

Conference Paper

The Effect of Adding Cinnamon Bark Oil to the Tyrosinase Inhibitory Activity of Emulgel Containing Cocoa Pod Husk Extract

Sani Ega Priani*, Soia Neriani Budiman, Sri Peni Fitrianiingsih, Livia Syafnir and Taufik Muhammad Fakh

Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Islam Bandung, Bandung, Indonesia

Abstract.

Our previous study showed that cocoa pod husk extract has tyrosinase inhibitory properties and the potential to prevent skin hyperpigmentation. Another natural source that is known to have tyrosinase inhibitory properties is cinnamon bark oil. This paper aims to analyze the tyrosinase inhibitory properties of cinnamon bark oil through molecular docking. It also aims to determine the effect of adding cinnamon bark oil to the tyrosinase inhibitory properties of emulgel containing cocoa pod husk extract. The constituents of cinnamon bark oil were determined using gas chromatography-mass spectrometry. The molecular docking was conducted using autodock. The emulgels were prepared by adding 2% of cocoa pod husk extract with and without the addition of cinnamon bark oil (1%). Tyrosinase inhibitory properties were analyzed using a colorimetric enzymatic assay and the dopachrome method. The GCMS result showed the cinnamon bark oil containing 53.37% cinnamaldehyde. The in-silico study showed cinnamaldehyde properties as a tyrosinase inhibitor, since it can bind on the active site of the enzyme with free binding energy at -4.88 kcal/mol. The addition of cinnamon bark oil (1%) to the emulgel preparation increased the tyrosinase inhibitory activity by 63.33% based on in vitro study.

Keywords: emulgel, cocoa pod husk extract, cinnamon bar oil, tyrosinase inhibitor

Corresponding Author: S. E.

Priani; email:

sani.ega.p@unisba.ac.id

Published 27 December 2022

Publishing services provided by
Knowledge E

© S. E. Priani et al. This article is distributed under the terms of the [Creative Commons](#)

[Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Selection and Peer-review under the responsibility of the SIRES Conference Committee.

1. INTRODUCTION

One of the most common skin problems is hyperpigmentation. Hyperpigmentation can be a serious problem for women so efforts to prevent or reduce this condition need to be done. Hyperpigmentation is a condition where the melanin content in the skin increases. The process of melanin synthesis is called melanogenesis [1,2]. This process was catalyzed by tyrosinase, the key *enzyme* involved in pigment production [3]. Therefore, inhibition of the activity of these enzymes can be a potential strategy to prevent skin hyperpigmentation. The compounds with that's activity are commonly called tyrosinase inhibitors [4].

OPEN ACCESS

Many natural substances are known to have tyrosinase inhibitory activity. Our previous research showed that one of the natural resources known to have tyrosinase inhibitory activity is cocoa pod husk. Cocoa pod husk is a waste from chocolate production which is known to have tyrosinase inhibitory activity with an IC₅₀ value was 199.98 ppm

[5]. Stilbenoids, flavonols, and phenolic acid in cocoa pod contributed to the tyrosinase inhibitory activity of cocoa pod husk extract [6].

For topical application, cocoa pod husk extract will be developed in an emulgel dosage form. Emulgel is a topical drug delivery system, has a dual release control system, i.e., gel and emulsion. Emulgel is commonly used for the delivery of various cosmetic formulations. The emulgel have many advantages like easily spreadable, easily removable, emollient, pleasing appearance, and cosmetically acceptable [7,8].

In the emulgel system, there is an oil phase and a water phase. In this study, cinnamon bark oil was added to the oil phase. The purpose of adding cinnamon bark oil was to increase the tyrosinase inhibitory activity of the emulgel preparations. Cinnamon bark is known to contain active compounds with tyrosinase inhibitory activity i.e. Cinnamaldehyde [9]. The objective of this research was to determine the tyrosinase inhibitory activity of a major active compound of cinnamon bark oil with molecular docking and to determine the effect of cinnamon bark oil on tyrosinase inhibitory activity of emulgel that containing cocoa pod husk extract.

