Thai plants with high antioxidant levels, free radical scavenging activity, anti-tyrosinase and anti-collagenase activity

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Abstract

Background: Ultraviolet radiation from sunlight induces overproduction of reactive oxygen species (ROS) resulting in skin photodamage and hyperpigmentation disorders. Novel whitening and anti-wrinkle compounds from natural products have recently become of increasing interest. The purpose of this study was to find products that reduce ROS in 14 Thai plant extracts.

Methods: To determine total phenolic and flavonoid content, antioxidant activity, anti-tyrosinase activity and anti-collagenase activity, we compared extracts of 14 Thai plants prepared using different solvents (petroleum ether, dichloromethane and ethanol). Antioxidant activities were determined by DPPH and ABTS assays.

Results: Total phenolic content of the 14 Thai plants extracts was found at the highest levels in ethanol followed by dichloromethane and petroleum ether extracts, respectively, while flavonoid content was normally found in the dichloromethane fraction. Scavenging activity ranged from 7 to 99% scavenging as assessed by DPPH and ABTS assays. The ethanol leaf extract of Ardisia elliptica Thunb. had the highest phenolic content, antioxidant activity and collagenase inhibition, while Cassia alata (L.) Roxb. extract had the richest flavonoid content. Interestingly, three plants extracts, which were the ethanolic fractions of Annona squamosa L., Ardisia elliptica Thunb. and Senna alata (L.) Roxb., had high antioxidant content and activity, and significantly inhibited both tyrosinase and collagenase.

Conclusion: Our finding show that the ethanol fractions of Annona squamosa L., Ardisia elliptica Thunb. and Senna alata (L.) Roxb. show promise as potential ingredients for cosmetic products such as anti-wrinkle agents and skin whitening products.

Keywords: Ardisia elliptica Thunb, Antioxidant content, Scavenging activity, Anti-tyrosinase activity, Anti-collagenase activity

Background

Ultraviolet radiation (UVR) from sunlight is the most significant risk factor for nonmelanoma and melanoma skin cancers [1]. Overexposure to sunlight, in particular UVA and UVB, induces the overexpression of reactive oxygen species (ROS) which damage lipids, proteins and deoxyribonucleic acids. Collagen is the major foundation of the extracellular matrix in the dermis layer of the skin. Excessive ROS increases expression of collagenase, a protease that degrades collagen which can result in photodamage and wrinkling of the skin [2]. In addition, UV exposure induces melanin production resulting in hyperpigmentation. Tyrosinase is the key enzyme initiating skin pigmentation. Firstly, L-tyrosine is hydroxylated to form 3,4-dihydroxyphenylalanine (L-DOPA) by tyrosinase. Subsequently, L-DOPA is oxidized to DOPA quinone by tyrosinase. DOPA quinone is further converted to DOPAchrome that can be converted to 5,6-dihydroxyindole (DHI) or 5,6-dihydroxyindole-2-carboxylic acid (DHICA) [3]. The current treatments for skin aging involves hydroxyl acid to peel the epidermal layer, retinoids to reduce rough skin, and skin filler administered by injecting collagen into the skin. However, these treatments have adverse effects, such as hyperpigmentation, inflammation, cytotoxicity,
irritation and bacterial infection [4]. The most popular skin whitening agent is hydroquinone, which inhibits tyrosinase, but its side effects include dermatitis, edema, allergic reactions and ochronosis [5]. Recently, researchers have focused on natural products that inhibit UV-induced ROS, suppress enzymes, and reduce melanin formation as alternatives to current treatments. For example, active phytocompounds, such as arbutin, aloesin, gentisic acid, flavonoids, hesperidin, licorice, niacinamide, yeast derivatives, and polyphenols, inhibit melanogenesis without cytotoxicity to melanocytes [6]. Thus, plants may reduce wrinkle formation and hyperpigmentation caused by sunlight exposure.

The aim of this study was to analyze 14 Thai plants extracted with three different solvents for their potential as anti-wrinkle and skin whitening ingredients. The quantity of antioxidant phenols and flavonoids was evaluated for a correlation with free radical scavenging activities, and anti-collagenase and anti-tyrosinase activities. The extracts had antioxidants that scavenged free radicals and inhibited enzymes involved in wrinkle and pigment formation. We identify *Ardisia elliptica* Thunb., *Annona squamosa* L. and *Senna alata* (L.) Roxb as very promising candidates for use in cosmetic products.

