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Research Article

Elastase Inhibitory Activity, Determination of Total Polyphenol and Determination of Total Flavonoids, and Pharmacognosy Study of Faloak Plant (*Sterculia quadrifida* R.Br) from East Nusa Tenggara-Indonesia

Sofiah Yunita Radjah[1], Kunia Sari Setio Putri[2], Berna Elya[1,*]

ABSTRACT

Introduction: Faloak (*Sterculia quadrifida* R. Br) is one of the typical plants of East Nusa Tenggara (NTT). Faloak contain flavonoid and polyphenol compounds, and show strong antioxidants activity which potentially correlated with its elastase inhibitory activity. Therefore, in this research, elastase inhibitory activity on various part of Faloak plant was investigated. **Objective:** The purpose of this research was to investigate the elastase inhibitory activity, determination of total polyphenol, determination of total flavonoids, and also pharmacognosy characterization of Faloak leaves, roots, stems and stem barks. **Methods**: Sample of leaves, roots, stems, and stem barks were extracted by 70% ethanol using ultrasound-assisted extraction (UAE). Phytochemical screening, microscopic identification and elastase inhibitory activity testing were performed on the leaves, roots, stems, and stem barks extract. This extract with the highest elastase inhibitory activity was then determined for its total polyphenol content and of total flavonoids content. **Result**s: UAE method with 70% ethanol successfully extracted active compounds from leaves, stems, roots, and stem barks of Faloak. Extract of all Faloak parts contained alkaloids, flavonoids, tannins, terpenes, and glycosides. The extract of Faloak stem barks showed the strongest elastase inhibitory activity as compared to the extract from other parts, with IC50 of 73.7 µg/mL. Alkaloid, flavonoid, tannin, terpene, and glycoside were detected as secondary metabolite in the extract of leaves, roots, stems and stem barks. The extract of Faloak stem barks showed the highest elastase inhibitory activity with IC_{50} 73.7 µg/mL. The total flavonoids and total polyphenol content of Faloak stem bark extract were respectively 28.75 mg/gram and 45.25 mg/gram extract. **Conclusion**: The 70% ethanol extract of leaves, roots, stems, and stem barks of Faloak showed elastase inhibitory activity, and stem barks extract showed the strongest activity. Faloak stem barks extract can be considered as potential to be developed as active compound in anti-aging product, both in cosmetic and pharmaceutical dosage forms.

Key words: Elastase inhibitory, Polyphenol, Flavonoids, *Sterculia quadrifida*.

Sofiah Yunita Radjah[1], Kunia Sari Setio Putri[2], Berna Elya[1,*]

[1]Department of Pharmacognosy, Phytochemistry, and Natural Products, Faculty of Pharmacy, Universitas Indonesia, Depok, INDONESIA.
[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, Universitas Indonesia, Depok, INDONESIA.

Correspondence

Berna Elya

Department of Pharmacognosy, Phytochemistry, and Natural Products, Faculty of Pharmacy, Universitas Indonesia, Depok, INDONESIA.

E-mail: berna.elya@farmasi.ui.ac.id

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INTRODUCTION

Sterculia quadrifida R. Br. of Sterculiaceae family is locally known as "Faloak" in East Nusa Tenggara Province, Indonesia. Some parts of Faloak contain flavonoid and polyphenol compounds, and show strong antioxidants activity which potential to be developed as a source of natural antioxidants.[1] Previous research was carried out by Grace and Siswadi (2019) on the extraction of Faloak stem bark using the maceration method with 70% ethanol solvent and showed a total flavonoid content (TFC) of 6.618 mg ± 0.123 mg quercetin equivalent/gram extract.

