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Evaluation of Antioxidant Activity and Inhibition of Tyrosinase Activity of *Raphanus sativus* var. *caudatus* Alef Extract

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Abstract

This study was aimed to determine antioxidant and tyrosinase inhibition effects of the pod of *Raphanus sativus* L. var. *caudatus* Alef extract. The compounds consisted in the extracts were identified by HPLC from standard peak comparison. Pod was extracted by using 2 different solvents-dichloromethane (DCM) and water. The antioxidant activity was evaluated based on free radical scavenging (DPPH) activity and ferric reducing ability (FRAP) assay. The lightening effect was determined from the inhibition of mushroom tyrosinase *in vitro*. The results showed that DCM extract contained sulforaphene, protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, and ferulic acid. The aqueous extract contained sulforaphene, protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, vanillic acid, and *p*-coumaric acid. The $DPPH^{\bullet}$ scavenging effect expressed as the IC_{50} values for DCM and aqueous extracts were 883.38 ± 22.9 and 1,160.49 ± 22.30 µg/mL, respectively. The reducing power of aqueous extract (218.27 ± 0.010 µM $FeSO_4$ equivalent) was greater than DCM extract (166.34 ± 0.018 µM $FeSO_4$ equivalent) at the same concentration (2,000 µg/mL). However, both extracts showed lesser antioxidant activity than gallic acid, a positive control. DCM extract showed higher tyrosinase inhibitory effect than aqueous extract but lesser than kojic acid, a positive control. Both extracts at 2,000 µg/mL concentration exerted 42 and 19 % tyrosinase inhibition, respectively. In conclusion, the extraction solvent yielded different chemical constituents and thus the activities. The DCM extract exerted greater antioxidant activity and tyrosinase inhibition activity than the aqueous extract. The extract fractionation is required to get higher yields of bioactive compounds prior to further study and for cosmetics product development.

Keywords: HPLC, Antioxidant, Antityrosinase, *Raphanus sativus* var. *caudatus* Alef, Thai rat-tailed radish

Introduction

In the present, the cosmetic formulated from natural ingredient has gained interest. The beauty facial products are mostly for reducing freckles or dark spot, curing acne or providing moisturizing effect for anti-aging. Some of these products may contain hazardous substances, such as hydroquinone; when it is used higher than the regulate amount (2 %), it may cause skin irritation and may develop cancer [1]. Several studies have reported the detection of mercury, vitamin A derivative and steroid in some cosmetic products [1]. Mercury can cause burning sensation of the skin, contact dermatitis, nail discoloration [1], irritant, renal damage and neuropsychiatric symptoms [2]. Vitamin A derivative causes erythema and desquamation [3]. While steroid causes facial erythema, telangiectasia, aggravation of existing lesions and hypertrichosis [4]. Therefore, the search for safer compounds has been conducted.

Thai rat-tailed radish (*Raphanus sativus* Linn var. *caudatus* Alef; RS) is an indigenous Thai vegetable. Previous research reported that RS comprises of isothiocyanate compounds such as sulforaphane and sulforaphene [5,6]. The extracts of RS have been reported to possess many biological effects, se.g. anticancer action in colon cancer [6,7], lung cancer [8] and liver cancer [9], antioxidant activity [10], antifungal activity [11], antidepressant-like activity [12], and anti-leishmanial activity [13]. Previously, the freeze-dried juice of *Raphanus sativus* root showed tyrosinase inhibition effect (IC_{50} = 3.09 mg/mL) higher than the methanolic extract (IC_{50} = 9.62 mg/mL) [14]. Since Thai rat-tailed radish is in the same species as *Raphanus sativus*, therefore, it is of interest to explore the inhibition of tyrosinase activity.

In addition, phenolics are primary compounds found in many plants including Brassicaceae [15] and act as antioxidant-a desirable antiaging effect for cosmetic purpose. However, these compounds have yet been reported in Thai rat-tailed radish. In the present study, we identified the presence of phenolics and isothiocyanates (i.e., sulforaphane and sulforaphene) in the RS pod extract. The *in vitro* antioxidant was investigated from free radical scavenging (DPPH) ability and ferric reducing ability (FRAP) assay, while the lightening effect was determined from the mushroom tyrosinase inhibition.

Materials and methods

Chemicals

The commercial grade of dichloromethane (DCM) and the HPLC grade of glacial acetic acid, tetrahydrofuran (THF) and acetonitrile were purchased from V.S. Chem House (Bangkok, Thailand). Dimethyl sulfoxide (DMSO) (analytical grade) was purchased from Fisher Scientific (Loughborough, UK). Deionized (DI) water for HPLC analysis was from PURELAB option-Q (England). Pure D, L-sulforaphane was from Calbiochem (EMD, Darmstadt, Germany). L-sulforaphene was purchased from Enzo Life Science (NY, USA). Standards gallic acid, 3,4-dihydroxybenzoic acid (protocatechuic acid), *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid and ferulic acid, mushroom tyrosinase enzyme and 3,4-dihydroxy-L-phenylalanin (L-DOPA), 2,2-Diphenyl-1-picrylhydrazyl, Iron(III) chloride and 2,4,6,-tris(2-pyridyl)-S-triazine were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Kojic acid was from TCI (Tokyo, Japan). Methanol (analytical grade) was from Ajax Finechem Pty Ltd (NSW, Australia), hydrochloric acid 37 % was from QReC (New Zealand). Sodium acetate trihydrate was from Okhla Industrial Area, Phase-I (New Delhi, India).

