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Free Radical Scavenging and Tyrosinase Inhibition Activities of *Hypoxis aurea* Lour. Tuberous Extracts

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ABSTRACT

The phytochemicals consisted in the *H. aurea* Lour. tuberous extracted by distilled water at 80ºC (HWH) and room temperature (27ºC) (HWR) were glycoside (deoxy sugers), and glycoside (deoxy sugers) and condensed tannins respectively, whereas condensed and hydrolyzed stable tannins was found by ethanolic extraction at room temperature (HER). In addition, the total phenolic contents from the HWH, HWR and HER extracts determined by Folin-Ciocalteu were 12.77 ± 0.05, 23.04 ± 0.42 and 18.63 ± 1.49 mg/g of extract respectively, which were compared with the standard gallic acid. The extracts at various concentrations were investigated for the free radical scavenging activity by DPPH assay and tyrosinase inhibition activity by the modified dopachrome method of those extracts. It was found that the HWR gave both the highest free radical scavenging activity (SC_{50} of 0.65 ± 0.06 mg/ml); and tyrosinase inhibition activity (IC_{50} of 0.12 ± 0.01 mg/ml) ($p < 0.05$), which was comparable with vitamin C as the anti-oxidant and tyrosinase inhibitor agent. This study has been suggested that the *H. aurea* Lour. tuberous extracted by distilled water at room temperature (27ºC) can be further developed as whitening cosmetic products.

Key words: Hypoxis aurea Lour., free radical scavenging, tyrosinase inhibition, phenolic compound

INTRODUCTION

Tyrosinase which is an enzyme-copper containing and catalyzes the two reactions, hydroxylation and oxidation, can change L-tyrosine to L-dopa and L-dopa to o-dopaquinone-H^+, and then pass the intermediates finally to melanin [6]. Melanin is the polyphenolic pigment, which is responsible for eye, hair and skin in animals [7]. Melanin plays the important role in protection the skin from ultraviolet (UV) radiation damages and removing reactive oxygen species (ROS). However, the high melanin accumulation and overproduction can cause the skin problems such as dark spots, melasma, freckles and several hyperpigmentation syndromes, which are the sign of aging [1]. In addition, ROS play a significant role in the regulation of the melanogenesis. Therefore, oxidation reaction and melanogenesis are the two major causes of skin problems such as wrinkles, dark spot and aging. Free radical scavenging and tyrosinase inhibition activity are widely used to determine the anti-oxidation and anti-melanogenesis, respectively, and are frequently used to evaluate the possibility of compounds for anti-aging and/or whitening effects in cosmetics [10]. Vitamin C and L-glutathione are a tyrosinase inhibitor and ROS scavenger, which can down-regulate melanogenesis [8]. As well, several a tyrosinase inhibitors and ROS scavengers from natural products can inhibit melanogenesis such as *Artocarpus lakoocha* heartwood extract which is the anti-tyrosinase and improve skin whitening [19].

Hypoxis aurea Lour. (or Star grass) is the plant from genus Hypoxidaceae (**Figure 1**), which is widely distributed in Torrid Zone in the world especially China, Japan and Southeast of Asia countries including Thailand. For the traditional medicine evidences, *H. aurea* Lour. has been used to treat hernia and warm kidney in China [3], while it can be used for treatment of acnes, dark spots and blemish in Thailand. It has been previously reported that the *H. aurea* Lour. extracted by ethanol contains quercetin-3-O-β-D-glucoside, kaemferol-3-O-β-D-glucoside, apigenin-5-O-β-D-glucopyranoside, a-spinasterol, 2,6-dimethoxy-benzoic acid, 1H-indole-3-carboxylic acid, (2S, 3R, 4E, 8E)-1-(β-D-ghicopyranosyloxy)-3-hydroxy-2 [((R)-2'-hydroxyeicosanoly)amino]-9-methy-4,8-

