Evaluation of Some Biological Activities, Total Phenol and Flavonoid Contents of Artocarpus Altilis

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Abstract — **Artocarpus altilis, commonly known as breadfruit, contains large amount of phenolic compounds, which is reported to possess various bioactivity. In this research, some extracts of breadfruit were determined total polyphenol and flavonoid contents, DPPH free radical scavenging activity, inhibition of tyrosinase and collagenase activity, and anti UV effect. The extracts of the leaves from Artocarpus altilis show good results in terms of total polyphenol and flavonoid contents as well as biological activities. Thus, the leaves of Artocarpus altilis are potential sources for development of skin whitening and anti-aging products.**

Keywords — **Artocarpus Altilis, Anti UV, Biological Activities, Collagenase, Total Polyphenol and Flavonoid Contents, Tyrosinase.**

I. Introduction

Breadfruit, a member of the genus Moraceae, has the scientific or Latin name Artocarpus altilis that is derived from Greek (artos = bread, karpos = fruit), and altilis means 'fat' [1]. Baked or roasted in a fire, the fruit has a starchy texture and fragrance that is reminiscent of fresh baked bread. In addition, breadfruit is widely used as wrapping fish by leaves to roast in an oven, building canoes and making utensils [2]. Besides, being reported to include triterpenes and flavonoid, Artocarpus altilis particularly is a rich source of phenolic compounds [3],[4]. Not only in nutrition, but breadfruit is also considered in pharmaceuticals for its anticancer, antioxidant, antibacterial and antifungal activity [3],[5],[8]. Moreover, some isolated compositions such as artocarpin, cudraflavone C, 6-prenylnaringenin, norartocarpin, and cudraflavone B from breadfruit could be good candidates for the development of skin-whitening agents due to showing favorable result in tyrosinase inhibitor, which is key enzyme for melanin biosynthesis in mammalian cells, and anti-melanogenesis via competitive and mixed inhibition.

Melanin is a natural skin pigment that is essential for protecting human skin against UV radiation. However, when melanin appears too much, it will cause various skin disorders, typically tan or brown spots. Melanogenesis is a complex process to produce melanin under the control of tyrosinase. Tyrosinase (EC 1.14.18.1) is a binuclear copper-containing monooxygenase, which catalyzes three different reactions in the melanin biosynthetic. Tyrosinase is the main factor causing some dermatological diseases including freckles, age spots, and melisma [9]. Hydroquinone, arbutin, kojic acid, azelaic acid and L-ascorbic acid are commercial tyrosinase inhibitors, which have been used as skin-whitening agents, but these compounds have certain drawbacks. Thus, the finding of new efficient and safe antityrosinase agents from nature is necessary for anti-hyperpigmentation product development.

Through references, it has been shown that Artocarpus altilis has many interesting biological activities. However, at present, in Vietnam, there are few scientific publications on breadfruit, therefore, this research was conducted with the objectives of preparing breadfruit extracts with containing amount of phenolic and flavonoid contents and evaluating DPPH free radical scavenging, tyrosinase and collagenase inhibitory activity, and anti UV effect of the extracts.

II. Materials And Methods

A. General Experimental Procedures

Optical values were measured on a Shimadzu UV-1800 spectrophotometer (Shimadzu Pte., Ltd., Singapore). L-dihydroxyphenylalanine (L-DOPA) and tyrosinase (EC 1.14.18.1) from mushroom (3933 U·mL−1) and were obtained from Sigma-Aldrich (Sigma-Aldrich Pte., Ltd., Singapore). Other chemicals were of the highest grade available.

B. Collection

Leaves and branches of Artocarpus altilis were collected in October 2020 from Dong Thanh Commune, Hoc Mon District, Ho Chi Minh City, Vietnam. The leaves were separated and naturally dried together with branches for 7 days. All of the dried materials are then grinded into small pieces separately and stored in normal condition.

C. Extraction

Reflux apparatus was used for extraction. Each material was also weighted 5 g with 250 mL 96% ethanol (E) and water (W) in different ratios in the reflux apparatus at boiling temperature replacing solvent 3 times after 3 boiling hours. The extracts were concentrated using a rotary evaporator and stored in the refrigerator for further use.