The extract preparation

The RS pod was collected from Northern Thailand in 2017 and prepared for the extraction [16]. After washing and dry on sheet, the RS pod was cut in small pieces and homogenized in DI water at a ratio of 1:1 (w/v). The homogenate was kept at room temperature for 2 h before filtration. The filtrate was divided in 2 portions. The first portion was filtered with Whatman™ No. 4 through Büchner funnel and vacuum pump. The water was removed from filtrate by a freeze dryer yielding dry aqueous crude extract (2.869 %w/w fresh weight). The extract was stored before the freezing process at –18 °C and subsequent decreasing the temperature to –45 °C in ethanol bath. The frozen extract was subjected to the freeze dryer at the temperature below –100 °C under pressure below 0 Pa until obtaining the freeze-dried product of extract. The second portion was partitioned by using DCM thrice with the same water phase at a ratio of 1:1 (v/v). The lower DCM layer was collected and the contaminated water was removed by adding sodium sulfate anhydrous and then filtered. The DCM was removed under vacuum by rotary evaporator. The dry residue was purged with nitrogen gas and dried by using vacuum drying oven to yield dry crude DCM extract (0.062 %w/w fresh weight). Both extracts were kept under –20 °C for further use.

Identification of phenolic compounds

Phenolics were identified by HPLC analysis comparing to commonly found nine phenolics standards [17]. In this study, we determined 2 types of phenolics: (I) hydroxybenzoic acid, which are (1) gallic acid, (2) 3,4-dihydroxybenzoic acid (protocatechuic acid), (3) *p*-hydroxybenzoic acid, (4) vanillic acid and (5) syringic acid; and (II) hydroxycinnamic acid, which are (6) chlorogenic acid, (7) caffeic acid,

p-coumaric acid and (8) ferulic acid. Reverse phase-HPLC analysis of aqueous and DCM crude extracts (50 mg/ml) were performed by using HPLC Agilent 1100 Series (G1316A column compartment) with an Agilent 1100 Series G1315B diode array detector (Waldbronn, Germany), and reverse phase HiQ sil C18W column (4.6×250 mm, i.d. 5 μm). The mobile phase consisted of purified water with acetic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5 to 9 % solvent B; from 5 to 15 min, linear gradient from 9 to 11 % solvent B; from 15 to 22 min, linear gradient from 11 to 15 % solvent B; from 22 to 30 min, linear gradient from 15 to 18 % solvent B; from 30 to 38 min, linear gradient from 18 to 23 % solvent B; from 38 to 43 min, linear gradient from 22 to 80 % solvent B; from 43 to 46 min, linear gradient from 80 to 90 % solvent B; from 46 to 55 min, linear gradient from 90 to 95 % solvent B; from 55 to 60 min, linear gradient from 95 to 5 % solvent B; from 60 to 65 min isocratic at 95 % solvent B and a re-equilibration period of 5 min with 5 % solvent B between individual runs. The column temperature was 38 °C and the injection volume was 20 μL. To confirm the presence of identified compound, UV-diode array detection was set at 280 nm, 320 nm and 370 nm and the co-injection between phenolics standard (10 mg/mL) and the crude extract (50 mg/mL) was performed.

Identification of isothiocyanates

Reverse phase-HPLC analysis of RS pod aqueous and DCM crude extracts (50 mg/mL) was performed by using HPLC with a diode array detector, HiQ sil C18W column (4.6 × 250 mm, i.d. 5 μm) [16]. The mobile phase was 5 % THF in ultrapure water (v/v). The flow rate was stable at 1 mL/min for 30 min. UV-diode array detection was set at 210 nm to confirm the presence of sulforaphane and sulforaphene. The concentration of sulforaphane and sulforaphene used in the experiment were 0.016 to 0.25 mg/mL, respectively.