There have been several studies on other plants with antioxidants activity which showed that antioxidant activity was potentially correlated with their elastase inhibitory activity. Research by Salem et al. (2020) reported that *R. officinalis* contains flavonoid compounds and high total polyphenol content which correlated with strong antioxidant and anti-aging activity. Thus, it can be used for further development of topical preparations with anti-aging properties. In the study, total polyphenol and total flavonoid levels were directly proportional to the inhibition of elastase activity. The higher the total polyphenol content and total flavonoid levels, the higher the activity inhibition against elastase.[2]

Elastase is an enzyme that is responsible for the breakdown of elastin.[3] Misregulation of the elastase enzyme is involved in the skin aging process.[4] Inhibitors of the elastase have the potential to be developed into anti-aging ingredients. Several natural compounds from plants have been shown to inhibit the activity of this enzyme and to promote synthesis of elastin[5]. In the previous research, *Rubus Fraxinifolius Stem* and *Rhus javanica* L. contain flavonoids and polyphenol which have antioxidant activity and correlate with elastase inhibitory activity.[6,7] Some of polyphenols found in plants such as catechin, epicatechin, epigallocatechin, boswellic acid, and flavonoid such as purpurin, quercetin,

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kaempferol and myricetin also showed inhibitory activity against elastase.[8-10]. Based on these data, the extract of the Faloak plant part is predicted to be a potential candidate for skin antiaging through the inhibitory effect of the elastase enzyme.

Ultrasound-Assisted Extraction (UAE) has the advantage to increase the yield of the extract with the use of fewer solvents and reduce extraction time[11,12]. Based on previous research, the combination of the UAE method and ethanol solvent has successfully used as an efficient extraction method for polyphenol compounds and flavonoids in several plants.[12-14] Therefore, in this research, four parts of the Faloak plant (leaves, roots, stems, and stem barks) were extracted with 70% ethanol solvent using the UAE method. Furthermore, phytochemical screening and elastase inhibitory activity tests were carried out on the extract of each part of the plant. Extracts of plant parts that showed the highest elastase inhibitory activity was then determined for its total polyphenol and total flavonoid content.

MATERIALS

Stem barks, stems, roots, and leaves of Faloak were collected from East Penfui village, Central Kupang district, Kupang District, East Nusa Tenggara Province and was determined at Herbarium Bogoriensis, Biological Research Center of the Indonesian Institute of Sciences in February 2020. The solvent used in research was pro-analysis solvent (ethanol p.a , methanol p.a), 70% ethanol , Trizma base (Sigma Aldrich No.T1503), N-Succinyl-Ala-Ala-Ala-p-nitroanilide / SANA (Sigma Aldrich SLBR 7591V), porcine pancreas elastase (Sigma Aldrich SLBV 9311), HCl, *Aqua demineralisata* (Brataco Chemika, Indonesia), DMSO (Merck, Germany), quercetin (Sigma Aldrich, India), Folin Ciocalteau (F9252 / F47641), *aqua destillata* (Brataco Chemika, Indonesia).

Microscopic observations by scanning electron microscope (SEM) and light microscope

Microscopic analysis of leaves, roots, stems, and stem barks of Faloak were performed using SEM Model: JSM – IT 200 in the Zoology Field of the Biology Research Centre – Indonesian Institute of Sciences (LIPI), Cibinong.

Extraction

Leaves, stem barks, stems, roots extract of Faloak were obtained by extraction using the UAE method using an ultrasonic bath and 70% ethanol as a solvent. Each powder of faloak was put into a container, then filled with 70% ethanol to the ratio powder (g): solvent (mL) = 1:20. The 5-gram of leaves, stem barks, stems, and roots powder was soaked in 100 mL of ethanol 70% and extracted by an ultrasonic bath with a frequency of 40 kHz, temperature 40^{0}C for 30 minutes. The extract of Faloak were filtered to take the supernatant, then evaporated using a rotary vacuum evaporator and using a water bath until a thick extract is obtained.

Phytochemical screening

The identification of compounds in the extract was carried out on 70% ethanol extract of stem barks, stems, roots and leaves of Faloak. The extracts were subjected to preliminary phytochemical investigation for the detection of following compounds; terpenoids, glycosides, flavonoids, alkaloids, tannins, and saponins. The procedures described by Indonesian Pharmacopoeia 4th edition and Harborne[15,16].