D. Total Phenolic and Flavonoid Contents

1) Total polyphenol content

The total polyphenol content was performed as Folin-Ciocalteau described method [10], [11]. Results were expressed as mg gallic acid equivalents (GAE) g-1 material.

2) Total flavone and flavonol content

The total flavanone and flavanonol content were determined by the 2,4-dinitrophenylhydrazine described

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method.[12] Results were expressed as mg (2S)-pinostrobin equivalents ((2S)-PTE) g-1 of material.

E. Biological Activities

1) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay

The DPPH scavenging activity assay was performed as previously described method [8]. Trolox, an antioxidant, was used as the positive control.

2) Tyrosinase inhibitory assay

The tyrosinase inhibitory activity assay was analyzed by using the described method. Kojic acid, a known tyrosinase inhibitor, was used as the positive control [9].

3) Collagenase inhibitory assay

The collagenase inhibitory activity assay was determined as previously described method [13]. Ethylene diamine tetraacetic acid (EDTA) was used as the positive control.

F. Anti UV Effect

Anti UV effect was performed as described method [14]-[16].

III. Result And Discussion

A. Extraction Efficiency

After evaporating the solvents, the collected extracts had a sticky texture, and its color was dark brown. The extraction efficiency ranges from 7.98 to 31.26%. In which, the E:W (1:1) leaves extract has the highest extraction efficiency.

B. Total Phenolic and Flavonoid Content

The results for total phenolic and flavonoid contents of extracts are presented in Table I. Leaves were extracted with E:W (1:1) solvent gave the richest total phenolic and flavonoid sources because of the highest extraction efficiency. For leaves, ethanol leaves extract also had the second highest total phenolic and flavonoid contents. On the other hand, branches were detected opposite trend that the higher the amount of ethanol in the extraction solvent, the richer the total phenolic and flavonoid sources. Leaves showed significantly greater content of total phenolic and flavonoid than branches when extracted with the same solvent.

Up to now, the publications on the total phenolic and flavonoid contents of breadfruit are still quite few in the world. Most international publications use solvents such as methanol, hexane, and dichloromethane for extraction [17]-[19]. Therefore, it is difficult to compare the results of total phenolic and flavonoid contents of Artocarpus altilis that the research has obtained with the results published in the world. In addition, the novelty of this research is also shown by providing the results of total phenolic and flavonoid contents obtained from breadfruit (leaves and branches) when extracted with 3 different solvents from 96% ethanol and water.

Total polyphenol content of the extracts ranged from 0.62 to 2.28 mg GAE g-1 material. For leaves, the data clearly outline the richest this content-E:W (1:1) extract (2.28 mg GAE g-1 material), following by ethanol extract (1.23 mg GAE g-1 material). Besides, for branches, total polyphenol content gradually increased from 0.62, 0.79 to 0.89 mg GAE g-1 material when the amount of ethanol in the solvent increased.

Total flavone and flavonol content of extracts ranged from 0.19 to 0.82 mg QE g-1 material. Similar to total polyphenol content, E:W (1:1) and ethanol leaves extract achieved the highest total flavone and flavononol content, 0.82 and 0.42 mg QE g-1 material, respectively. Branches showed the same trend with total polyphenol content, in which content of total flavone and flavononol was found to be 0.31 mg QE g-1 material in ethanol extract that equal to E:W (7:3) leaves extract.

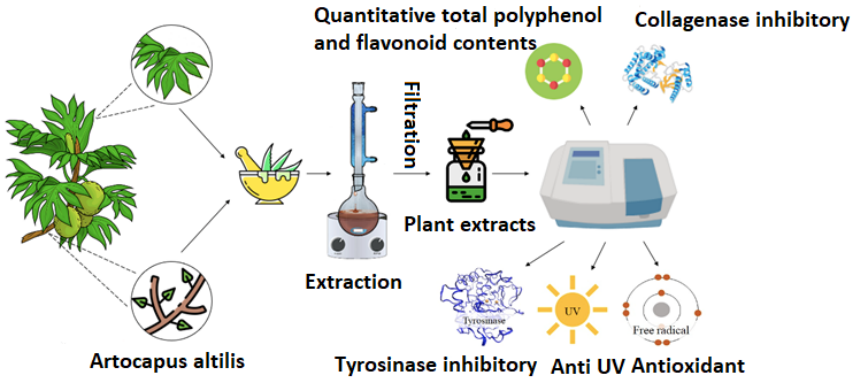

Fig. 1. Graphic of TOC.

