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Studies on Antioxidant and Antityrosinase Properties of *Premna integrifolia* Linn. (Taung-tangyi) in Myanmar

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Abstract

This research studies dermatological properties (the antiaging and inhibition of melanin systhesis) of Taung-tangyi. 2-hydroxy sesamin (**1**), 1-hydroxy sesamin (**2**), 4-hydroxy sesamin (**3**), 1,4-dihydroxy sesamin (**4**), 4,8-dihydroxy sesamin (**5**), apigenin 6,8-diglycoside (**6**) were isolated by column chromatography. Among the isolated compounds, five exhibited radical scavenging property. Taung-tangyi was also exhibited tyrosinase inhibitory activity (inhibition of melanin synthesis). Radical scavenging activity (Antioxidant) of EtOH extract was more potent (IC_{50}=44.2mg/ml) than H_2O extract (IC_{50}=55.03mg/ml). Percent inhibition of ethanolic extract (60.5%) was greater than that of water extract (41.5%) at 30 mg/ml concentration. All the isolated compounds at 0.5 mg/ml concentration level were found to inhibit the melanin synthesis. This is the first report for the presence of these compounds in *Premna integrifolia* Linn.

Key words: *Premna integrifolia*, antioxidant, antityrosinase

Introduction

Non-toxic natural products are useful in the formulation of cosmetics of considerable interest. Recent efforts have focused on the identification of substances that inhibit tyrosinase activity or suppress formation of reactive oxygen species (ROS) in skin cells. Since tyrosinase is the rate-limiting enzyme in the synthesis of melanin, the pigment responsible for the colour of human skin, tyrosinase inhibitors may have skin-whitening effects. Since ROS have been implicated in the aging of human skin, agents that suppress the production of ROS may retard such aging (Afanas'ev, 2005). Natural cosmetics have been used since times immemorial. It refers to the cosmetics produced from things obtained from nature. They may either involve using the products directly or derivatives of these products, which can be wisely utilized in preparing different type of cosmetics. Plants products are the most widely used ingredients in natural cosmetics. Thus, they are also often referred to as herbal cosmetics. Natural cosmetics have a host of benefits. Being natural, they are considered to be quite harmless on the skin. They contain time tested ingredients with proven efficiency. A judicious combination of potent herbs not only produces cosmetic effect but also helps cure skin ailments and hair problems. The natural products such as leaves, roots, fruits etc. supply several essential nutrients to the skin too. Natural cosmetics use various parts of plants – the leaves, the bark, and the roots, the oil extracted from seeds, the fruits and also at times the whole plant (http://www.allnaturalbeauty.us). Nowadays, people have become more interested in natural products as they believe that synthetic products provide some adverse effect. Most researchers try to evaluate the valuable things from nature. As an example, Kawamura, 1994 (in: http://www.ezilon.infobase.com) studied the efficiency in UV protection of skin cosmetic or hair preparation consists bark or stem extract of *Premna integrifolia* Linn. (cited by http://www.ezilon.infobase.com). However, none of the study have been undertaken from the aspect of dermatology.

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Therefore, the present research is aimed to isolate the chemical constituents from the heartwood of Taung-tangyi and to verify its effectiveness in dermatological properties.

Materials and Method

Plant materials

Heartwood of *Premna integrifolia* Linn. (Taung-tangyi) was collected from Myeik Township, Taninthayi Region. It was cut into small pieces and air-dried at room temperature. Then, it was ground into powder and stored in air-tight containers to prevent moisture changes and contamination.

Extraction of phytoconstituents from heartwood of *Premna integrifolia* Linn.

Taung-tangyi powder (100) g was percolated with (350 cm^3) of pet-ether for 24 hours to get pet-ether extract. Then, the residue was percolated with 95% EtOH (350 cm^3) to get EtOH extract. Both extracts were evaporated under reduced pressure to obtain constant weight.