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octadeeadiene, n-dotriacontanol, 14,15-eicosenic acid, lignoceric acid, β-sitosterol, daucosterol, aureaside A, aureaside B, curculigoside I, orcinol glucoside, curcapital, cimifugin prim-*O*-*β*-D-glucopyranoside, 2-*O*-*β*-D-apiofuranosyl (1→6)-*O*-*β*-D-glucopyranoside, (*2R*,*5S*)-bornane-2,5-diol-2-*O*-*β*-Dglucopyranoside, and bornyl 7-*O*-*β*-D-apio-D-furanosyl(1→6)-*O*-*β*-D-glucopyranoside [3,4]. However, there is no report for bioactivity of *H. aurea* Lour. especially the bioactivity claiming for cosmetic science. This present study was investigated the free radical scavenging and tyrosinase inhibition activities as well as phytochemicals and total phenolic compounds of the *H. aurea* Lour. tuberous extracts, in order to evaluate the possible use of the extracts for cosmetic products.

Fig. 1: Tubers of the *Hypoxis aurea* Lour. or Star grass.

Materials and Methods

Extraction of the H. aurea Lour. Tubers:

The tubers of *H. aurea* Lour. were collected from Kanchanaburi, Thailand during October-November, 2012. The specimen was authenticated by Mr. Tanongsak Jonganurak, a botanist at Forest Herbarium-BKF, Department of National Parks, Wildlife and Plant Conservation, Thailand. The fresh tubers were died at 60°C, ground to powder and kept at dry place. The tuberous powder was extracted by boiling with controlled temperature at 85-90°C for 2 hrs (HWH), and macerated with distilled water (HWR) or 95% (v/v) ethanol (HER) at room temperature (27°C) for 24 hr. The extract was filtered through Whatman no.1 filter paper connected with a vacuum pump. The filtrates were concentrated by a rotary evaporator (R-205, Buchi, England) and lyophilized by a freeze dryer. The dried extracts were kept in an amber vial until use. The percentage yields were calculated on the dry weight basis.

Phytochemical analysis:

Phytochemicals contents in the extracts such as alkaloids, saponins, flavonoids, anthocyanins, glycosides and tannins, were investigated. Phytochemical tests of the extracts were assayed as previously described [20].

Total phenolic compounds:

The total phenolic compounds contents in the extracts were determined by the modified Folin-Ciocalteu assay [13]. Briefly, 50 µl of the samples at 1 mg/ml and 75 µl of Folin-Ciocalteu reagent (Sigma-Aldrich, USA) (0.5 mg/ml in ethanol) were put into each well of a 96-well microplate (Z-TEX 340r, Austria). Then 75 µl of 7.5% sodium carbonate solution was added into each well. The absorbances were measured by a microplate reader at 725 nm after 90 min of the reaction at 25ºC. The distilled water was used as control group. The amount of phenolic contents in the extracts were calculated from the standard curve of gallic acid (Sigma-Aldrich, USA) at various concentrations. The total phenolic compounds of the extracts were calculated as the following:

$$C\ (mg/ml) = c \times [V/m]$$

Where, C was the total phenolic compounds of the extracts (mg/ml); c was the amount of gallic acid (mg/ml); V was the volume of the extracts (ml); and m was the mass of the extracts (g).

Free radical scavenging activity:

Free radical scavenging activity of the extracts was determined by DPPH assay as previously described [12]. Briefly, 50 µl of the samples at the various concentrations and 50 µl of DPPH (Sigma-Aldrich, USA) solution (0.5 mg/ml in ethanol) were put into each well of a 96-well microplate. The absorbances were measured by a microplate reader at 515 nm after 30 min of the reaction at 25ºC. Vitamin C (Sigma-Aldrich, USA) was used as a standard. The percentages of the DPPH radical scavenging activity were calculated as the following:

$$\%\ Scavenging = [(A_0 - A_1)/A_o] \times 100$$

Where, A_0 was the absorbance of the control and A_1 was the absorbance of the treated sample. The concentrations providing 50% scavenging (SC_{50})

were calculated from the graph plotted between the free radical scavenging percentages and the sample concentrations.