DPPH radical-scavenging activity

The antioxidant activity of extracts was determined based on the basis of the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical [18]. Briefly, DPPH was dissolved in methanol and was added into each wells of 96-well plate (a final concentration of 200 μM). Various concentrations of DCM and aqueous extracts dissolved in DMSO was mixed in the ratio 1:1 with the $DPPH^{\bullet}$ solution in a 96-well microplate. The reaction mixture was incubated in the dark for 30 min at room temperature and measured at 517 nm using a microplate reader. The scavenging activity of DPPH radical represented as IC_{50} value was calculated in term of mean ± SD. The positive control was gallic acid and standard compounds were sulforaphene and sulforaphane. Gallic acid was used in the concentration ranged from 0.1 to 5 μg/mL.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was based on the reduction of ferric-tripyridyltriazine complex (Fe^{3+}-TPTZ) to a blue colored ferrous-tripyridyltriazine complex (Fe^{2+}-TPTZ) [19]. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer pH 3.6, 10 mM TPTZ 2,4,6,-tris(2-pyridyl)-S-triazine solution in 40 mM HCl, 20 mM $FeCl_3$ at a ratio of 10:1:1 (v/v/v). Various concentrations of DCM and aqueous extract solution were prepared in DMSO. Gallic acid was used as a positive control. Gallic acid was used in the concentration ranged from 0.1 to 10 μg/mL. The $FeSO_4 \bullet 7H_2O$ (ranged from 3.60 to 89.92 μM) was used as the standard solution. The reaction mixture was incubated in the dark 30 min at room temperature and measured at 593 nm using a microplate reader. The antioxidant potential of the extracts was determined from ferric reducing antioxidant power (FRAP). The standard curve was plotted and the $FeSO_4$ linear regression equation was used to calculate the FRAP values of sample represented as molar concentration of $FeSO_4$.

Determination of mushroom tyrosinase inhibition activity

To assess the lightening effect of the extracts, the inhibition of tyrosinase activity-an enzyme in melanogenesis-was evaluated *in vitro* [20]. Mushroom tyrosinase in phosphate buffer pH 6.8 was added into each wells of a 96-well plate (a final concentration of 54 units/mL). DCM and aqueous extracts

dissolved in DMSO were pipetted into each well with the final concentrations ranged 0.2 - 4,000 µg/mL. The L-DOPA substrate in phosphate buffer pH 6.8 was added to the reaction mixture yielding final concentration of 4.5 mM. All of the reaction mixture was incubated at room temperature for 10 min and measured for dopachrome at 475 nm using a microplate reader. The concentration possessing 50 % tyrosinase inhibition was calculated. The positive control was kojic acid and standard compounds were sulforaphene and sulforaphane. Kojic acid was used in the contraptions ranging from 4 to 20 µg/mL.

Statistical analysis

All experiments were done in 3 - 5 replicates. Data is expressed as average and standard deviations (mean ± SD). The data was analyzed to compare between groups and control group by one-way ANOVA at 95 % confidence intervals (p-value < 0.05).

Results and discussions

Phytochemical identification

HPLC chromatograms (**Figure 1**) and retention time (**Table 1**) are shown for phenolic standards. The DCM extract was found to contain (2) protocatechuic acid, (3) *p*-hydroxybenzoic acid, (6) caffeic acid, and (9) ferulic acid (**Figure 2**). The aqueous extract was found to contain (2) protocatechuic acid, (3) *p*-hydroxybenzoic acid, (5) caffeic acid, (6) vanillic acid, and (8) *p*-coumaric acid (**Figure 3**). Ferulic acid was only detected in the DCM extract, while vanillic acid and *p*-coumaric acid were only detected in the aqueous extract. It should be noted that the other unknown peaks were also appeared in the DCM extract and yet to be identified because their retention times were not matched with the standard phenolics at 3 wavelengths (280, 320 and 370 nm), and especially after the co-injection of the standard in the extract was performed. The amount of each phenolics per gram crude extract were calculated from peak height and summarized in **Table 1**.