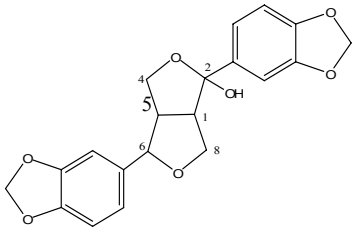
Isolation of chemical constituents from *Premna integrifolia* Linn. (Taung-tangyi)

(100) g of defatted dried powder was extracted with 95% ethanol (3 x 350 cm^3) for 4 hour at room temperature. After removal of solvent, (11g) of crude extract was obtained and transferred into silica gel column (100 g; 2.0 cm in diameter) packed in EtOAc. The column was eluted with EtOAc : EtOH (19:1), EtOAc : EtOH (9:1), EtOAc: EtOH (8:1), EtOAc : EtOH (7:1), EtOAc : EtOH (6:1), EtOAc : EtOH (4:1), EtOAc : EtOH (1:1) and finally with EtOH. The fractions were checked and combined to provide two major fractions (**F-I** and **F- II**).

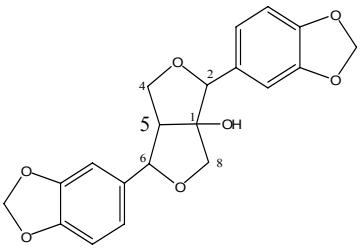
Crystallization of **F-I** in chloroform provided Compound **1 (2-hydroxy sesamin)** (colorless needles, 300 mg, 0.3% in yield, m.p. 150°C, R_f = 0.36 in $CHCl_3$). The mother liquor after crystallization was subjected to silica gel column chromatography (30 g, 1.0 cm in diameter) packed in PE : $CHCl_3$ (1:1). The column was eluted with PE : $CHCl_3$, (1:2); P.E : $CHCl_3$, (1:3); P.E : $CHCl_3$, (1:4); $CHCl_3$: CH_3OH (19:1); and finally with $CHCl_3$: CH_3OH (9:1). The fractions were checked by TLC and developed colour by spraying with 5% H_2SO_4 followed by heating. Fractions that provided similar TLC pattern were combined. In this way five major fractions were obtained.

Crystallization of $\mathbf{F_1}$ in $CHCl_3$-EtOAc provided Compound **2 (1-hydroxy sesamin)** (pale yellow crystals, 92 mg, m.p. 82°C, R_f = 0.45 in PE : $CHCl_3$, 1 : 2). Crystallization of $\mathbf{F_2}$ in $CHCl_3$: EtOAc provided Compound **3 (4-hydroxy sesamin)** (white crystals, 25 mg, m.p. 154°C, R_f = 0.42 in PE : $CHCl_3$, 1:2). Crystallization of $\mathbf{F_3}$ in $CHCl_3$-EtOAc provided Compound **4 (1,4-dihydroxy sesamin)** (pale yellow crystals, 47 mg, m.p. 130°C, R_f = 0.36 in PE : $CHCl_3$, 1:3). Crystallization of $\mathbf{F_4}$ in $CHCl_3$-EtOAc gave Compound **5 (4,8-dihydroxy sesamin)** (pale yellow crystals, 32 mg, m.p.160°C, R_f = 0.26 in PE : $CHCl_3$ 1:3).

Slowly evaporation of Fraction **F-II** at room temperature provided Compound **6** apigenin 6,8 diglycoside (yellow powder, 15 mg, 0.03% in yield, m.p. 209-210 °C, R_f = 0.32 in EtOAc : MeOH, 19:1) (Figure 1).



2-hydroxy sesamin (1)



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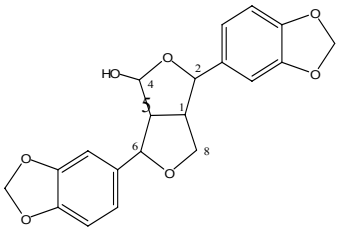
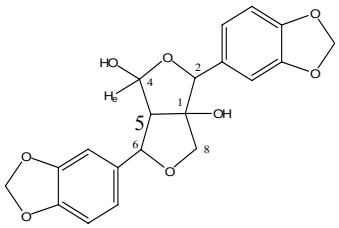
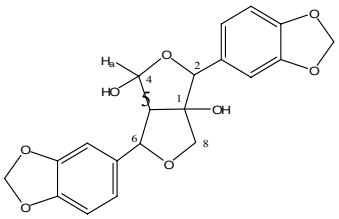