Elastase inhibitory activity

Roots, stem barks, stems, leaves extracts were prepared and diluted into various concentrations. Elastase inhibitory activity assay was performed using an established protocol from Sigma Aldrich with slight modifications according to the previous research. [8,17,18] Plant extract 20 µl in DMSO solution, 130 µl Tris HCl buffer pH 8.0 and, 0.022 unit PPE were incubated within 15 minutes at 25^{0}C. 0.29 mM SANA substrate was then added and mixed immediately and the mixture was then reincubated within 15 minutes with light protection at room temperature. Quercetin was used as a standard control. The absorbance was measured at 401 nm using microplate reader versamax. The experiments were performed in triplicate. Elastase inhibitory activity was calculated using the following formula:

$$\text{Percentage of inhibition (\%)} = \frac{(A-B)-(C-D)}{(A-B)} \times 100$$

Where A= Blank absorbance; B=Blank control absorbance; C=Sample absorbance; D=Sample control absorbance

Determination of total polyphenol

Determination of polyphenol content was measured on extracts with the highest elastase inhibitor activity (stem barks). The procedures described by Indonesian Herb Pharmacopoeia[19]. Methanol P was added into 200 mg extract up to 25 ml. 1.0 mL of this sample solution is taken, then added with 5 mL diluted Folin-Ciocalteu LP (7.5% in aqua destillata), 4 mL 1% NaOH then incubated at room temperature for 1 hour. The absorbance of this solution was measured using UV-Vis.

Determination of total flavonoids

Determination of flavonoids content was measured on extracts with the highest elastase inhibitor activity (stem barks). The procedures described by Indonesian Herb Pharmacopoeia.[19] Ethanol P was added into 200 mg extract up to 25 ml. 0.5 mL of this sample solution is taken, then added with 1.5 mL of ethanol P, 0,1 mL 10% AlCl3 P, 0.1 mL, Na-acetate 1M, and, 2.8 mL aqua destilata. The total volume of the solution is 5 mL. The solution was shaken and and then incubated at room temperature for 30 min. The absorbance of this solution measurement using UV-Vis.

RESULTS AND DISCUSSION

Microscopic characterization of plant powder

The microscopic fragment using SEM were shown in Figures 1- 4.

Extraction

Faloak were extracted using UAE with 70% ethanol as solvent.

The extraction results are shown in Table 1.

UAE method on Faloak resulted the highest extract yield from stem barks, followed with extract from roots, leaves and stems. The higher yield of extract indicates the higher concentration of extracted component.

Phytochemical screening

Phytochemical screening on the extracts showed the presence of flavonoids, glycosides, alkaloids, tannins, terpenes, and saponins, as described in Table 2.

Ethanol extract from all parts of Faloak plant contained flavonoids which potentially exhibit antioxidant activity as well as elastase inhibitory activity. The tannin compound was also detected in all of the extracts, thus we further measured total phenolic concentration.

Elastase inhibitory activity

The elastase inhibitory activity of extracts from four parts of Faloak plant were investigated, and quercetin was used as the standard compound. The result was shown in Table 3.

Table 3 showed that stem bark extract exhibited the strongest elastase inhibitory activity with IC50 73.7 µg/mL as compared to the extract

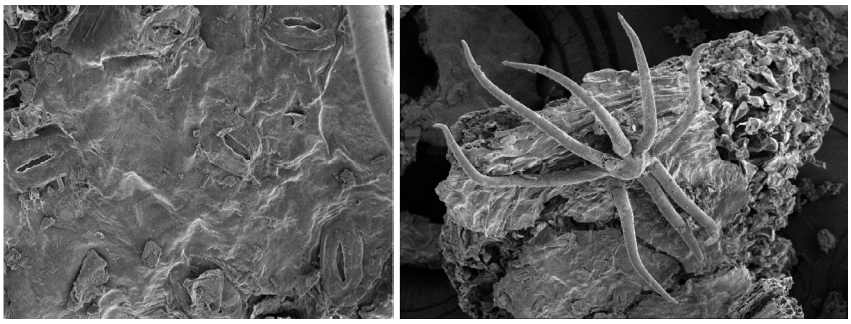


Figure 1: Microscopic of Faloak leaves powder. A= Stomata, B= Star shape trichoma.