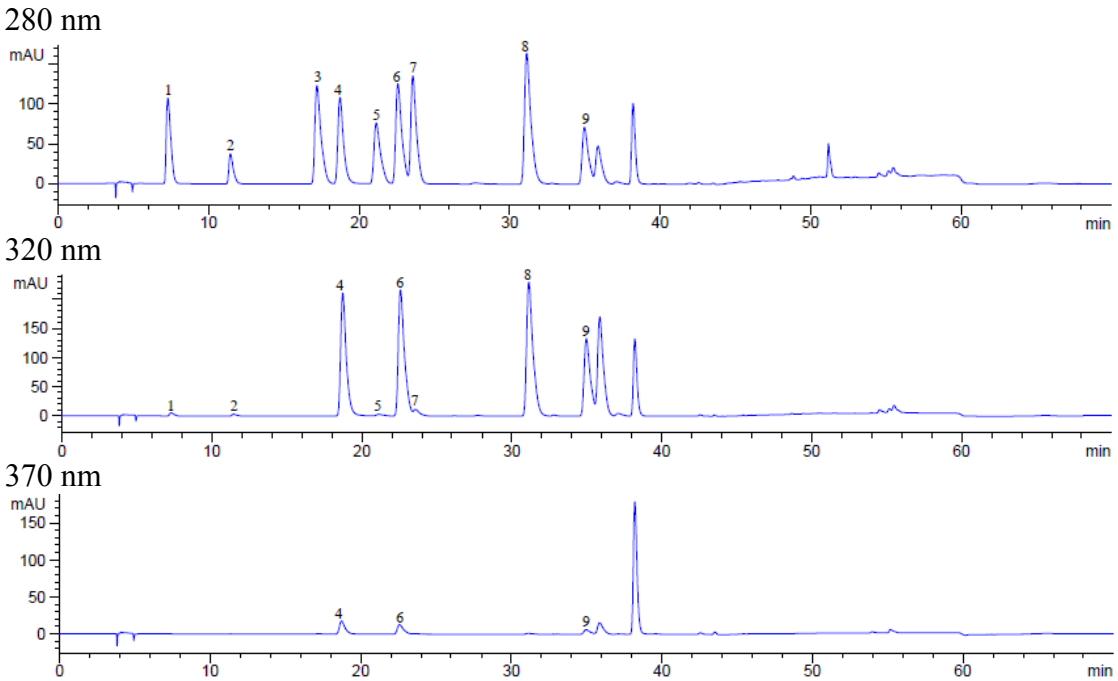


Figure 1 HPLC chromatograms of 9 phenolic standard compounds: (1) gallic acid (2) protocatechuic acid, (3) *p*-hydroxybenzoic acid, (4) chlorogenic acid, (5) vanillic acid, (6) caffeic acid, (7) syringic acid, (8) *p*-coumaric acid and, (9) ferulic acid.

Moreover, the isothiocyanates contents were also determined because these compounds are commonly found in the Brassicaceae plant. HPLC chromatograms (**Figure 4**) of sulforaphene and sulforaphane standards at 210 nm showed the retention time at 23.84 ± 0.10 min and 26.15 ± 0.03 min, respectively. DCM extract contained both sulforaphene and sulforaphane. Aqueous extract contained only sulforaphene. Sulforaphene content in DCM and aqueous extracts were 5.11 ± 0.23 mg/g extract and 0.72 ± 0.02 mg/g extract, respectively. Although sulforaphane was identified, the content could not be calculated based on the poor resolution of peak area.

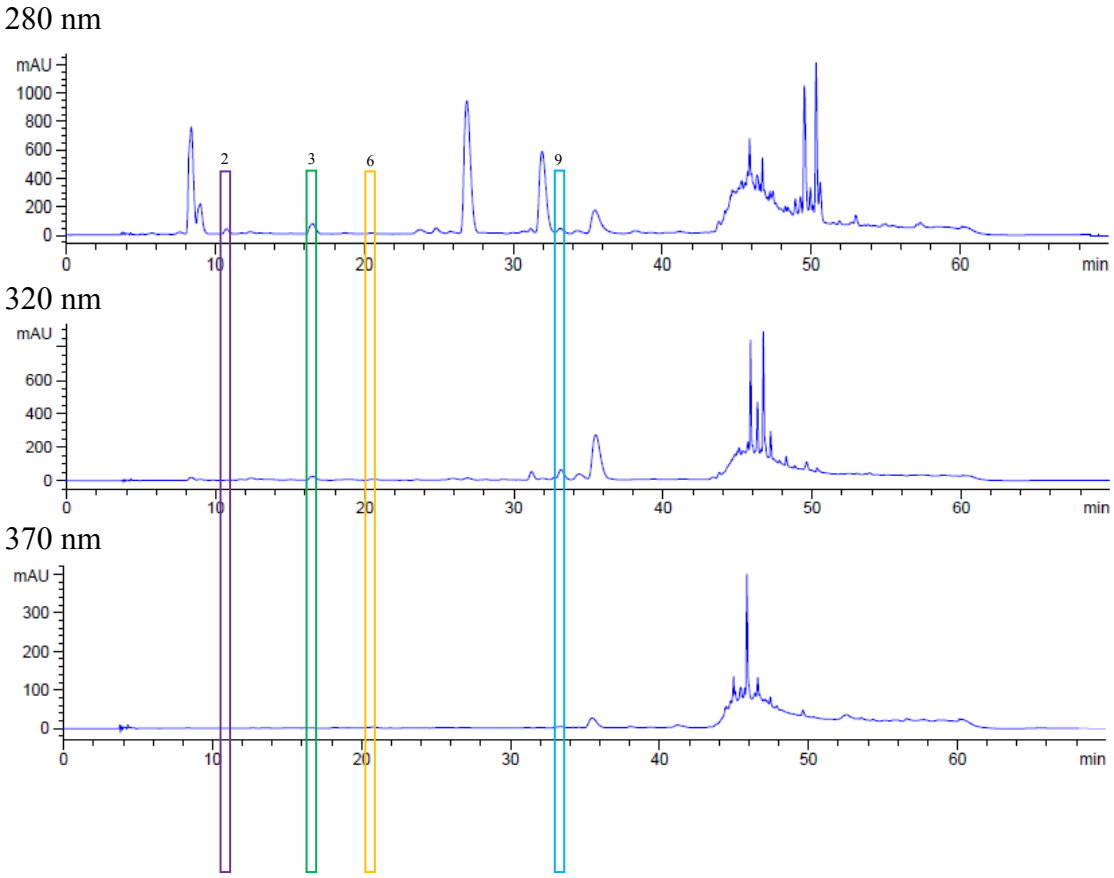


Figure 2 HPLC chromatograms of the RS pod dichloromethane extract revealed the presence of (2) protocatechuic acid, (3) *p*-hydroxybenzoic acid, (6) caffeic acid and, (9) ferulic acid.

