Type of Manuscript: ***Research Article***

Analysis of *Capsicum annuum* L. Methanolic Extract and Its Potential as a Hepatoprotector

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Abstract

ThinLayerChromatography(TLC) method performed to purify capsaicin compound in C. annum L. and test the bioactive component of *Capsicum Annum* L on MAPK1 target proteins using the Immunohistochemistry (IHC) method to determine the reactions between receptor-ligand (antigen-antibody) complexes formed and become a reference *in vivo* tests. 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 active ingredient of capsaicin is efficacious as a stimulant of gastric acid secretion and prevents infections in the digestive system.The immunohistochemical examination is intended to determine the expression of MAPK1 (ERK) on cells of mice hepatocytes (Mus musculus). MAPK 1 (ERK) expression score data were obtained using the modified Remmele method is the result of multiplying the percentage score of immunoreactive cells with the color intensity score on immunoreactive cells. The abnormal target protein signaling pathway contributes to cell proliferation, differentiation, survival, and uncontrolled cell apoptosis.Analysis of amino acid residues, Capsaicin to MAPK1haverelativelysimilarresiduessothatitcanbeconcludedthat the binding sites of the two compounds are close to similarities and affect receptors at relatively similar sites, namely oncogenicreceptors/ proteins. Concluded that the ethanol extract of Capsicum. annum L. had a greater in the powder form , TLC method showed the capsaicin standard was 1020 µg/ml, that capsaicin in chilies (*Capsicum Annum* L.) can synergistically inhibit cell through reactions in the anti-apoptotic MAPK 1 potentiates the antiproliferative action that can promote hepatoprotector, a finding that demands further clinicaltesting.

Keyword : *TLC, Immunohistochemistry, MAPK 1 expression, Hepatoprotector.*

Background

Chili(Capsicumannum Linn)isanherbandastraditional medicine. Its production is utilized domestically and exportedin dryproducts.[1]C. annum L.isused asa stimulant, carminative, tonic[2]alsototreatasthma,impotence,[3]symptoms of fever, colds,influenza, kolera, anthelmintic,[4]antiflatulent, expectorant, antitussive, antifungal, andcholesterol medication.[5]

Oneof themostimportant compounds in C. annum L. is capsaicin which is a secondary metabolite.[6]In other studies, chemicalcompounds. Capsaicinoidsincludenordihydrocapsaicin, capsaicin, dihydrocapsaicin,norcapsaicin, homodihydrocapsaicin, homocapsaicin, and nonivamide.[7]

Chili has natural compounds that can provide enormous benefits for humans and animals. One of the most important compounds in chili is capsaicin. Capsaicin (8-methyl-N-vanillyl-6-none amid) is an

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active component of chili which is irritant to mammals including humans and causes burning and heat in any tissue that is touched.

In the pharmaceutical field in addition to relieving pain or pain, capsaicin is also known to have anticancer activity. High potential in the pharmaceutical field as anticancer, anti-arthritis and analgesia in addition to having a commercial value in the food industry.[8,9]

In the pharmaceutical field in addition to relieving pain or pain, capsaicin is also known to have anticancer activity because it inhibits certain oncogenic proteins. Immunohis to chemical examination between capsaicincompoundsand oncogenic proteins produced in the signaling of hepatoprotector pathogenesis.

From this background, this study aims to, the Thin Layer Chromatography (TLC) method performed to purify capsaicin compound in C. annum L. and test the bioactive component of *Capsicum Annum* L on MAPK1target proteins using the Immunohistochemistry (IHC) method to determine the reactions between receptor-ligand (antigen-antibody) complexes formed and become a reference *in vivo* tests.

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 IK®A Rotary Evaporator RV10 Digital V with a water bath temperature of 55ºC. Then the extraction results were weighed (g) and packed in a microtube1.0

Determination of capsaicin levels

The extract obtained was placed on a 60 F254 silica gel plate and spread 2 and 5 µl including standart 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 [10,11]

Immunohistochemical sample preparation

The preparations are immersed in xylol 2 times, sequential alcohol (96%, 90%, 80%, 70%) for the hydration process. Washed in PBS pH 7.4 3 times each for 5 minutes. Soaked in 3% hydrogen peroxide (in distillate water) for 20 minutes. Washed in PBS pH 7.4 for 3x5 minutes. Soak in 1% BSA for 10-30 minutes at room temperature. Washed in PBS pH 7.4 for 3x5 minutes. Primary antibodies are added for 1 hour at room temperature. Then incubated overnight. Then washed in PBS pH 7.4 for 3x5 minutes. Then added a secondary antibody labeled Strep avidin horseradish peroxidase (SA-HRP) for 1 hour at room temperature. Washed in PBS pH 7.4 for 3x5 minutes. Preparations are added chromogen DAB (3,3-diaminobenzidine tetrahydrochloride) for 10-20 minutes at room temperature. Wash in distilled water for 3x5 minutes. Counterstained with hematoxylin for 5 minutes at room temperature. Wash in distilled water for 3x5 minutes. Mounting with insert. Observation using a microscope at magnification 100 and 400 times.