TABLE I: Total Phenolic And Flavonoid Contents Of Extracts

Extracts	Total polyphenol content (mg GAE g^{-1} material)	Total flavone and flavonol (mg QE g^{-1} material)	Total flavanone and flavanonol (mg (2S)-PTE g-1 material)
Leaves, E	1.23	0.42	1.47
Leaves, E:W (7:3)	1.13	0.31	0.94
Leaves, E:W (1:1)	2.28	0.82	2.11
Branches, E	0.89	0.31	0.88
Branches, E:W (7:3)	0.70	0.22	0.69
Branches, E:W (1:1)	0.62	0.19	0.70

Content of total flavanone and flavanonol in extracts ranged from 0.69 to 2.11 mg (2S)-PTE g-1 material. For leaves, the same was true that E:W (1:1) and ethanol leaves extract showed the richest of this content, 2.11 and 1.47 mg (2S)-PTE g-1 material, respectively. Ethanol branches extract still reached the highest total flavanone and flavanonol content (0.88 mg (2S)-PTE g-1 material), while the result for E:W (7:3) and E:W (1:1) branches extract was quite similar.

C. Biological Activities

The results for DPPH radical scavenging, tyrosinase and collagenase inhibitory activity of extracts are presented in Table II. All extracts had the ability to inhibit tyrosinase, collagenase activity and scavenge DPPH free radical, except E:W (1:1) leaves extract activities than extracts from the other two solvents. Therefore, these extracts were selected to evaluate anti UV effect.

1) DPPH radical scavenging activity

Antioxidant activity of plant extract was mainly due to presence of phenolic compound that may exert an antioxidant effect as free radical scavengers. Both materials, when extracted with ethanol, showed higher DPPH radical scavenging activity than other solvents, IC50 = 63.3 and 75.6 µg mL-1 for leaves and branches, respectively. However, Artocarpus altilis leaves and branches extracts had moderate antioxidant activity which might be due to their phenolic constituents.

2) Tyrosinase inhibitory activity

All extracts had the ability to inhibit tyrosinase activity with the IC_{50} value ranged from 67.1 to 7.3 µg mL^{-1}. E:W (7:3) branches extract provided interesting result, which exhibited remarkable inhibitory effect with the IC_{50} value of 7.3 µg mL^{-1} that equal to 1.15 times of kojic acid. For leaves, ethanol extract showed the most potent activity with an IC_{50} value of 16.8 µg mL^{-1}. In this research, extracts of *Artocarpus altilis* had lesser absorbance than possitive control, suggesting that extracts possessed less tyrosinase inhibitory activity than kojic acid.

Until now, there have been quite a few publication on the ability to inhibit tyrosinase enzyme of *Artocarpus altilis*. However, most of the these publications focus on the core of breadfruit wood and mainly use methanol and diethyl ether solvents for extraction. It give IC_{50} values of methanol extracts as 29.9 and 7.1 µg mL^{-1} for heartwood and branches; 10 µg mL^{-1} for core wood diethyl ether extract.[19,20] Because of the difference of experimental procedures, it is difficult to compare the IC_{50} value showing the ability to inhibit tyrosinase enzyme of *Artocarpus altilis* that this research obtained with the results published in the world.

This is the first time reported on the ability to inhibit tyrosinase enzyme of breadfruit on leaves and branches when extracted with 3 different solvent with the lowest IC_{50} value of 7.3 µg mL^{-1}.

3) Collagenase inhibitory activity

The percentage of inhibition activity (I%) illustrated the different between that of the three distinct concentrations of extracts comparing with EDTA are presented in Table 2. Leaves showed the higher effects in collagenase inhibition than that of branches. Comparing with EDTA, all extracts had less collagenase inhibitory activity than positive control because EDTA was found to possess inhibitory effect with the I% value of 71.64 ± 1.22 at 1000 µg mL-1, which was higher than the I% value of all extracts.