4-hydroxy sesamin (3)

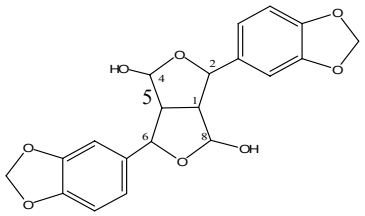


4a

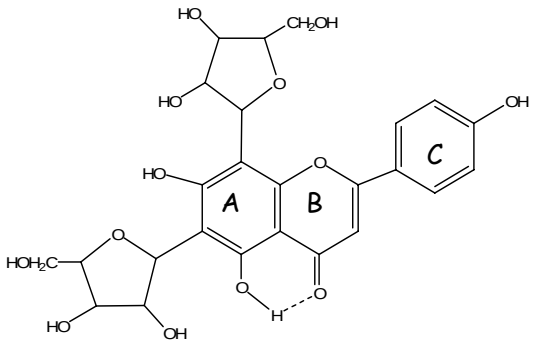


4b

1-4-dihydroxy sesamin**(4)**



4-8-dihydroxy sesamin(5)



Apigenin 6-8 diglycoside (6)

Figure 1. Structure of isolated compounds

A study on free radical scavenging activity of *Premna integrifolia* Linn.

The radical scavenging activity of EtOH extract, H_2O extract and isolated compounds were studied. Control solution was prepared by mixing 60mg DPPH solution (1.5cm^3) and 95% EtOH (1.5cm^3) using vortex mixer. Similarly the ***blank solution*** was also prepared by mixing test solution (1.5 cm^3) and 50% ethanol (1.5 cm^3). The ***sample solution*** was also prepared by mixing the test solution (1.5 cm^3) with 60 μM DPPH solutions (1.5 cm^3). All these solutions were allowed to stand at room temperature for 30 minutes. Then, the absorbance was measured at λ 517 nm recorded on spectrophotometer UV-160 1PC(P/N 206-6750) (Wong *et al*., 2006).

A study on skin whitening effect of *Premna integrifolia* Linn.

The inhibition of melanin synthesis of EtOH, H_2O and isolated compounds were determined by dopacrome method. Individual test sample solutions (each 0.1 cm^3) were placed into the test tubes. Tyrosinase (30 units) was added to each test tube and mixed by vortex mixer. Then, they were kept for 10 minutes. 10 mM Phosphate buffer (1.1 cm^3) and 0.55 mM tyrosine (1 cm^3) were added to individual tubes and mixed on vortex mixer. These tubes were incubated at 37°C for 10 minutes. After incubation, the absorbance was taken at 475 nm on spectrophotometer. Blank solution was prepared by mixing test sample solutions (0.1 cm^3) and tyrosine (1 cm^3). Negative control reaction was carried out without using test sample (inhibitor) and positive control was carried out using kojic acid as standard inhibitor (Tada *et al.,* 1996).

Results and Discussion

EtOH extract of Taung-tangyi showed greater radical scavenging activity (IC_{50}=44.2mg/ml) than H_2O extract (IC_{50}=55.03mg/ml). Among the isolated compounds, five were found to exhibit radical scavenging activity. They all possess mild radical scavenging activity. However, compound **1** and **3** have lower IC_{50} value of 22.01 and 22.35 μg/ml followed by compound **4** and **5** (22.35 and 25.1 μg/ml) (Table 1 and Figure 2). Test results clearly demonstrated that "Taung- tangyi" possesses the antioxidant property as it has the ability to trap free radical that generates in the skin. Therefore, it was inferred that using "Taung-tangyi" as a cosmetic would prevent photo-aging (aging caused by free radical).