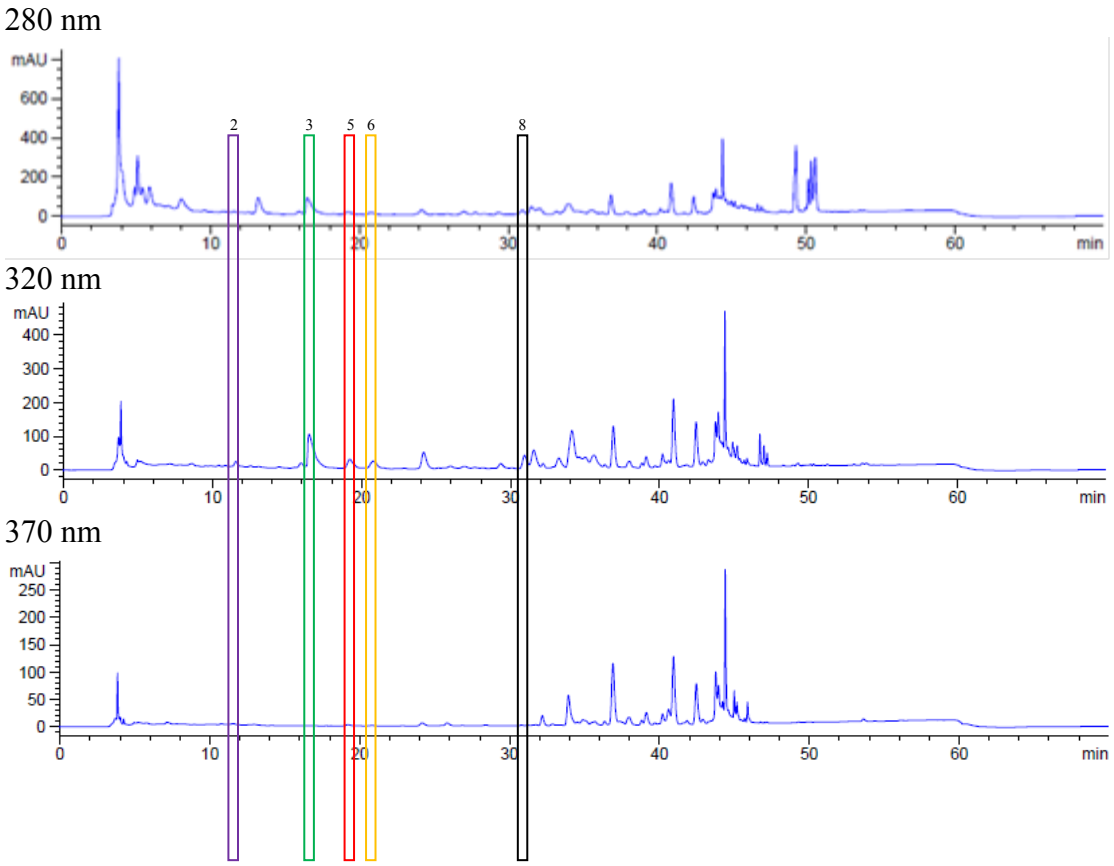


Figure 3 HPLC chromatograms of the RS pod aqueous extract illustrated the existence of (2) protocatechuic acid, (3) *p*-hydroxybenzoic acid, (5) vanillic acid, (6) caffeic acid and, (8) *p*-coumaric acid.