TABLE II: BIOLOGICAL ACTIVITIES OF EXTRACTS

Sample		DPPH radical scavenging activity	Tyrosinase inhibitory activity	Collagenase inhibitory activity	
		IC_{50} (µg mL^{-1})		Concentration (µg mL^{-1})	I%
Positive control	Trolox	2.7	-	-	-
	Kojic acid	-	6.3	-	-
	EDTA	-	-	1000	71.64 ± 1.22
Extracts	Leaves, E	63.3	16.8	50	10.94 ± 6.91
				100	52.39 ± 1.22
				200	92.15 ± 0.60
	Leaves, E:W (7:3)	64.8	67.1	50	3.05 ± 1.90
				100	16.05 ± 1.44
				200	34.48 ± 5.35
	Leaves, E:W (1:1)	> 100	25.8	50	5.55 ± 0.71
				100	15.68 ± 3.50
				200	35.37 ± 0.16
	Branches, E	75.6	35.8	50	8.71 ± 2.93
				100	17.79 ± 2.02
				200	34.91 ± 3.52
	Branches, E:W (7:3)	89.1	11.0	50	16.01 ± 1.72
				100	32.82 ± 2.61
				200	59.47 ± 0.40
	Branches, E:W (1:1)	85.0	7.3	50	3.61 ± 2.31
				100	15.14 ± 2.49
				200	65.83 ± 1.35

TABLE III: UV-PF, UVA-PF AND Λ_c OF ETHANOL EXTRACTS

	Leaves				Branches			
Concentration (µg mL^{-1})	125	150	200	250	275	300	350	400
UV-PE (SPF)	10.08±0.69	15.09±1.03	37.19±2.55	124.51±7.98	23.22±0.15	32.19±0.20	54.67±0.32	97.37±0.55
UVA-PF	6.49±0.46	8.96±0.63	18.34±1.30	36.32±2.14	13.32±0.35	17.60±0.05	26.92±0.07	42.78±0.12
χC	381.5±0.5	381.00±1.00	381.00±0.50	378.50±0.5	382.00±0.10	382.00±0.10	382.00±0.10	382.00±0.10

D. Anti UV Effect

From the same material weight, ethanol leaves and branches extracts had high total phenolic and flavonoid contents and exhibited better biological UV-PF and UVA-PF values of ethanol extracts were increased along with the rise of the concentration. However, the increasing rates between concentration were different, the increase between 200 and 250 µg mL-1 for leaves and 350 and 4000 µg mL-1 for branches were highest. All of the concentrations of extracts that had UV-PF and UVA-PF in standard range ($15 \leq$UV-PF ≤ 100 and $5 \leq$ UVA-PF ≤ 33) and critical wavelength greater than 370 nm so those concentrations were concluded as broad spectrum. Thus, for checking the ideal range of UV-PF ($35 \leq$ UV-PF ≤ 60) and UVA-PF ($10 \leq$ UVA-PF ≤ 25) both ethanol extracts had potential in anti-UV and the concentration would be in the range from equal or greater than 200 to less than 250 µg mL-1 for leaves and equal or less than 300 to less than 350 µg mL-1 for branches.

IV. Conclusion

Artocarpus altilis leaves and branches were selected for this research. Reflux apparatus was used for extraction. Phenolic compound was also present in these extracts. Some biological activities were done by using DPPH, tyrosinase, collagenase and anti UV method. Artocarpus altilis extracts showed moderate antioxidant activity as compared to trolox and possessed less tyrosinase inhibitory activity than kojic acid. For collagenase inhibitory activity, all extracts had less inhibition activity than EDTA. However, ethanol breadfruit extracts had potential in anti-UV because of UV-PF and UVA-PF values in the standard range and broad spectrum with critical wavelength greater than 370 nm. It may be due to the presence of a modest amount of phenolic compounds in the leaves and branches of the plant. Hence, the extracts of this plant may not act as first-line antioxidants and anti-UV defense but may be used as a supportive agent.

The extracts of the leaves from Artocarpus altilis show good results in terms of total polyphenol and flavonoid contents as well as biological activities than that branches. In addition, branches had low extraction yield and sample collection is difficult, so breadfruit leaves are potential sources for the development of skin whitening and anti-aging products.

In the future, further investigations are required to enrich phenolic compounds for their biological activities. This plant is proven to have antioxidant, inhibition activity of tyrosinase, collagenase and anti-UV effect potential, so further investigation on various pharmacological activities supported by these biological activities is recommended.

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