2. METHODOLOGY

2.1. Preparation and Characterization of Cocoa Pod Husk and Cinnamon Bark Oil

Coarse powder of cocoa pod husk was extracted using ethanol 70% and then concentrated using vacuum rotary evaporator. The extract then subjected to phytochemical screening. Cinnamon bark oil was obtained from pavetia essential oil, west java. Active compounds of the oil were determined using Gas chromatography-mass spectrometry (GC-MS).

2.1.1. Tyrosinase Inhibitory Activity of Cinnamon Bark Oil Active Compound Using Molecular Docking Method

The crystal structure of the tyrosinase enzyme macromolecule was downloaded from the Protein Data Bank, then prepared using MGL Tools 1.5.6 software equipped with

AutoDock 4.2. The enzyme macromolecules then identified, evaluated, and explored which part of the binding active site plays a role in biological activity using the BIOVIA Discovery Studio 2020. Molecular docking simulations were carried out using MGLTools 1.5.6 software equipped with AutoDock 4.2 to observe and identify the affinity and interactions that occur between the macromolecules of the tyrosinase enzyme and cinnamaldehyde. The distance between the surface of the enzyme macromolecule and the test compound molecule is limited to a maximum radius limit of 0.375 Å. All simulations were carried out using a grid box size of 64 x 60 x 60, then the Lamarckian Genetic Algorithm method with 100 conformations was used [10].

2.1.2. Preparation of Emulgel Containing Cocoa Pod Husk Extract and Cinnamon Oil

The emulgels were prepared with and without the addition of cinnamon bark oil. The formulations of the emulgels were shown in table 1. The emulgel preparations were made by heating the oil phase (olive oil, span 80) and also the water phase (aquadest, tween 80) at a temperature of 70°C. Next, the water and oil phases were mixed. Cinnamon oil, alpha-tocopherol, lesgard natural, and propylene glycol were added to the previous mixture and mix using high-speed homogenizer until homogeneous [11,12].

2.1.3. In Vitro Tyrosinase Inhibitory Activity Determination of the Emulgel Preparation

Tyrosinase inhibitory activity test was performed using dopachrome methods. The emulgels were dissolved in DMSO, then diluted using potassium phosphate buffer (pH 6.5) to achieve a final concentration from 625 to 10000 ppm. 70 µL of each sample was combined with 30 µL of tyrosinase in triplicate. After incubation at room temperature (5 minutes), 110 µL of a substrate (L-Dopa) was added to each well. The sample was then incubated for 30 min at room temperature. Absorbance in each well was read using a microplate reader. % inhibition of the samples was calculated [13].

FE = Cocoa pod husk emulgel without addition cinnamon bark oil; FEM = Cocoa pod husk emulgel with addition cinnamon bark oil.

TABLE 1: Formulation of emulgel.

Ingredients	Formulation (% w/w)	
	FE	FEM
Cinnamon bark oil	-	1
Olive oil	15	15
Cocoa pod extract	2	2
Tween 80	5.3	5.3
Span 80	4.7	4.7
Lexgard Natural	1	1
Tocopherol	0.1	0.1
Propylene glycol	15	15
Viscolam Mac 100	7	7
Triethanolamine (TEA)	Qs	Qs
Distilled water ad	100	100

3. RESULTS AND DISCUSSION

The cocoa pod husk extract was known to contain many phytochemical constituents. Phytochemical screening showed that cocoa pod husk extract containing alkaloids, flavonoids, saponins, quinones, terpenoids, steroids, tannins, and polyphenols. Flavonoids and polyphenols compounds of cocoa pod husk extract contributed to tyrosinase inhibitory activity. GC-MS characterization of cinnamon bark oil showed that the most abundant constituent of the oil is cinnamaldehyde (53.33%). The GC-MS result showed in fig. 1. That compound is the aldehyde that gives cinnamon its flavor and odor. Some research showed that cinnamaldehyde has tyrosinase inhibitory activity.