Results and Discussion

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).

Extraction is a method for obtaining active compounds from natural ingredients using suitable solvents[12]. 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[13].

Table 1: The capsaicinlevel in Capsicum annum L.analyzed usingTLC method

Sample	Weight	Amount spotting in		Capsaicin level		
	the sample					
	(g)	(µg)	Area	(µg)	(%)b/b	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	

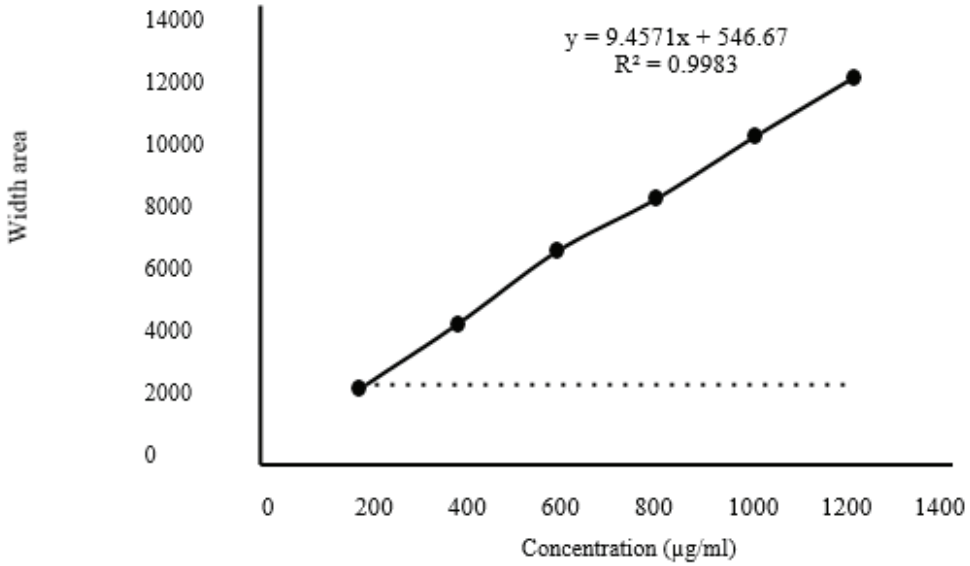

Figure 1 : Linier regression curve with capsaicin standard1020µg/ml

The active ingredient of capsaicin is efficacious as a stimulant of gastric acid secretion and prevents infections in the digestive system.[14] Other elements in C. annum L. are capsicol which has analgesic properties[15], reduces asthma, and itching.[16]

The immunohistochemical examination is intended to determine the expression of MAPK1 (ERK) on cells of mice hepatocytes (*Mus musculus*). MAPK 1 (ERK) expression score data were obtained using the modified Remmele method.[17] Remmele scale index (Immuno Reactive Score / IRS) is the result of multiplying the percentage score of immunoreactive cells with the color intensity score on immunoreactive cells. Data for each sample is the average value of the IRS observed in 5 (five) Field View (FV) at 400x magnification (Figures 2).