Tyrosinase inhibition activity:

Tyrosinase inhibition activity was assayed by the modified dopachrome method using tyrosine as a substrate as previously described [11]. Briefly, 50 µl of the samples at various concentrations, 50 µl of 0.1 mg/ml L-tyrosine (Sigma-Aldrich, USA), 50 µl of 0.1 mg/ml mushroom tyrosinase (Sigma-Aldrich, USA), and 50 µl of 0.1mM phosphate buffer were added in 96-well microplates. Vitamin C was used as a standard. The mixture was incubated at 37°C for 60 min. Before and after incubations, the absorbances were measured at 450 nm by a microplate reader. The percentages of tyrosinase inhibition were calculated according to the following equation:

% Inhibition activity = [(A-B) - (C-D)]/(A-B) x 100

Where, A was the absorbance of the blank after incubation, B was the absorbance of the blank before incubation, C was the absorbance of the samples after incubation, and D was the absorbance of the samples before incubation. The concentrations providing 50% inhibition (IC_{50}) was calculated from the graph plotted between % inhibition activity and the concentrations.

Statistical analysis:

The results were presented as the mean ± SD of three independent experiments (n = 3). ANOVA was used for the analysis of the test results (LSD test) at the significance level of *p*-value <0.05.

Results and Discussion

The yields and characteristics of the H. aurea Lour. tuberous extracts:

The tubers of *H. aurea* Lour. have been known to improve skin problems in Thailand long times ago. The yields and characteristics of the tuberous extracts were showed in **Table 1**. The yield of the tuberous extract by maceration with distilled water at 27ºC (HWR) showed the highest extraction yield with 11.84%. All extracts were viscous-powder, brown and slightly odor.

Phytochemical contents and total phenolic compounds of the tuberous extracts:

Table 2 showed the phytochemical contents and total phenolic compounds of the *H. aurea* Lour. tuberous extracts. The phytochemical constituents in the tuberous extract by maceration with distilled water at 27ºC (HWR) were glycosides and tannins, whereas only glycoside and tannin were found in the tuberous extract by maceration with distilled water at 80-95ºC (HWR) and the tuberous extract by maceration with ethanol at 27ºC (HER), respectively. Phytochemicals found in the extracts depend on kinds of plants, solvent and; temperature and time used in the extraction process [12]. The tannins, a class of polyphenol is heat-labile, which might be degraded by heat process with long time extraction [14]. Thus, the absence of tannin in the HWH extract might be from boiling at high temperature (80-95ºC) with the 2 hr extraction time, whereas the glycoside content was still presence. Tannins have a lot of bioactivity such as anti-oxidation, anti-proliferative and apoptosis in many cancers [15,17]. The absence of tannins might affect with various bioactivities such as anti-oxidative and tyrosinase inhibition activities of the HWH extract.

Table 1: The percentages of yields and characteristics of the *H. aurea* Lour. tuberous extracts.

Extracts	Yields (%)	Characteristics
HWR	11.84	Brown Viscous powder Slight odor
HWH	9.86	Deeply brown Viscous powder Slight odor
HER	6.00	Brown Viscous powder Slight odor

Note: HWR was the *H. aurea* Lour. tuberous extracts prepared by maceration with distilled water at 27ºC; HWH was the *H. aurea* Lour. tuberous extracts prepared by maceration with distilled water at 85-90ºC; and HER was the *H. aurea* Lour. tuberous extracts prepared by maceration with 95% ethanol

Table 2: Phytochemical contents and total phenolic compounds of the *H. aurea* Lour. tuberous extract.