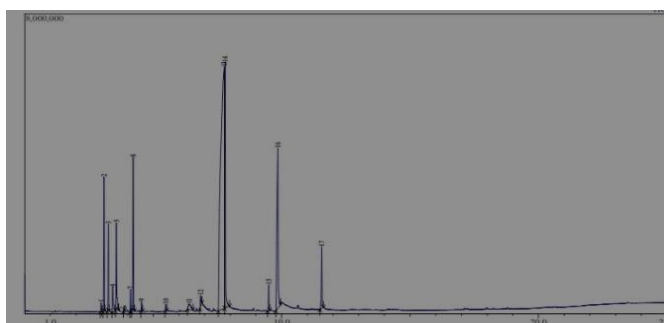


Figure 1: GC-MS results of Cinnamon bark Oil.

Tyrosinase enzyme is an enzyme that plays a role in the synthesis of melanin. The natural substrate of this enzyme is tyrosine, so in this study, the tyrosine molecule is used to identify the active site of the enzyme. Furthermore, the molecular docking test was carried out to see the ability of the cinnamaldehyde compound to interact with the active site of the tyrosinase enzyme. The results can be seen in Fig. 2. From the result,

it can be seen that cinnamaldehyde can interact with the active site of the tyrosinase enzyme with binding free energy is 4.88 kcal/mol. Cinnamaldehyde can bind to the active site of the enzyme due to the formation of hydrophobic interactions. The ability of cinnamaldehyde to bind to the active site of the enzyme makes it a potential to act as a competitive inhibitor of the enzyme [14].

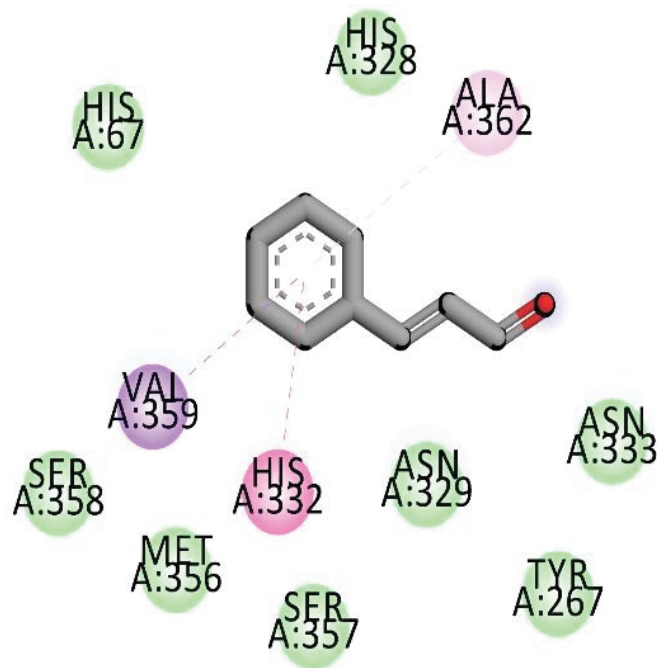


Figure 2: Molecular interaction between cinnamaldehyde and active site of the enzyme.

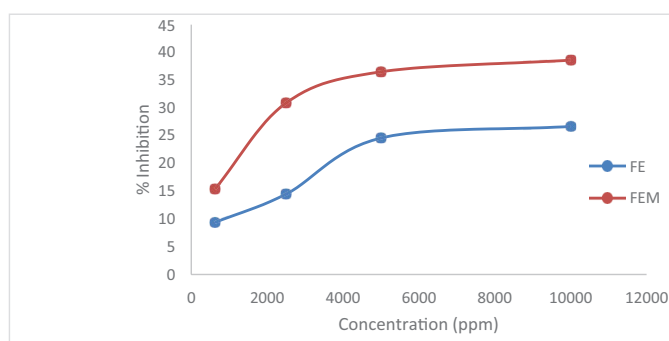


Figure 3: Tyrosinase inhibitory activity of emulgels (FE: without oil, FEM: containing 1% of cinnamon Oil).

