.67, with R^2= 0.9983, the capsaicin standard of 1020 µg/ml. It can be concluded that the extract solution of *C. annum L.* had a greater capsaicin level than in the powder form.

Keywords: Capsaicin, *Capsicum annum Linn*, Thin Layer Chromatography

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INTRODUCTION

Indonesia has a source of biodiversity that is supported by geological conditions, soil fertility, and climate. The cultivation of several types of horticultural crops has an economic value in the plantation sector. On the other hand, natural ingredients for medicines need to be investigated for their efficacy to support phytochemicals studies.[1]

Chili (*Capsicum annum Linn*) is an herb and as traditional medicine. Its production is utilized domestically and exported in dry products.[2] *C. annum L.* is used as a stimulant, carminative, tonic,[3] also to treat asthma, impotence,[4] symptoms of fever, colds, influenza, cholera, anthelmintic,[5] antiflatulent, expectorant, antitussive, antifungal, and cholesterol medication.[6]

C. annum L. has natural compounds that can provide benefits to humans. One of the most important compounds in *C. annum L.* is capsaicin which is a secondary metabolite.[7] In other studies, chemical compounds associated with secondary metabolites i.e. alkaloids, terpenoids, steroids, and saponins.[8] *C. annum L.* has a pungent taste and characteristic odor that is mediated by capsaicinoid compounds. Capsaicinoids include nordihydrocapsaicin, capsaicin, dihydrocapsaicin, norcapsaicin, homodihydrocapsaicin, homocapsaicin, and nonivamide.[9]

In the present study, the Thin Layer Chromatography (TLC) method performed to purify capsaicin compound in *C. annum L.* This type chromatography to separate the adsorption on the thin layer of the adsorbent.

MATERIALS AND METHODS

C. annum L. ethanol extract

A total of 300 g of *C. annum L.* were dried and ground into powder form. 1500 ml of ethanol were added and mixed with *C. annum L.* powder until it is homogeneous. Furthermore, the mixture was mixed with Ultra-Turrax® digital T25 for 30 minutes then incubated for 24 h. The extraction process was carried out using IKA® Rotary Evaporator RV10 Digital V with a water bath temperature of 55°C. Then the extraction results were weighed (g) and packed in a microtube.[10]

Determination of capsaicin levels

The extract obtained was placed on a 60 F254 silica gel plate and spread 2 and 5 µl including standard capsaicin. Furthermore, the plates were incubated in the filled space (toluene, chloroform, acetone) for (4s, 2s, 30s), respectively. Plates containing dry solution were measured at a wavelength of 228 nm and an Rf value of 0.59.[11,12]

RESULTS AND DISCUSSION

This study analyzed capsaicin quantitatively with TLC-spectrophotodensitometer. TLC is a method of separating substances by tracing them through the stationary phase in the media plate. Substances can be separated by the TLC technique based on differences in the affinity of each component between the active and stationary phases. The amount of absorption of substances that have been separated can be measured by a spectrophotodensitometer.[13] The sample contents can be determined from the ratio between the absorption of the sample and its raw material. In this study, the results of capsaicin levels in the powder were 0.36% and ethanol extracts were 1.84% (Table 1).

In the present study, showed that the capsaicin level using a linear regression equation y= 9.4571x + 546.67, with R^2= 0.9983, the capsaicin standard of 1020 µg/ml (Figure 1 and 2). The capsaicin area in ethanol extracts (3816.9) (Figure 4) higher than in powder form (2422.7) (Figure 3).

Extraction is a method for obtaining active compounds from natural ingredients using suitable solvents.[14] Natural ingredients need to be extracted to get some or all of the active ingredients used to synthesize the right dosage, easily stored, and maintain for a long time. The extracted material can be in the form of fresh ingredients or powder.[15]

C. annum L. originates from tropical and subtropical regions of America, especially Colombia, South America, and continues to spread to Latin America, Europe and Asia, including Indonesia.[16] *C. annum L.* contains capsaicin, dihydrocapsaicin, vitamin A, vitamin C, capsanthin, carotene, capsorubin, zeaxanthin, and cryptoxanthin dyes. Micro minerals, such as iron, potassium, calcium, phosphorus, and niacin are also contained in *C. annum L.*[17] The active ingredient of capsaicin is efficacious as a stimulant of gastric acid secretion and prevents infections in the digestive system.[18] Other elements in *C. annum L.* are capsicol which has analgesic properties,[19] reduces asthma, and itching.[20]

Capsaicin (*8-methyl-N-vanilyl-6-nonenamide*) is an active component of *C. annum L.* Capsaicin can irritate and cause a burning sensation in tissues when contact occurs. In the pharmaceutical field, capsaicin is also known to have anticancer,[21] diabetic, anti-arthritis and analgesic activity besides having commercial value in the food industry.[19]