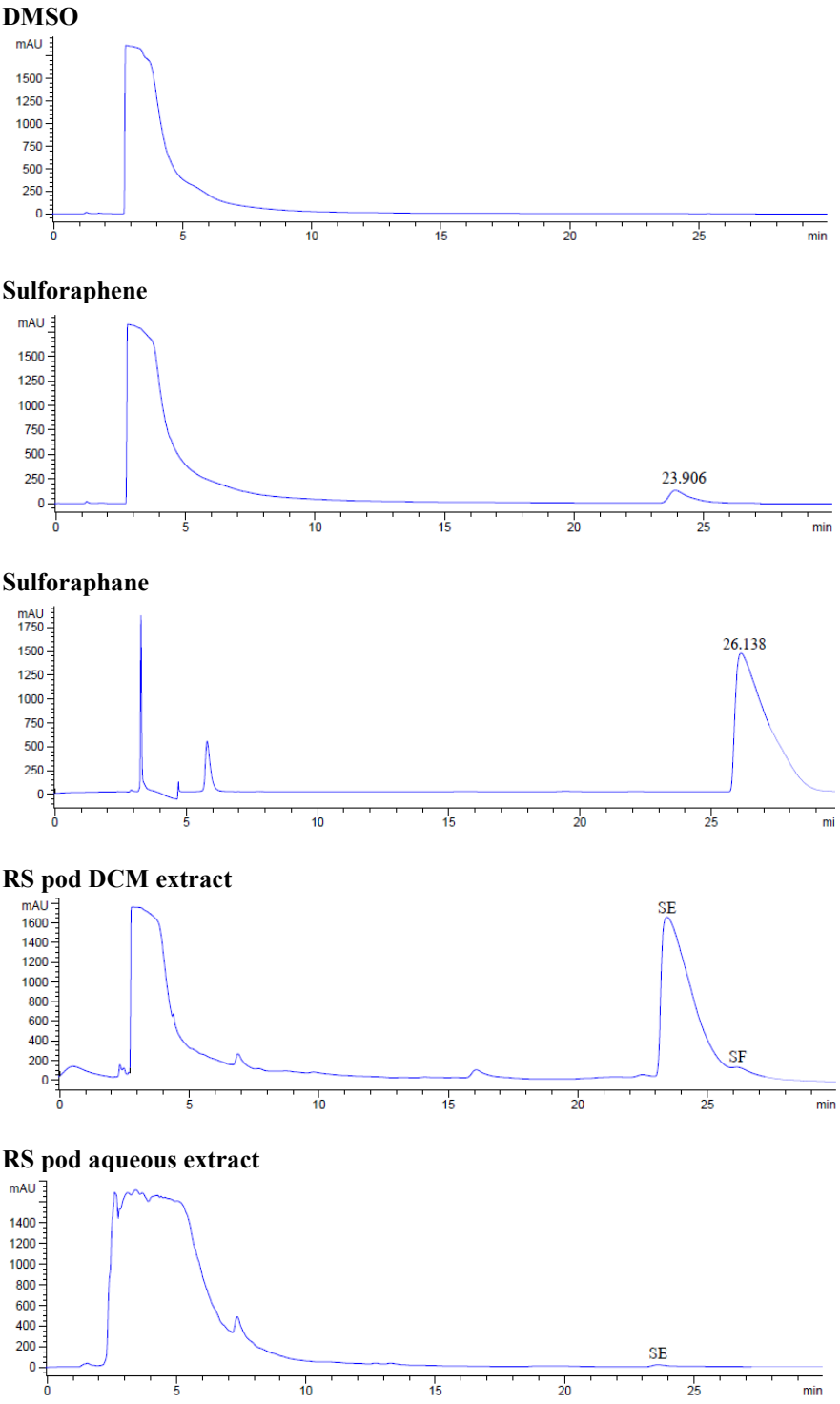
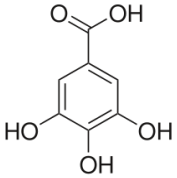
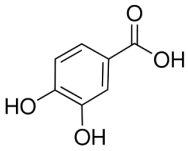
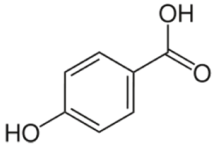
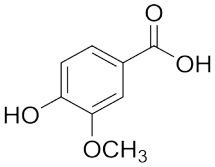
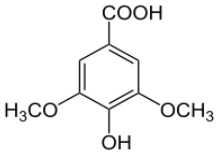
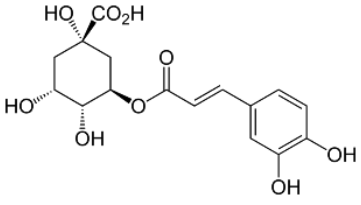
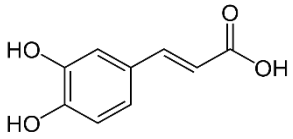
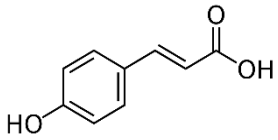
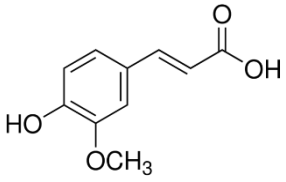
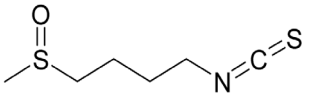
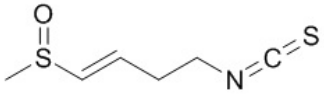


Figure 4 HPLC chromatogram of dimethyl sulfoxide (DMSO), standard sulforaphene, standard sulforaphane (0.25 mg/mL), and RS pod dichloromethane and aqueous extracts (50 mg/mL) detected at 210 nm.

Table 1 Quantification of phenolic compounds and isothiocyanates in the RS pod DCM and aqueous extracts.