Skin whitening effect of "Taung-tangyi" was studied by inhibition of it on tyrosinase activity in melanin synthesis. It was determined by using "dopacrome" method with reference to kojic acid (standard whitening agent). In this method, tyrosine was used as a substrate. Inhibition by kojic acid was used as a positive control and enzyme solution without test sample was used as a negative control. Decrease in absorbance of tyrosinase after exposing with the test sample revealed the anti-tyrosinase activity of that test sample. Of these two extracts, EtOH extract showed more pronounce suppression in tyrosinase activity (60.5%) than H_2O extract (41.5%). In addition, all isolated compounds showed antityrosinase activity (Table 2 and Figure 3). The minimum concentration to exhibit such activity was 0.5 mg/cm^3. Increase in concentration of test compounds provided greater inhibition as expected.

Table 1. Absorbance of DPPH, % Inhibition and IC_{50} of Different Samples Isolated from *Premna integrifolia* Linn and Ascorbic Acid.

Test Samples	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
Ethanol extract	20	0.18	36.84	44.2
	40	0.154	45.96	
	60	0.093	67.36	
	80	0.033	70.42	
Water extract	20	0.21	26.31	55.03
	40	0.172	39.64	
	60	0..121	57.54	
	80	0.074	74.03	
2-Hydroxy sesamin (**1**)	5	0.251	20.31	22.01
	10	0.207	34.28	
	20	0.164	47.93	
	40	0.119	62.22	
1-Hydroxy sesamin (**2**)	5	0.258	18.09	28.62
	10	0.208	33.65	
	20	0.189	40.00	
	40	0.109	65.88	
4-Hydroxy sesamin (**3**)	5	0.255	19.09	22.35
	10	0.203	35.55	
	20	0.163	48.25	
	40	0.093	65.08	
1,4-Dihydroxy sesamin (**4**)	5	0.265	20.01	24.56
	10	0.24	30.74	
	20	0.162	46.57	
	40	0.085	63.20	
4,8-Dihydroxy sesamin (**5**)	5	0.265	20.01	25.1
	10	0.24	29.74	
	20	0.162	4357	
	40	0.085	63.2	
Ascrobic acid	1.25	0.085	33.2	1.88
	2.50	0.034	66.61	
	5.00	0.037	69.59	
	10.00	0.029	77.13	

Table 2. % Inhibition of Different Concentrations of Crude Extracts and Isolated Compounds of *Premna integrifoli* Linn. on Tyrosinase Activity.

No.	Test Samples	Concentration (mg/cm^3)	Inhibition (%)
1	Ethanol Extract	20	-
		30	60.5
		40	63.2
2	Water Extract	20	-
		30	41.5
		40	42.4
3	2-Hydroxy sesamin (**1**)	0.25	-
		0.5	48.5
		1.0	52.3
4	1-Hydroxy sesamin (**2**)	0.25	-
		0.5	43.0
		1.0	51.8
5	4-Hydroxy sesamin (**3**)	0.25	-
		0.5	39.0
		1.0	45.2
6	1,4-Dihydroxy sesamin (**4**)	0.25	-
		0.5	48.8
		1.0	53.6
7	4,8-Dihydroxy sesamin (**5**)	0.25	-
		0.5	43.0
		1.0	50.2
8	Apigenin C-diglycoside (**6**)	0.25	-
		0.5	25.0
		1.0	32.0
9	Kojic acid (standard)	0.25	21.8
		0.5	52.2
		1.0	100.9

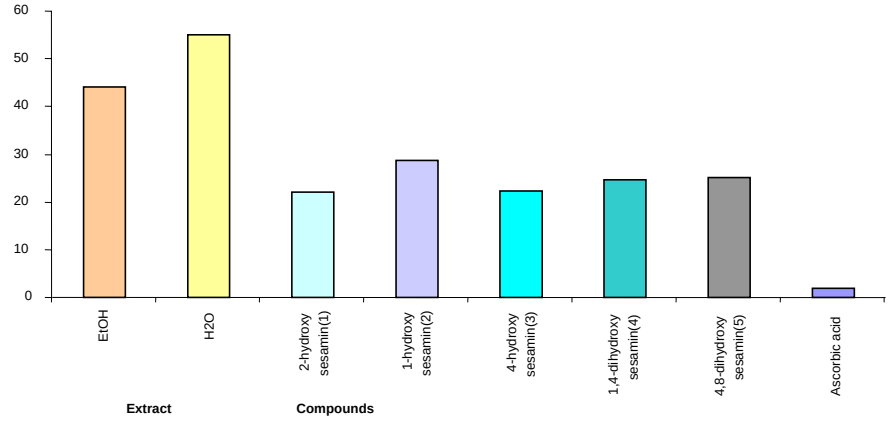


Figure 2. Radical scavenging activities of crude extracts and isolated compounds from Taung-tangyi and ascorbic acid