Ethanol solvents are used because capsaicin can dissolve in polar solvents and have polar varieties. Capsaicin is an alkaloid that has a high solubility in alcohol but is low in the water. Capsaicin is considered an oil with lipophilic properties and is also fat-soluble. Capsaicin has a melting point at 62-65ºC and boiling point 210-220ºC. Capsaicin is odorless, colorless, and belongs to a group that is soluble in oil, fat, methanol, ethyl acetate, and ethyl alkali.[22]

Capsaicin levels are influenced by the level of chili maturity. During the chili ripening process, capsaicin levels increase until the synthesis period. In this study, capsaicin levels were more in ethanol extract than in powder form.[23] In the chili powder, there are still many compounds and other residues that can reduce levels of capsaicin. Meanwhile in the ethanol extract obtained high levels because it has been separated and there is no residue from other compounds.[24]

CONCLUSION

It can be concluded that the ethanol extract of *C. annum L.* had a greater capsaicin level than in the powder form. The TLC method showed the capsaicin standard was 1020 µg/ml.

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CONFLICT OF INTERESTS

All authors declare that they have no competing interests.

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ABBREVIATIONS

g: gram; TLC: thin layer chromatography; µg: microgram; µg/ml: microgram/milliliter

Table 1: The capsaicin level in *C. annum L.* analyzed using TLC method

Sample	Weight (g)	Amount spotting in the sample		Capsaicin level		
		(µg)	Area	(µg)	(%)	Average (%)
Powder	0.1028	51.40	2419.88	0.186	0.36	0.36
	0.1031	51.55	2422.74	0.186	0.36	
Ethanol extract	0.1063	21.26	4684.53	0.437	2.06	1.84
	0.1049	20.98	3813.93	0.341	1.63	

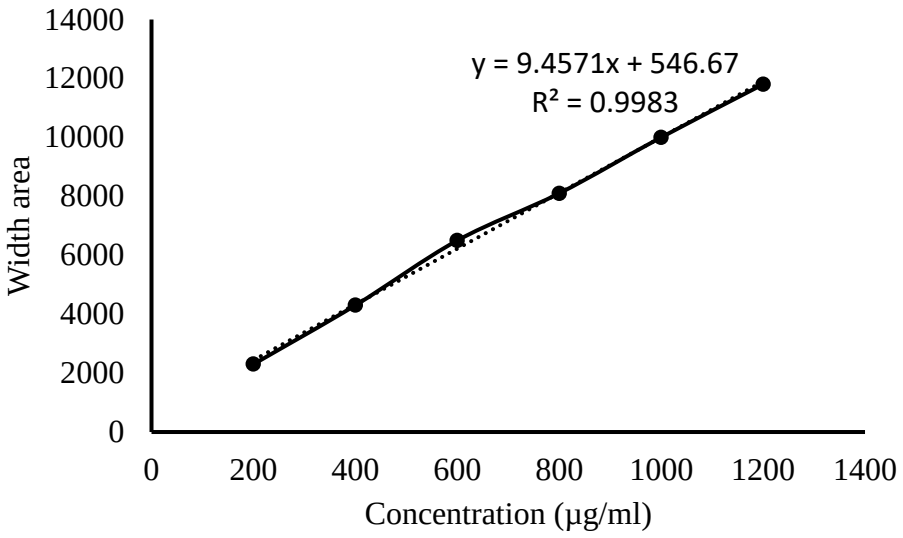
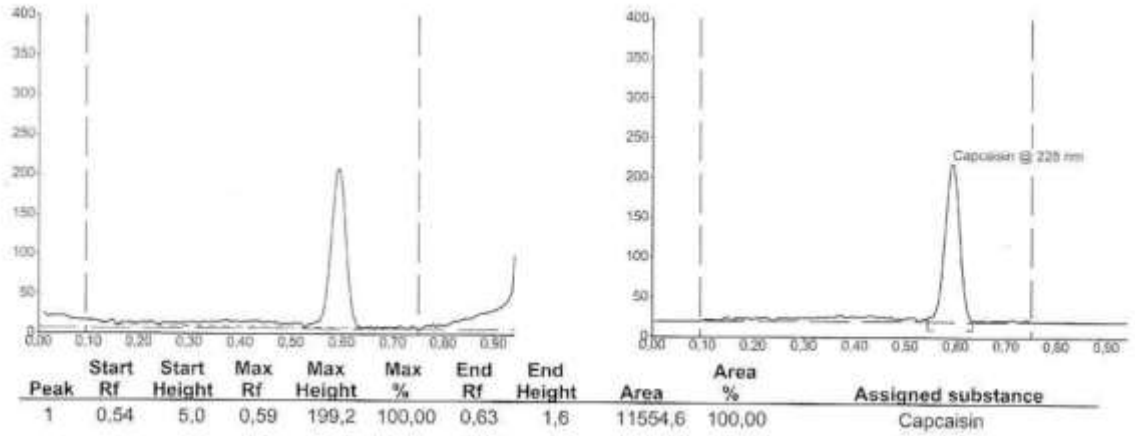
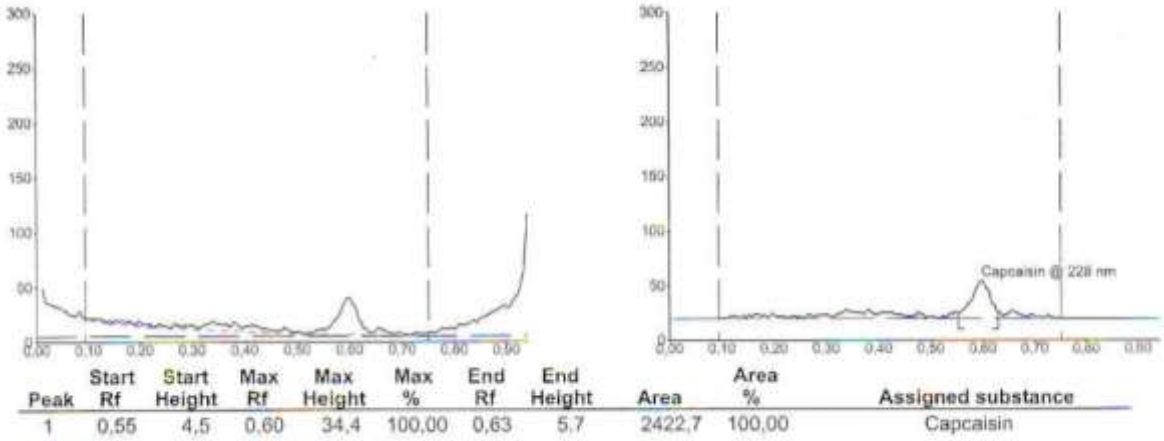