Cinnamon oil was added to the emulgel preparation containing cocoa pod husk to increase the tyrosinase inhibitory activity. The tyrosinase inhibitory activity was conducted using the colorimetric enzymatic assay. The effect of cinnamon oil addition to the tyrosinase inhibitory activity of the emulgel can be seen in Fig. 3. It can be concluded that the addition of 1% cinnamon oil can significantly increase the tyrosinase inhibitory activity of the emulgels, based on the percent inhibition parameter. The percent

inhibition increase by 63.33 %, caused by the addition of cinnamon oil. The ability of cinnamaldehyde and other compounds of cinnamon oil to bind on the active site of tyrosinase enzyme support that activity (Table 2).

TABLE 2: % inhibition of tyrosinase enzyme.

Sample Concentration (ppm)	% inhibition		The percentage increase (%)
	FE	FEM	
625	9.48	15.50	63.47
2500	14.59	30.85	111.50
5000	23.72	36.59	35.57
10000	26.67	38.07	42.76
	Average		63.33

FE = Cocoa pod husk emulgel without addition cinnamon bark oil; FEM = Cocoa pod husk emulgel with addition cinnamon bark oil.

4. CONCLUSIONS

Cinnamon oil contains cinnamaldehyde compounds at a concentration more than 50%. Molecular docking testing showed that cinnamaldehyde can bind to the active site of the tyrosinase enzyme with binding free energy is 4.88 Kcal/mol. The addition of cinnamon bark oil (1%) on the emulgel preparation increasing the tyrosinase inhibitory activity by 63.33 %.

References

- [1] Nieuweboer-Krobotova L. Hyperpigmentation: types, diagnostics and targeted treatment options. *J Eur Acad Dermatol Venereol*. 2013 Jan;27 Suppl 1:2–4.
- [2] Desai SR. Hyperpigmentation therapy: a review. *J Clin Aesthet Dermatol*. 2014 Aug;7(8):13–7.
- [3] D'Mello SA, Finlay GJ, Baguley BC, Askarian-Amiri ME. Signaling Pathways in Melanogenesis. *Int J Mol Sci*. 2016 Jul;17(7).
- [4] Zolghadri S, Bahrami A, Hassan Khan MT, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, et al. A comprehensive review on tyrosinase inhibitors. *J Enzyme Inhib Med Chem*. 2019 Dec;34(1):279–309.
- [5] Priani SE, Aprilia S, Aryani R, Purwanti L. Antioxidant and tyrosinase inhibitory activity of face serum containing cocoa pod husk phytosome (*Theobroma cacao* L.). *J Appl*

- Pharm Sci. 2019;9(10):110–5.
- [6] Karim AA, Azlan A, Ismail A, Hashim P, Abd Gani SS, Zainudin BH, et al. BMC Complement Altern Med. 2014;14(1):1–13.
- [7] Priani SE, Humanisya H, Darusman F. Int J Sci Res. 2014;3(13):2338–2339.
- [8] Sreevidya VS. Journal of Pharmaceutical and Phytopharmacological Research. 2019;9(1):92–97.
- [9] Chang CT, Chang WL, Hsu JC, Shih Y, Chou ST. Chemical composition and tyrosinase inhibitory activity of Cinnamomum cassia essential oil. Bot Stud (Taipei, Taiwan). 2013;54(1):1–7.
- [10] Senol FS, Khan MT, Orhan G, Gurkas E, Orhan IE, Oztekin NS, et al. In silico approach to inhibition of tyrosinase by ascorbic acid using molecular docking simulations. Curr Top Med Chem. 2014;14(12):1469–72.
- [11] Mohamed MI. Optimization of chlorphenesin emulgel formulation. AAPS J. 2004 Oct;6(3).
- [12] Priani SE, Lestari IA, Lestari F. Jurnal Ilmiah Ibnu Sina. 2019;4(1):66–76.
- [13] Batubara I, Darusman LK, Mitsunaga T, Rahminiwati M, Djauhari E. J Biol Sci. 2010.
- [14] Asadzadeh A, Fassihi A, Yaghmaei P, Pourfarzam M. Biotech. Res. Asia. 2015;12.