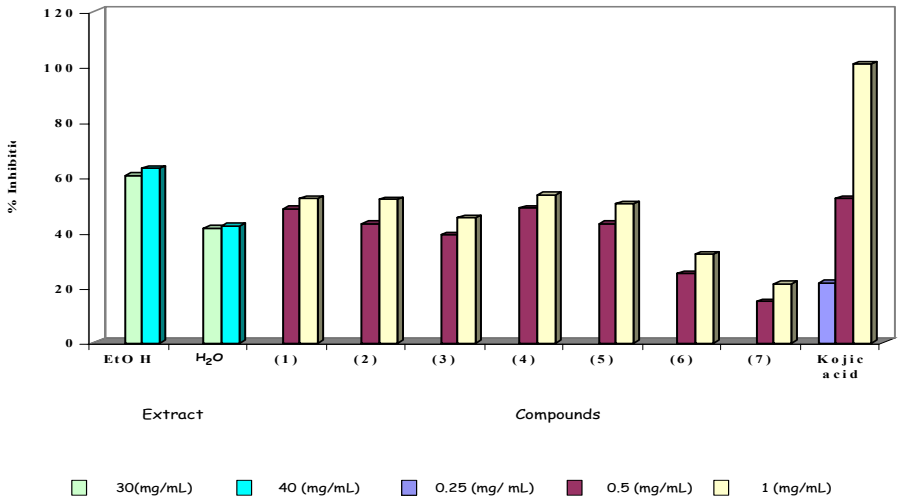


Figure 3. Comparison on tyrosinase inhibition percentage of crude extracts and isolated compounds of *Premna integrifolia* Linn.

Conclusion

2-hydroxy sesamin(**1**), 1-hydroxy sesamin(**2**), 4-hydroxy sesamin(**3**) , 1,4-dihydroxy sesamin(**4**), 4,8-dihydroxy sesamin(**5**) and apigenin 6-8-diglycoside(**6**) were isolated from heartwood of *Premna integrifolia* Linn. (Taung-tangyi in Myanmar). It was the first report for the presence of these compounds in *Premna integrifolia* Linn. It possesses mild radical scavenging activity when compared to standard antioxidant, ascorbic acid (IC_{50} = 1.88 mg/ml). Activity of ethanolic extract was more potent (IC_{50} = 44.2 mg/ml) than that of water extract (IC_{50} = 55.03 mg/ml). Five compounds were found to show radical scavenging property. Decreasing order of antioxidant activity of isolated compounds is **1** (IC_{50} = 22.01 mg/ml) > **3** (IC_{50} = 22.35 mg/ml) > **4** (IC_{50} = 24.56 mg/ml) > **5** (IC_{50}= 25.1 mg/ml) > **2** (IC_{50} =28.62 mg/ml). *P. integrifolia* Linn. exhibited antityrosinase activity (i.e. inhibition of melanin synthesis). Percent inhibition of ethanolic extract (60.5%) was greater than that of water extract (41.5%) at 30 mg/ml concentration. All the isolated compounds at 0.5 mg/ml concentration level were found to inhibit the melanin synthesis. Decreasing order of

inhibition property is **4** (48.8%) > **1** (48.5%) > **2** (43.0%) > **5** (43.0%) > **3** (39.0%) > **6** (25.0%).

It can be inferred that *Premna integrifolia* Linn. is an invaluable natural cosmetic as it has anti-aging property, sun protective effect and skin whitening effect. Therefore, it could be used as a natural ingredient in cosmetic products.

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Online Materials

1. http:// www.allnaturalbeauty.us, (2004)
2. http://www.ezilon.infobase.com, (2006)