Figure 1: Linear regression curve with capsaicin standard 1020 µg/ml



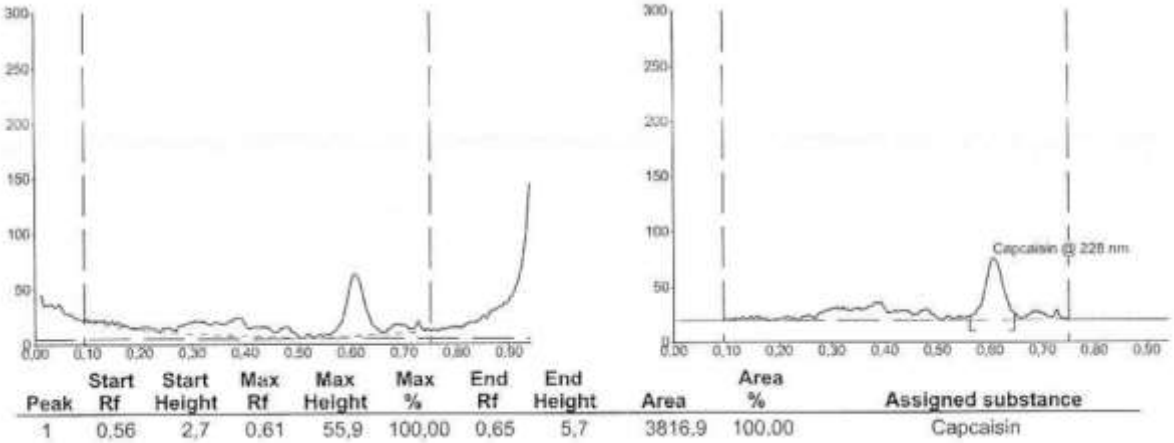
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0,54	5,0	0,59	199,2	100,00	0,63	1,6	11554,6	100,00	Capcaisin

Figure 2: The capsaicin area in *C. annum L.* standard using TLC method



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0,55	4,5	0,60	34,4	100,00	0,63	5,7	2422,7	100,00	Capcaisin

Figure 3: The capsaicin area in *C. annum L.* powder using TLC method



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0,56	2,7	0,61	55,9	100,00	0,65	5,7	3816,9	100,00	Capcaisin

Figure 4: The capsaicin area in *C. annum L.* ethanol extract using TLC method