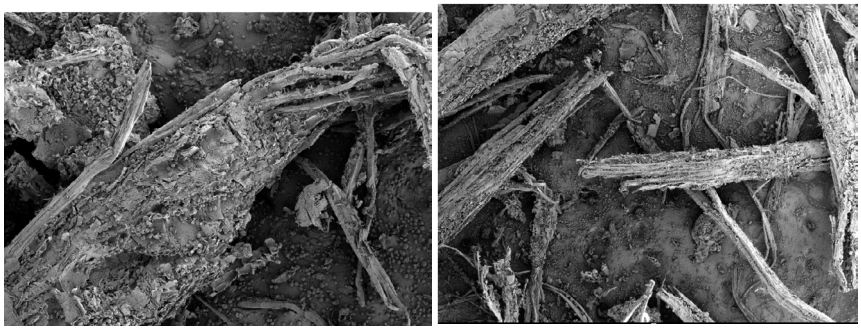


Figure 2: Microscopic of Faloak roots powder. A=epidermis, B= Xylem.

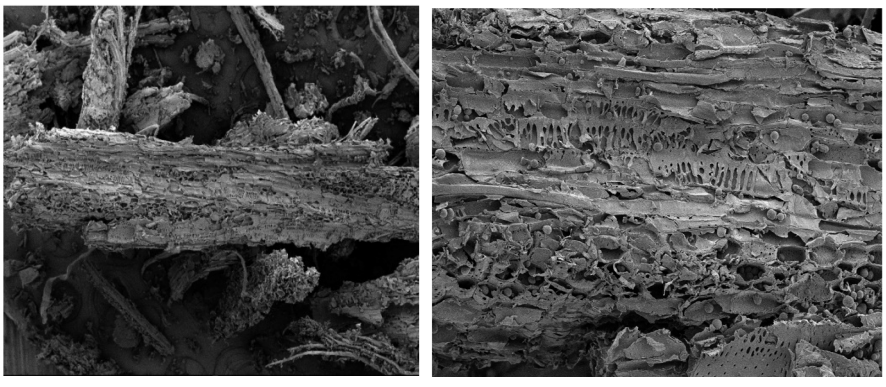
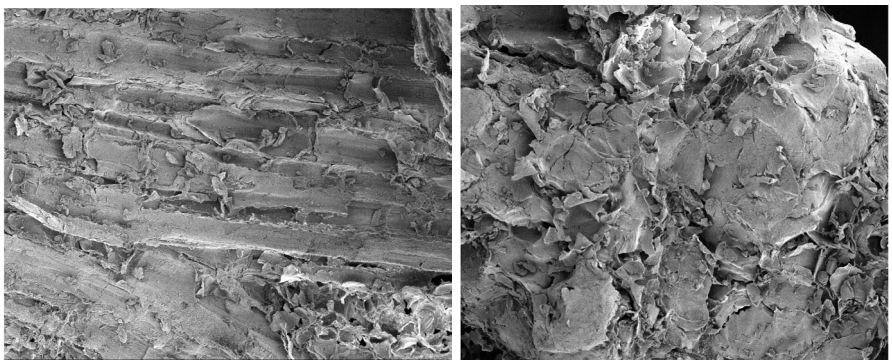


Figure 3: Microscopic of Faloak stems powder. A= Xylem, B= Parenchim.



A B

Figure 4: Microscopic of Faloak stem barks powder. A= Epidermis, B= Parenchim.

Table 1: The yield of Faloak extraction.

Result	Leaf	Root	Stem	Stem bark
Extract weight (g)	14.4	15.6	12.6	18
Yield (%)	12	13	10,5	15

Table 2: Phytochemical screening of extract 70% ethanol of Faloak plant.

Chemical compound	Results			
	Leaves	Roots	Stems	Stem barks
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Terpenes	+	+	+	+
Saponins	-	-	-	-
Glycosides	+	+	+	+

Table 3: Elastase inhibitory activity of Faloak extracts from different part.