Extracts	Phytochemical contents						Total Phenolic Compounds (mg/g Extract)
	Alkaloids	Saponins	Flavonoids	Anthocyanins	Glycosides	Tannins	
HWR	-	-	-	-	+	+	23.04 ± 0.42^{a}
HWH	-	-	-	-	+	-	12.77 ± 0.05^{b}
HER	-	-	-	-	-	+	18.63 ± 1.49^{c}

Note: The symbols - was negative test; and + was positive test. HWR was the *H. aurea* Lour. tuberous extracts prepared by maceration with distilled water at 27ºC; HWH was the *H. aurea* Lour. tuberous extracts prepared by maceration with distilled water at 85-90ºC; and HER was the *H. aurea* Lour. tuberous extracts prepared by maceration with 95% ethanol; Superscript asterisks (a, b and c) in the columns indicate significant differences ($p < 0.05$).

In addition, it was found that the HWR extract exhibited the highest total phenolic compound of 23.04 ± 0.42 mg/g Extract ($p < 0.05$) determined by Folin-Ciocalteu colorimetric method. This might be due to the phytochemical contents (tannins and glycosides) in the extract. It has been reported that phenolic compounds showed several bioactivities such as anti-oxidation [22], anti-inflammatory [18] and anti-bacterial activity [5]**.** Thus, the presence of total phenolic compounds in the *H. aurea* Lour. tuberous extracts might have some bioactivities.

Free radical scavenging and tyrosinase inhibition activities of the tuberous extracts:

The free radical scavenging and tyrosinase inhibition activities of the tuberous extracts were represented in the SC_{50} and IC_{50} values (mg/ml), respectively. All tuberous extracts (HWR, HWH and HER) gave both free radical scavenging activity by DPPH assay and the tyrosinase inhibition activity by the modified dopachrome method (**Figure 2**). The SC_{50} and the IC_{50} values of the tuberous extracts were 0.65 ± 0.06, 8.60 ± 0.83 and 3.21 ± 0.21 mg/ml; and 0.12 ± 0.01, 1.66 ± 0.19 and 0.15 ± 0.02 mg/ml, respectively. The tuberous extract by maceration with distilled water at 27ºC (HWR) exhibited the highest free radical scavenging activity, but it was lower than that of vitamin C of 13 folds ($p<0.05$). Also, the HWR extract demonstrated the highest tyrosinase inhibition activity, which was comparable to vitamin C ($p<0.05$). It has been reported that tannins and glycosides, is an antioxidant and tyrosinase inhibitor which is the same with vitamin C [9,16,22]. For example, the tannins of *Aruncus silvester* Kostel. ex Maxim and *Potentilla alba* L. extracts showed strong anti-oxidative activity [15]. Oladele *et al*. [14] reported that tannins inhibit some enzyme activities such as trypsin, amylase and lipase by forming insoluble complexes, and divalent ions such as Fe^{2+} and Zn^{2+} ions. Thus, tannins from the tuberous extracts should inhibit the tyrosinase activity by divalent ions because tyrosinase is an enzyme-Cu^{2+} containing. Thus, the HWR extract which showed the highest activities might be due to the synergistic effect of phytochemicals, since the presences of both tannins and glycosides in the HWR extract. **Table 3** exhibited the correlation (*R*) between the total phenolic compounds and biological activities, the free radical scavenging and tyrosinase inhibition activities of the tuberous extracts. There is good positive correlation between the total phenolic compound (*PC*) and the free radical scavenging (*RS*) with the *R* of 0.7698; and tyrosinase inhibition activities (*TI*) of 0.8983, respectively, which indicating that these two activities of the tuberous extracts may be due to their total phenolic compounds. On the other hand, the correlation between the free radical scavenging (*RS*) and tyrosinase inhibition activities (*TI*) was excellent with the positive *R* of 1.000, which meant if the free radical scavenging of the tuberous extracts was increased, the tyrosinase inhibition activity would be increased as well. Therefore, the decreasing of tyrosinase activity as well as the presence of radical scavenging activity of the H. aurea Lour. tuberous extracts should directly to down-regulate on the melanogenesis [2]. At present, the anti-melanogenesis of the tuberous extracts on human melanocytes is in progress at our laboratory, Thai traditional medicine college (TMC), Rajamangala University of Technology Thanyaburi, Thailand.