**Methods**

**Chemicals and reagents**

Folin Ciocalteu’s phenol reagent, sodium carbonate (Na₂CO₃), gallic acid, quercetin, 10% aluminium chloride, ethanol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium persulfate, kojic acid, mushroom tyrosinase (EC 1.14.18.1), 3,4-dihydroxy-L-phenylalanine (L-DOPA), N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA), collagenase from *Clostridium histolyticum* (EC 3.4.24.3), epigallocatechin gallate (EGCG), sodium chloride, calcium chloride and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Petroleum ether, dichloromethane, absolute ethanol, methanol, disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany). All chemicals and reagents were analytical grade.

**Plant materials and extraction**

Thirteen species of Thai leaves were collected from the HRH Princess Sirindhorn Herb Garden, Rayong province, Thailand. Mangosteens were obtained from Chanthaburi province, Thailand. These plants were authenticated and deposited at the Herbarium, Department of Botany, Faculty of Science, Chulalongkorn University, Thailand. The scientific names, voucher numbers and plant parts are shown in Table 1. The plants were extracted by using the Soxhlet apparatus. In brief, 10 g of dried plant was extracted separately with petroleum ether, dichloromethane and ethanol. Solvents were removed using a vacuum rotary evaporator under reduced pressure using the MiVac Quattro concentrator. Concentrated samples were dissolved in DMSO at 100 mg/ml and stored at -20 °C until used. Yields of dry extracts are presented in Table 1 as % w/w dry plant materials.

**Determination of total phenolic content**

Total phenolic content of plant extracts was evaluated using the Folin-Ciocalteu method [7]. Briefly, 50 μl of extracts at 1 mg/ml in distilled water was mixed with 50 μl of 0.1 M

| Voucher number | Parts used | Scientific name                  | Yield % (w/w) | Petroleum ether | Dichloromethane | Ethanol |
|----------------|------------|----------------------------------|---------------|----------------|----------------|--------|
| A 015122 (BCU) | Leaf       | *Ardisia elliptica* Thunb.       | 19.89         | 3.25           | 31.11          |
| A 015123 (BCU) | Leaf       | *Stemona curtisii* Hook.f.       | 7.55          | 4.10           | 6.34           |
| A 015124 (BCU) | Leaf       | *Gynura pseudochina* (L.) DC.    | 8.00          | 2.76           | 3.79           |
| A 015125 (BCU) | Leaf       | *Senna alata* (L.) Roxb.         | 5.84          | 3.52           | 7.63           |
| A 015126 (BCU) | Leaf       | *Croton roxburghii* N.P.Balak.    | 7.50          | 4.82           | 8.17           |
| A 015127 (BCU) | Leaf       | *Croton sublyratus* Kurz.        | 7.33          | 4.03           | 3.32           |
| A 015128 (BCU) | Leaf       | *Phyllanthus acidus* (L.) Skeels | 5.70          | 2.86           | 4.20           |
| A 015129 (BCU) | Leaf       | *Rhinacanthus nasutus* (L.) Kurz  | 4.43          | 2.86           | 5.35           |
| A 015130 (BCU) | Leaf       | *Hibiscus mutabilis* L.          | 6.30          | 2.79           | 0.73           |
| A 015131 (BCU) | Leaf       | *Streblus asper* Lour.           | 3.87          | 2.53           | 3.56           |
| A 015132 (BCU) | Leaf       | *Annona squamosa* L.             | 8.69          | 3.81           | 5.47           |
| A 015133 (BCU) | Leaf       | *Datura metel* L.                | 6.44          | 4.13           | 14.15          |
| A 015250 (BCU) | Leaf       | *Ipomoea pes-caprae* (L.) R.br.  | 6.38          | 4.50           | 3.98           |
| A 015279 (BCU) | Pericarp   | *Garcinia mangostana* Linn.      | 4.94          | 11.07          | 18.64          |
Experiments were undertaken in triplicate. The scavenging ability was calculated as scavenging activity (\(\%\)) = 100 × \((\frac{A_{734}\text{ of control} - A_{734}\text{ of sample}}{A_{734}\text{ of control}})\). The percentages of ABTS scavenging activity of the extracts were compared with those of ascorbic acid, and are presented as mg vitamin C equivalent antioxidant capacity (VCEAC) per g dry plant material. IC50 was determined from a graph of percent inhibition against concentration (from 15.62–1000 μg/ml of each extract).