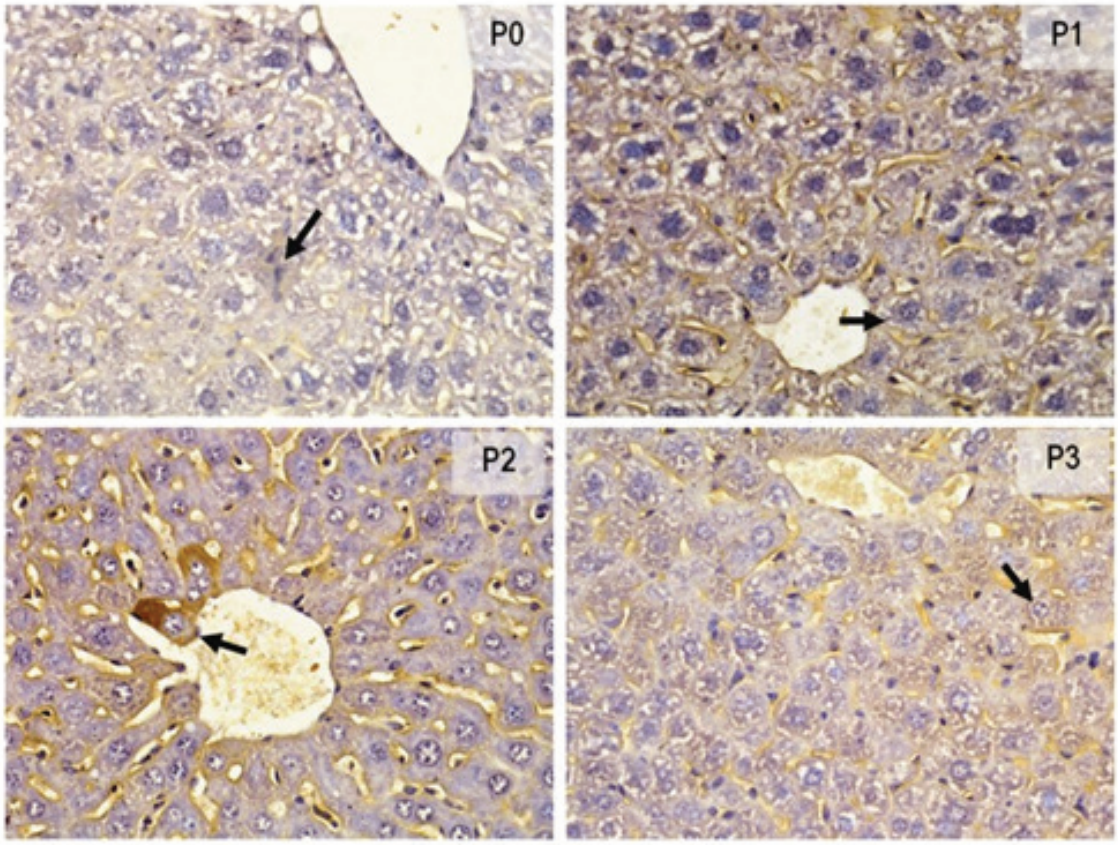

Figure 2: Immunohistochemical staining MAPK 1, 1000x magnification; Nikon H600L microscope; DS Fi2 300-megapixel camera.

Comparison of MAPK1 (brown chromogen) expression in hepatocyte (arrow) cells between treatment groups. MAPK1 is expressed in both the cytoplasm and the hepatocyte cell nucleus. The results of the examination showed that the expression of MAPK1 in the treatment groupGivingAFB1(3mg/KgBB)(P2) andGivingAFB1(3mg/KgBB) and Capsaicin (10 mg / Kg BB) (P3) looked the same but wasstronger compared to the control treatment group (P0) and the treatment group givingCapsaicin(10mg/KgBW)(P1)(Figures2).

The data obtained in the form of a Remmele Scale Index score (Immuno Reactive Score / IRS) results from the multiplication score of immunoreactive cells with a color intensity score, were analyzedby Kruskal Wallisfollowed by the ZTest, statistical analysis using the IBM SPSSCorp. computerstatistical programReal21.

Statisticalanalysis, data are presentedas mean (mean)± standard error. Differences between groups were assessed for statistical significance using the Kruskal-Wallis test or the Multiple comparison test with the Ztest (MultiplecomparisonsbyZtest), dependingondatadistribution. Pvalues<0.05were considered statistically significant.

Based on the results of testing using Kruskal Wallis can provide information that Capsaicin can inhibit the expression of MAPK1onmicehepatocytesinducedby AFB1*invivo* administration (Figure 3).

MAPK overexpression are increasingly being studied in carcinogenesis. The abnormal target protein signaling pathway contributes to cell proliferation, differentiation, survival, and uncontrolled cell apoptosis are biomarkers of carcinogenic processes.[18]

Analysis of amino acid residues, Capsaicin to MAPK1haverelativelysimilarresiduessothatitcanbe

concluded that the binding sites of the two compounds are close to similarities and affect receptors at relatively similar sites, namely oncogenicreceptors/ proteins.[19,20,21.]

Uji Z untuk Skor MAPK 1

Mean Rank		$R_{i.1}$	$R_{i.2}$	Differents of Mean Rank ($R_{i.1} - R_{i.2}$)	λ^1	p^2
Kontrol	CAP	8.75	11.42	-2.66667	-0.65	ns
	AFB1	8.75	18.42	-9.66667	-2.37	s
	CAP+AFB1	8.75	11.42	-2.66667	-0.65	ns
CAP	AFB1	11.42	18.42	-7	-1.71	s
	CAP+AFB1	11.42	11.42	0	0.00	ns
AFB1	CAP+AFB1	11.42	11.42	0	0.00	ns

1) $\lambda=(R_{i.1}-R_{i.2})/4{,}082$; 2) Z_{tabel} Distribusi Normal, s = signifikan ($p<0{,}05$), ns = non signifikan ($p>0{,}05$)

Figure3 : MAPK-1Ztestresults.

Conclusion

It can be concluded that the ethanol extract of Capsicum. annum L. had a greaterin the powder form, TLC method showed the capsaicin standard was 1020 µg/ml, that capsaicin in chilies (*Capsicum Annum* L.) can synergistically inhibit cell through reactions in the anti-apoptotic MAPK 1 potentiates the antiproliferative action that can promote hepatoprotector, a finding that demands further clinicaltesting.

Ethics and Consent:

All applicable institutional guidelines for the care and use of animals were followed. This research received ethical clearance approval from Animal Care and Use Commitee, Faculty of Veterinary Medicine, Universitas Airlangga Surabaya, East Java, Indonesia (No. 1. KE. 198. 12.2019).

Confilct of Interests: The authors declare that they have no conflict interests

Funding: The research was funded by Directorate of Research and Community Service, Deputy of Strengthening Research and Development Ministry of Research and Technology/BRIN of the Republic of Indonesia-Penelitian Disertasi Doktor (PDD), Universitas Airlangga Surabaya (letter of appointment agreement number:755/UN3.14/PT/2020.

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