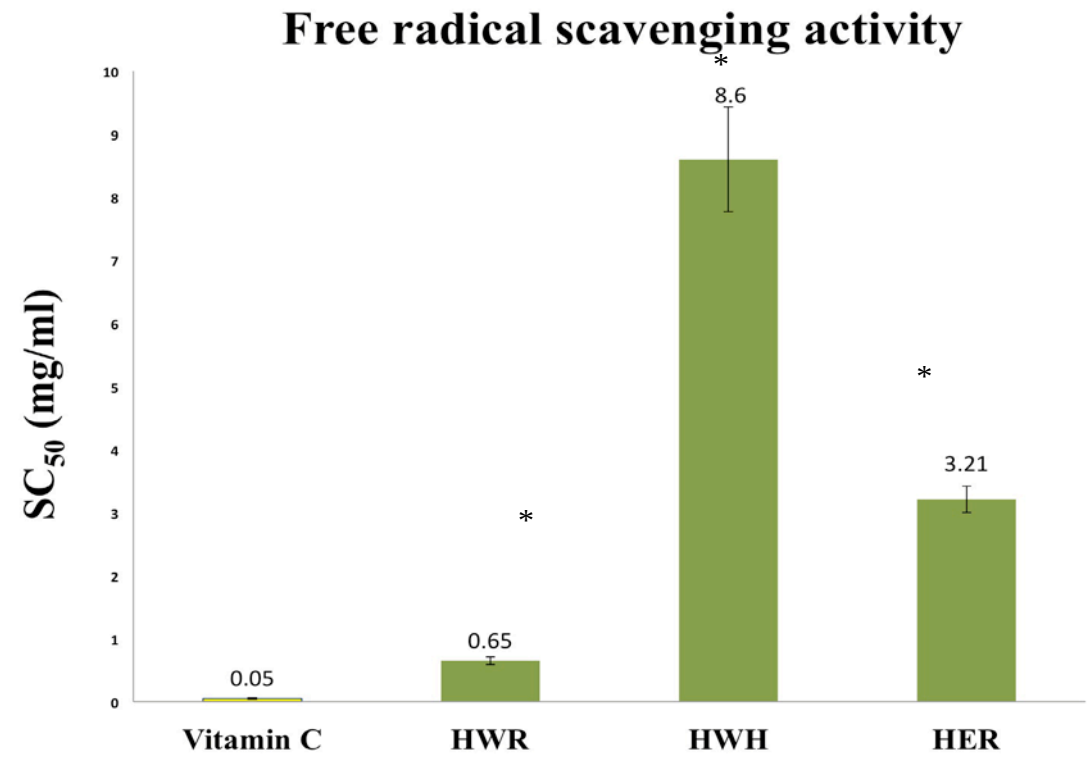
Conclusion:

The tuberous extract from *H. aurea* Lour. prepared by the maceration with distilled water at 27ºC (HWR) exhibited the tannins and glycosides with the total phenolic compounds of 23.04 ± 0.42 mg/g.Extract, and demonstrated the free radical scavenging and the good inhibition of tyrosinase activity, which was comparable to vitamin C. However, because of their phytochemical stability problem in heat and the basic environments, the proper forms such as the entrapment in nanovesicles are recommended. This study has suggested that the HER extract can be further developed as a novel tyrosinase inhibitor for whitening cosmetics.