No.	Standard	Retention time (min)	Quantification (mg/g crude extract)	
			RS pod DCM extract	RS pod aqueous extract
Phenolics	**(I) hydroxybenzoic acid**			
1	Gallic acid 	6.77 ± 0.04	nd	nd
2	Protocatechuic acid 	10.46 ± 0.12	2.33 ± 0.03	4.21 ± 1.60
3	*p*-Hydroxybenzoic acid 	15.37 ± 0.04	6.01 ± 0.06	1.06 ± 0.18
4	Vanillic acid 	19.12 ± 0.18	nd	26.15 ± 8.97
5	Syringic acid 	21.17 ± 0.02	nd	nd
	(II) hydroxycinnamic acid			
6	Chlorogenic acid 	16.93 ± 0.04	nd	nd

No.	Standard	Retention time (min)	Quantification (mg/g crude extract)	
			RS pod DCM extract	RS pod aqueous extract
7	Caffeic acid 	19.99 ± 0.03	0.43 ± 0.02	0.63 ± 0.05
8	*p*-Coumaric acid 	31.21 ± 0.11	nd	0.66 ± 0.06
9	Ferulic acid 	32.30 ± 0.02	2.20 ± 0.01	nd
Isothiocyanates				
	Sulforaphane 	26.15 ± 0.03	nd	nd
	Sulforaphene 	23.84 ± 0.10	5.11 ± 0.23	0.72 ± 0.02

Data are represented as mean ± SD (n = 3).
nd = not determined
DCM = dichloromethane

Antioxidant activity

DPPH radical scavenging activity

DPPH is a stable chromogen radical with purple color detected at 517 nm. DPPH scavenging assay is based on electron donation of antioxidants to neutralize DPPH radical. The decrease in color intensity in the presence of antioxidant can thus be measured [21]. The DPPH scavenging action of gallic acid, a positive control showed the IC_{50} value of 2.60 ± 0.12 µg/mL. The DCM extract (IC_{50} value of 883.38 ± 22.9 µg/mL) had greater DPPH scavenging activity than the aqueous extract (IC_{50} value of 1,160.49 ± 22.30 µg/mL) but lower than gallic acid. However, our study reports greater DPPH scavenging activity of the DCM extract than previous research which used the same solvent but with different extraction process [10]. In the previous study, RS pod was macerated in hexane to get hexane extract [10]. The dry residue

was macerated with the respective dichloromethane and ethanol under the same steps as hexane extract preparation to obtain the dichloromethane extract and ethanol extract. One study reported that the phenolic compound which had high to low DPPH radical scavenging activity were gallic acid, caffeic acid, protocatechuic acid, ferulic acid, vanillic acid, *p*-coumaric acid, and *p*-hydroxybenzoic acid [22]. Thangboonjit *et al*. [23] reported the radical scavenging of DPPH for gallic acid to be higher than ferulic acid, caffeic acid, and p-coumaric acid. Ferulic acid, only found in the DCM extract, was previously reported to exert much greater activity than vanillic acid and *p*-coumaric acid, only found in the water extract. Therefore, ferulic acid may be partly attributed to radical scavenging of the DCM extract.

Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine $[Fe(III)(TPTZ)_2]^{3+}$ complex and producing a reduced form and blue colored ferrous tripyridyltriazine $[Fe(II)(TPTZ)_2]^{2+}$ at low pH [19]. The reducing properties of antioxidants are based on their ability to break the free radical chain through donating a hydrogen atom [24]. Results in **Table 2** showed that gallic acid (0.1 to 10 µg/mL) possessed FRAP value between 3.50 ± 0.004 to 232.24 ± 0.005 µM $FeSO_4$ equivalent. The FRAP value of the DCM extract was lower than the aqueous extract. At the same 2,000 µg/mL concentration, the DCM extract had FRAP value of 166.34 ± 0.018 µM $FeSO_4$ equivalent, while the aqueous extract had 218.27 ± 0.010 µM $FeSO_4$ equivalent. However, both extracts exerted lower FRAP value than gallic acid, a positive control. Phenolics have been known to act as antioxidant especially the 3-hydroxyl-containing phenolic compound [25,26]. Therefore, the higher detection of phenolics in the aqueous extract may play major role for its reducing property.

Table 2 The FRAP value of gallic acid, sulforaphane and sulforaphene, and the RS pod DCM and aqueous extracts.

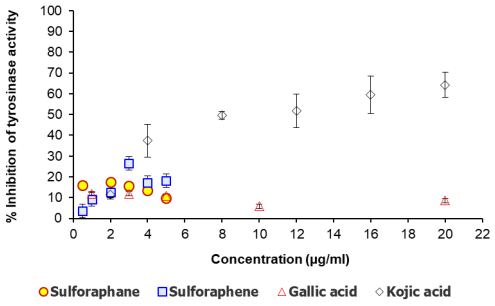
Compound	Concentration (µg/mL)	FRAP value (µM $FeSO_4$ equivalent)
Gallic acid	0.1	3.50 ± 0.004
	0.5	8.21 ± 0.006
	1	23.83 ± 0.006
	2	42.93 ± 0.001
	3	72.90 ± 0.003
	4	100.14 ± 0.004
	10	232.24 ± 0.00523
Sulforaphene		inactive
Sulforaphane		inactive
DCM extract	100	0.93 ± 0.022
	250	10.60 ± 0.008
	500	37.07 ± 0.026
	1,000	85.17 ± 0.033
	2,000	166.34 ± 0.018
Aqueous extract	100	19.36 ± 0.004
	250	46.54 ± 0.006
	500	86.90 ± 0.004
	1,000	176.71 ± 0.002
	2,000	218.27 ± 0.010

Data is represented as mean ± SD (n = 5).
Linear regression fit of FRAP standard curve (Y = 0.0128X + 0.0721, R^2 = 0.9996) was obtained for $FeSO_4$ at concentration ranged 3.6 to 89.9 µM.