Sample	Elastase inhibitory (IC50)
Quercetin	29.14 µg / mL
Faloak stem barks	73.7 µg / mL
Faloak stems	141.7 µg / mL
Faloak roots	166.3 µg / mL
Faloak leaves	183.04 µg / mL

from other part of the plant. Elastase is an enzyme which responsible for the breakdown of elastin[3], thus a compound with inhibitory activity toward elastin can be considered to be developed into anti-aging ingredients. This result of this research indicated that the stem bark extract of Faloak plant with concentration of 73.7 µg/mL can inhibit 50% of elastase activity, thus this extract showed potential as anti-aging component. As comparison to the Faloak stem bark extract, quercetin as standard showed stronger elastase inhibitory activity with IC_{50} of 29.14 µg/mL.

Determination of total flavonoids and total polyphenol

Since the extract of Faloak stem bark extract showed the strongest elastase inhibitory activity, total flavonoid and polyphenol content of this stem bark extract were further measured.

Quercetin levels were calculated as total flavonoid and total polyphenol levels in the sample. Standard curve of quercetin for total flavonoid measurement showed linear regression equation y = 0.0019x+0.0345 and value of relation coefficient (r) =0.9979. Based on the measurement, the average flavonoids content in each gram of extract was 28.75±0.002 mg. Meanwhile, standard curve of quercetin for total polyphenol measurement showed linear regression equation y = 0.0052x+0.0304 and value of relation coefficient (r) =0.9952. Based on the measurement results, the average polyphenol content in each gram of extract was 45.25±0.002 mg. These results indicated that Faloak stem barks extract contained relatively high polyphenol and flavonoid components, and these flavonoids and phenolic are possible to have a role as an elastase inhibitor.

Previous studies showed that flavonoids, such as quercetin, kaempferol, and myricetin, can significantly inhibit elastase proteolytic activity[8,10] Other studies also showed that polyphenol inhibit elastase proteolytic activity by forming hydrogen bonds between their hydroxyl group with amino acids in the enzyme, causing a hydrophobic effect that results in a complex or aggregate, insoluble precipitate, which decreases catalytic activity and denatures the enzyme. Polyphenol form hydrogen bonds with amino acids in PPE: serine, histamine, and aspartate residues known as the catalytic triad.[20] Another plant study reported that contains polyphenol and flavonoids that have the potential to inhibit elastase is *Rhus javanica*.[21] The major polyphenol compounds of

Rhus Javanica have been reported as were gallic acid, methyl gallate, syringic acid, pentagalloylglucose, and protocatechuic acid.[22] Due to its chemical compound, polyphenols had potent antioxidants to scavenge Reactive Oxygen Species (ROS). Polyphenols may have inhibited activity of proteolytic enzyme such as elastase.[8] Based on previous research, species of *Rhus javanica* in Jeju Island had strong anti-aging ability by inhibiting elastase 80.8±0.5 at 80% ethanol leaf extract 500 µg/ ml and IC50 = 70.5 µg / mL.[21] Research conducted by Mardhiyah et al., 2020 reported that the water fraction of *A. carambola* Depok leaves with a total polyphenol and flavonoid content of 115.68 mg gallic acid equivalent g extract and 9.15 mg equivalent quercetin / g extract can inhibit proteolytic activity elastase and prevent premature skin aging.[2]

Based on the result on this research on elastase inhibitory activity, total flavonoid and total polyphenol content, it is considered that Faloak stem bark extract can be considered as potential to be developed as active compound in anti-aging product, both in cosmetic and pharmaceutical dosage forms.