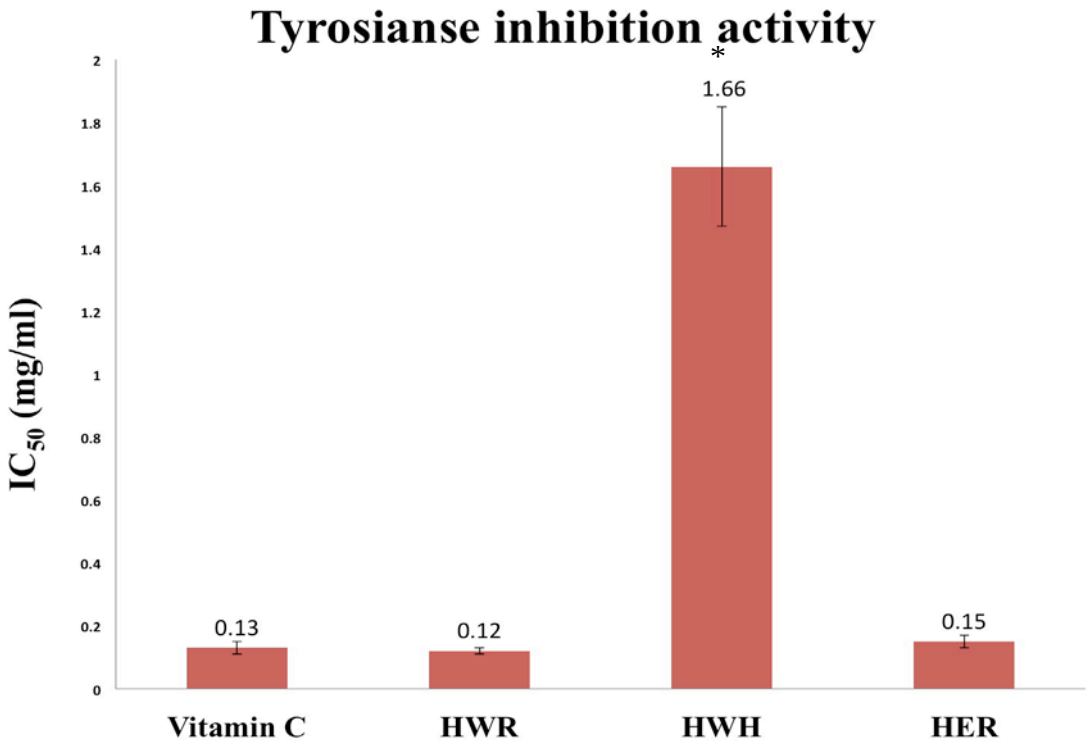
Table 3: Correlation (*R*) between the total phenolic compounds and biological activities, the free radical scavenging and tyrosinase inhibition activities of the tuberous extracts.

Extracts	Total Phenolic Compounds (*PC*)	Biological activities	
		Free Radical Scavenging (*RS*)	Tyrosinase Inhibition (*TI*)
HWR	23.04	1.54	8.33
HWH	12.77	0.11	0.60
HER	18.63	0.31	6.67

Note: total phenolic compounds (*PC*) was phenolic content (mg/g.Extract). Free cavenging activity (*RS*), and tyrosinase inhibition (*TI*) were calculated from 1/SC5 (mg/ml) and 1/IC50 (mg/ml), respectively.



(A)



(B)

Fig. 2: Free radical scavenging activity by DPPH assay (SC_{50} values) (A) and tyrosinase inhibition activity by the modified dopachrome method (IC_{50} values) (B) of the *H. aurea* Lour. tuberous extracts. HWR was the *H. aurea* Lour. tuberous extracts prepared by maceration with distilled water at 27ºC; HWH was the *H. aurea* Lour. tuberous extracts prepared by maceration with distilled water at 85-90ºC; and HER was the *H. aurea* Lour. tuberous extracts prepared by maceration with 95% ethanol Superscript asterisk (*) in the same columns indicate significant differences when compared with vitamin C ($p < 0.05$).

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