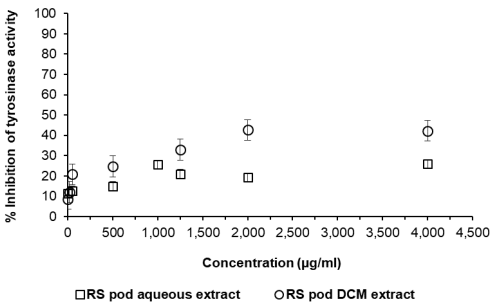
Determination of tyrosinase inhibition activity

Tyrosinase is the key enzyme in the first 2 steps of melanogenesis by activating the conversion of L-tyrosine to 3,4-dihydroxy-L-phenylalanin (L-DOPA) and to dopachrome [27]. The antimelanogenic activity was evaluated based on the *in vitro* inhibition of mushroom tyrosinase activity. Results (**Figure 5**) showed that kojic acid, a positive control possessed 50 % inhibitory activity (IC_{50} value) of 10.43 ± 3.15 µg/mL. In consideration of the rank order from strong to weak tyrosinase inhibitor at a range of initial concentration (less than 2 µg/mL), sulforaphane possessed stronger inhibitory action than sulforaphene, kojic acid, gallic acid, DCM extract, and aqueous extract, respectively. The standard compounds-sulforaphene, sulforaphane and gallic showed low inhibitory activity and even using higher concentration no more inhibition was observed under the condition studied. The results showed that the DCM extract displayed greater % inhibitory action than the aqueous extract. The percentage inhibitory action of DCM extract at 2,000 µg/mL was 42 % and for aqueous extract at 4,000 µg/mL was 25 %. Due to high lipophilicity of sulforaphane and sulforaphene, their concentration could not be increased, not to mention their limit solubility under the condition studied. Therefore, the IC_{50} could not be determined.

A)



B)



C)

Compound	Concentration (µg/mL)	% Tyrosinase inhibition	IC_{50} (µg/mL)
Sulforaphane	2	17.5 ± 5.2	nd
Sulforaphene	2	12.5 ± 7.7	nd
Kojic acid	2	11.6 ± 1.7	10.43 ± 3.15
Gallic acid	3	11.9 ± 1.5	nd
RS pod DCM extract	2	11.4 ± 0.9	nd
RS pod aqueous extract	2	8.6 ± 1.3	nd

Figure 5 Percent tyrosinase inhibitory activity of A) sulforaphane, sulforaphene, gallic acid, and kojic acid, B) RS pod DCM extract and aqueous extract, and C) the inset table expressed the %tyrosinase inhibitory activity of each test samples at the same concentration except gallic acid. Data are expressed as mean ± SD from triplication (nd = cannot be determined).

Previously, sulforaphane exerted its anti-melanogenesis and inhibition of tyrosinase expression in B16 mouse melanoma cells [28]. Sulforaphane inhibited tyrosinase activity up to 80 % at the maximum concentration 5 µM [28]. Sulforaphane inhibited phosphorylated p38 activated MITF which further decreased melanogenesis. Moreover, sulforaphane increased the expression of ERK protein that further suppressed the melanogenesis [28]. Several studies reported the tyrosinase inhibition of phenolics such as ferulic acid and *p*-coumaric acid [23], but not for vanillic acid [29]. It should be noted that sulforaphane, ferulic acid was only found in DCM extract, while vanillic was only found in aqueous extract. Hence, sulforaphane along with the other phenolics such as ferulic acid may attribute to tyrosinase inhibitory effect in the DCM extract. Although aqueous extract contained vanillic acid which did not have any

tyrosinase inhibitory effect, there might be other undetected substances that may attribute to its relative low activity.

Conclusions

The DCM extract of *Raphanus sativus* L. var. *caudatus* has greater DPPH radical scavenging and anti-tyrosinase activity than the aqueous extract. The aqueous extract possesses greater ferric reducing/antioxidant power than the DCM extract. It is evident that different solvent extraction of RS yields different phytochemical constituents leading to different degree of activity tested. This is the first report of tyrosinase activity of the RS pod extract. It confirms that RS is a potential source of compound that possess antioxidant and anti-tyrosinase activity which may offer the reduction of wrinkles and lightening effect in cosmetic product. However, more separation or fractionation to get the concentrated active ingredient may be required to increase the strength of action.

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