**Determination of mushroom tyrosinase inhibition**

The dopachrome method was performed with slight modification [10]. Briefly, 20 μl of plant extracts or DMSO (as control), 20 μl of 203.3 units/ml mushroom tyrosinase and 140 μl of 20 mM phosphate buffer at pH 6.8 were pre-incubated for 10 min at 25 °C. After pre-incubation, 20 μl of 2.5 mM L-DOPA was added and samples were then incubated for an additional 20 min at 25 °C. The amount of dopachrome was measured at 492 nm with a microplate reader. Kojic acid (KA) served as a positive control for inhibition. The percent inhibition of tyrosinase activity (\(\%\)) was expressed as \(\%\) tyrosinase inhibition = 100 × \((\frac{A_{492}\text{ of sample} - A_{492}\text{ of control}}{A_{492}\text{ of control}})\). The final concentrations of the extracts and kojic acid were 1 and 0.1 mg/ml, respectively. IC50 was determined from a graph of percent tyrosinase inhibition against concentration (from 15.62–1000 μg/ml of each extract).

**Determination of collagenase inhibition**

Collagenase inhibition was determined by a previously described method [11]. Briefly, 40 μl of collagenase from Clostridium histolyticum at 0.25 units/ml in 50 mM Tricine buffer containing 10 mM CaCl₂ and 400 mM NaCl, and 10 μl of 50 mM Tricine buffer were mixed with 10 μl of the extracts or DMSO (as control). Epigallocatechin gallate (EGCG) was used as a positive control. After a 15-min incubation at room temperature, 50 μl of N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA) was added. The absorbance was measured at 340 nm immediately and continually for 20 min. Enzyme activity was evaluated by decreased absorbance during the time interval. The percent inhibition of collagenase activity was calculated as 100 × \((\frac{\text{Activity of control} - \text{Activity of sample}}{\text{Activity of control}})\). Final concentrations of the extracts and epigallocatechin gallate were 1 and 0.1 mg/ml, respectively. IC50 was determined from a graph of percent collagenase inhibition against concentration (from 15.62–1000 μg/ml of each extract).

**Statistical analyses**

All experiments were carried out in triplicate and results are expressed as mean ± standard error. The correlation coefficient (R²) between antioxidant contents and Na₂CO₃. The reaction mixture was incubated for 1 h at room temperature in the dark. Absorbance at 750 nm was measured with a microplate reader (Biotek, USA.). Gallic acid from 1.56 to 100 μg/ml was used as the standard. Total phenolic content of the extracts is expressed as mg gallic acid equivalents (GAE) per g dry plant material. All samples were analyzed in triplicate.

**Flavonoid content determination**

Total flavonoid content (TFC) was determined using the aluminium chloride (AlCl₃) colorimetric assay [7]. Briefly, 50 μl of the extracts at 1 mg/ml in 80% ethanol was mixed with 50 μl of 2% AlCl₃ solution in the well of a 96 well-plate. The plate was incubated for 15 min at room temperature. The absorbance at 435 nm was measured using a microplate reader. Quercetin from 1.56 to 100 μg/ml was used as a standard. Total flavonoid content is expressed as mg quercetin equivalents (QE) per g dry plant material. Samples were analyzed in triplicate.

**DPPH scavenging activity**

DPPH scavenging activity assay was performed as described by Yamasaki et al. [8]. DPPH solution was freshly prepared for each assay. Briefly, 100 μg/ml extracts or 1.56 to 100 μg/ml ascorbic acid standard in absolute methanol was mixed with 180 μl of DPPH reagent in a 96 well-plate. The reaction mixture was incubated for 30 min at room temperature in the dark. Then, the absorbance at 517 nm was measured with a microplate reader. The experiments were undertaken in triplicate. The absorbance at 517 nm of DPPH was 0.70 ± 0.02, and decreased absorbance measured scavenging activity. The scavenging ability was calculated as scavenging activity (\(\%\)) = 100 × \((\frac{A_{517}\text{ of control} - A_{517}\text{ of sample}}{A_{517}\text{ of control}})\). Percentages of DPPH scavenging activity of the extracts were compared with those of ascorbic acid, and are expressed as mg vitamin C equivalent antioxidant capacity (VCEAC) per g dry plant material. IC50 was determined from a graph of percent inhibition against concentration (from 0.78–100 μg/ml of each extract).

**ABTS scavenging activity**

ABTS free radical scavenging activity was performed as previously described [9]. The ABTS⁺⁺ working reagent was prepared by mixing 7 mM ABTS⁺ and 2.45 mM potassium persulfate at 8:12 volume/volume ratio. The working solution was kept for 16 to 18 h at room temperature in the dark. The ABTS⁺⁺ solution was diluted with absolute ethanol to give an absorbance at 734 nm of 0.70 ± 0.02. Then, 100 μg/ml extracts or 1.56 to 100 μg/ml ascorbic acid standard in absolute ethanol was added to 180 μl of ABTS⁺⁺ working reagent in the wells of a 96 well plate. The plate was incubated for 45 min at room temperature, and absorbance was measured at 734 nm.
antioxidant activities was determined by using SigmaPlot version 12.2 software. Difference between two means was evaluated using Student’s *t*-test. Differences were considered significant when the *P*-value was less than 0.05.