CONCLUSION

UAE method with 70% ethanol successfully extracted active compounds from leaves, stems, roots and stem barks of Faloak. Extract of all Faloak parts contained alkaloids, flavonoids, tannins, terpenes, and glycosides. The extract of Faloak stem barks showed the strongest elastase inhibitory activity as compared to the extract from other parts, with IC_{50} of 73.7 µg/mL. The total flavonoid and total polyphenol content of faloak stem barks extract were respectively 28.75 mg/gram and 45.25 mg/gram extract.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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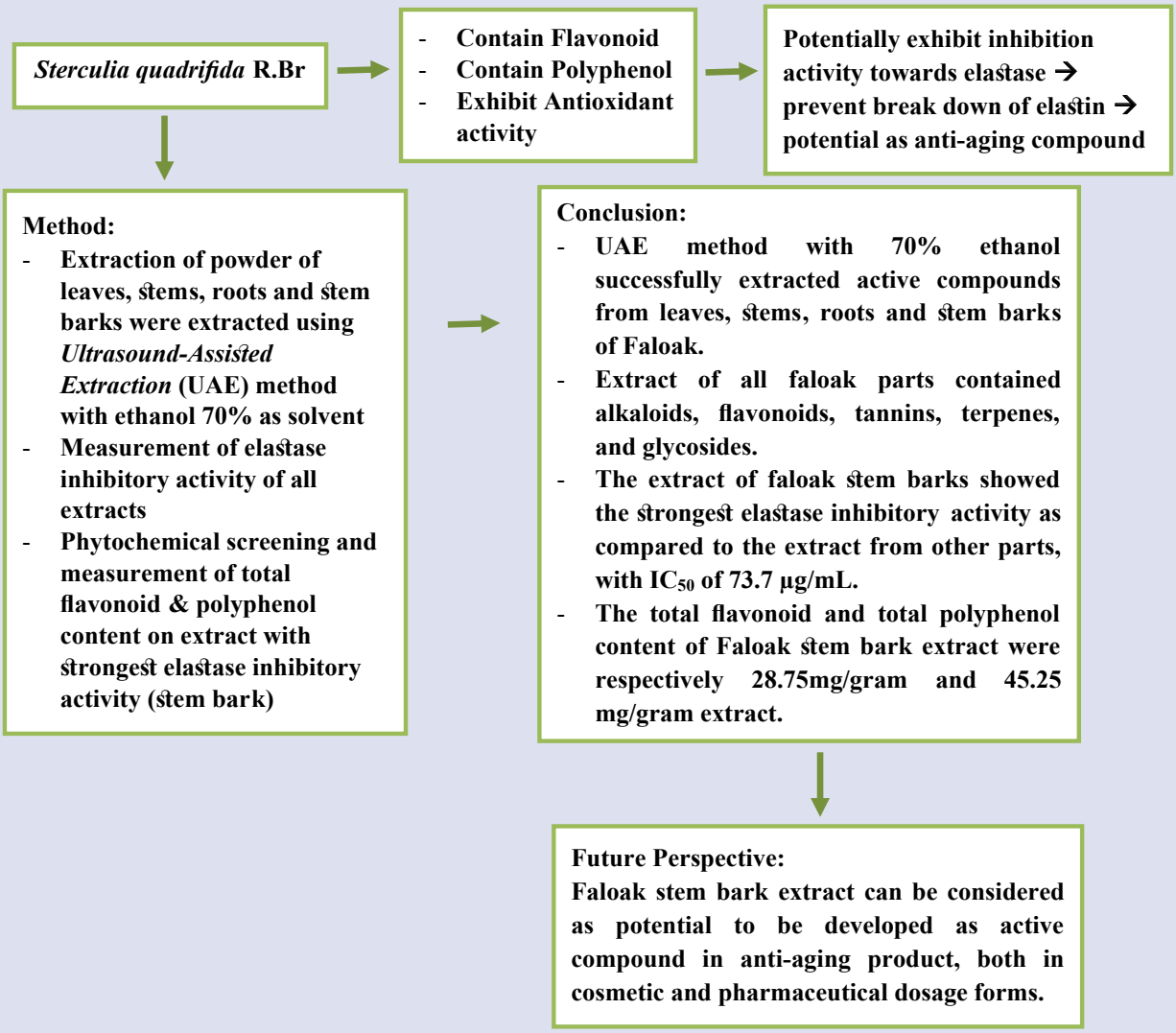
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Sofiah Yunita Radjah is a Pharmacist and Magister Pharmacy Student at the Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, West Java 16424, Indonesia. She conducted research on pharmacognosy study of natural product and biotechnology of pharmacy.

Kurnia Sari Setio Putri is a lecturer at Faculty of Pharmacy, Universitas Indonesia. She gained her doctoral degree from University of Groningen, The Netherlands in 2019. Her main research interest are in pharmaceutical technology, especially in developing modified/ advanced pharmaceutical dosage forms.

Berna Elya is a Professor and Lecturer at the Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, West Java 16424, Indonesia. She develops works in the area of Pharmacognosy and Phytochemistry (Natural Product).

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