**Results**

**Extraction yields**

Table 1 shows the scientific names, voucher numbers and plant parts of the 14 Thai plants used in this study. The percent yields of the extracts ranged from 0.73% to 31.11% by weight (Table 1). *Ardisia elliptica* Thunb. had the highest yield in the petroleum ether (19.89%) and ethanol extracts (31.11%), whereas *Garcinia mangostana* L. had the highest percent yield from dichloromethane extraction (11.07%).

**Phenolic content of 14 Thai plants**

Therefore, total phenolic content in the plants was determined by the Folin-Ciocalteu method. The extracts had a wide range in the quantity of phenols as shown in Table 2, and values varied by 33-fold among the extracts. *Ardisia elliptica* Thunb. had the highest phenol content in all three types of extracts, whereas the lowest phenolic content was present in the *Stemona curtissii* Hook.f. petroleum ether extract.

**Flavonoid content of 14 Thai plants**

Similar to phenols, total flavonoid content varied substantially among the plant species, ranging from 2.04 ± 0.16 to 31.38 ± 0.81 mg QE per g dry material (Table 2). In general, dichloromethane extraction yielded the highest flavonoid level compared with the other solvents. Of all extracts, the highest flavonoid quantity was found in the ethanol extract from *Senna alata* (L.) Roxb leaves (31.38 ± 0.81 mg QE per g dry material). On the other hand, *Ardisia elliptica* Thunb. (23.14 ± 1.10 mg QE per g dry material) had the richest flavonoid content in the dichloromethane fraction. Moreover, *Ipomoea pes-caprae* (L.) Rbr. had the highest flavonoid content among the petroleum ether extracts (27.48 ± 2.59 mg QE per g dry material). The lowest detectable flavonoid level was in the ethanol extract from *Datura metel* L. By stark contrast, flavonoids were not found in the petroleum ether and dichloromethane extracts from *Stemona curtissii* Hook.f., and petroleum ether extracts from *Streblus asper* Lour. and *Phyllanthus acidus* (L.) Skeels. Total flavonoid content did not correlate with total phenolic content (R² = 0.0284, Fig. 1a).

**DPPH radical scavenging activity in different extracts from 14 Thai plants**

Free radical scavenging activity using DPPH as the indicator is a basic antioxidant assay [12]. As shown in Table 3, scavenging activities of the extracts varied greatly, ranging from 7.11 ± 0.59% to 96.17 ± 0.05%. The *Ardisia elliptica* Thunb ethanol extract had the highest scavenging activity at 96%. Moreover, the next strongest antioxidant activities (> 90%) were observed in ethanol fractions from *Stemona curtissii* Hook.f., *Annona squamosa* L., *Phyllanthus acidus* (L.) Skeels. and *Garcinia mangostana* Linn. In terms of the other solvents, *Ardisia elliptica* Thunb, also had the richest scavenging activity among the petroleum ether fractions, and *Garcinia mangostana* L. had the highest antioxidant activity in the dichloromethane fractions. The lowest scavenging ability was detected in *Croton sublyratus* Kurz.

**Table 2** Total phenolic and flavonoid contents of 14 Thai plants obtained from different solvents

| Extract                  | Total phenolic content (mg GAE/g dry material) | Total flavonoid content (mg QE/g dry material) |
|--------------------------|-----------------------------------------------|-----------------------------------------------|
|                          | Petroleum ether | Dichloromethane | Ethanol | Petroleum ether | Dichloromethane | Ethanol |
| *Annona squamosa* L.     | 4.13 ± 0.38     | 9.26 ± 0.29     | 62.67 ± 2.32       | 8.91 ± 0.77     | 9.70 ± 0.24     | 12.99 ± 0.65 |
| *Ardisia elliptica* Thunb| 22.26 ± 1.77    | 59.97 ± 2.90    | 84.00 ± 6.23       | 19.87 ± 1.26    | 23.14 ± 1.10    | 18.56 ± 1.45 |
| *Crotanoxburghii* N.P.Balik | 3.57 ± 0.25    | 9.60 ± 0.46     | 19.41 ± 0.81       | 4.25 ± 0.35     | 12.34 ± 0.29    | 7.54 ± 0.35  |
| *Croton sublyratus* Kurz | 4.73 ± 0.38     | 6.74 ± 0.51     | 16.28 ± 0.29       | 18.55 ± 0.53    | 20.78 ± 1.49    | 14.86 ± 0.95 |
| *Datura metel* L.        | 8.30 ± 0.29     | 11.43 ± 0.17    | 18.92 ± 1.50       | 17.65 ± 1.62    | 16.77 ± 1.30    | 2.04 ± 0.16  |
| *Garcinia mangostana* Linn. | 19.75 ± 1.44   | 31.07 ± 2.30    | 80.79 ± 2.94       | 5.35 ± 0.10     | 11.13 ± 0.37    | 3.20 ± 0.05  |
| *Gynura pseudochina* (L.) DC. | 3.26 ± 0.11    | 9.18 ± 0.65     | 12.76 ± 0.81       | 4.87 ± 0.35     | 18.60 ± 1.06    | 3.69 ± 0.21  |
| *Hibiscus mutabilis* L.  | 3.44 ± 0.14     | 7.14 ± 0.48     | 17.05 ± 0.64       | 3.71 ± 0.24     | 18.79 ± 1.78    | 3.71 ± 0.09  |
| *Ipomoea pes-caprae* (L.) Rbr. | 4.72 ± 0.29    | 11.18 ± 0.53    | 37.91 ± 3.36       | 27.48 ± 2.59    | 18.68 ± 0.66    | 17.66 ± 0.29 |
| *Phyllanthus acidus* (L.) Skeels | 4.65 ± 0.46    | 10.05 ± 0.74    | 50.52 ± 2.66       | NA              | 15.80 ± 1.04    | 11.74 ± 0.74 |
| *Rhinacanthus nasutus* (L.) Kurz | 5.04 ± 0.30    | 9.14 ± 0.39     | 17.09 ± 1.44       | 16.98 ± 0.40    | 19.88 ± 1.98    | 9.53 ± 0.26  |
| *Senna alata* (L.) Roxb. | 4.59 ± 0.21     | 9.48 ± 0.44     | 36.83 ± 2.30       | 7.71 ± 0.36     | 13.97 ± 1.10    | 31.38 ± 0.81 |
| *Stemona curtissii* Hook.f. | 2.51 ± 0.22    | 7.76 ± 0.30     | 59.67 ± 3.28       | NA              | NA              | 14.50 ± 0.86 |
| *Streblus asper* Lour.   | 4.19 ± 0.30     | 8.22 ± 0.39     | 23.10 ± 1.84       | NA              | 18.66 ± 1.28    | 11.29 ± 1.04 |

Each value is mean ± S.D. of triplicate independent analyses. GAE Gallic Acid Equivalent, QE Quercitin equivalent, NA Not Available.
Fig. 1 Correlation Analyses. Values in Tables 2 through 4 were evaluated by linear regression analysis, and correlation coefficients expressed as $R^2$ are shown in the panels. 

- **a**: Total flavonoid content in mg QE/g dry material versus Total phenolic content in mg GAE/g dry material.
- **b**: DPPH percent scavenging activity versus Total phenolic content.
- **c**: DPPH percent scavenging activity versus Total flavonoid content.
- **d**: ABTS percent scavenging activity versus Total phenolic content.
- **e**: ABTS percent scavenging activity versus Total flavonoid content.
- **f**: ABTS percent scavenging activity versus DPPH percent scavenging activity.

**Table 3** Free Radical Scavenging activity by DPPH assay

| Extract                  | Percent Scavenging Activity (%) | mg VCEAC/g dry weight |
|-------------------------|---------------------------------|-----------------------|
|                         | Petroleum ether | Dichloromethane | Ethanol | Petroleum ether | Dichloromethane | Ethanol |
| *Annona squamosa* L.    | NA                | 17.91 ± 0.88     | 94.01 ± 0.40 | NA              | 3.08 ± 0.29     | 23.60 ± 0.62 |
| *Ardisia elliptica* Thunb. | 49.29 ± 1.29   | 71.35 ± 6.11     | 96.17 ± 0.05 | 12.09 ± 0.94    | 20.23 ± 0.99    | 24.93 ± 0.19  |
| *Croton roxburghii* N.P.Balakr | 8.34 ± 0.57   | 15.79 ± 1.21     | 36.89 ± 1.37 | 1.03 ± 0.09     | 2.38 ± 0.13     | 8.45 ± 0.84   |
| *Croton sublyratus* Kurz | 7.11 ± 0.59     | 14.34 ± 0.65     | 27.64 ± 0.91 | 0.67 ± 0.07     | 1.85 ± 0.06     | 5.29 ± 0.30   |
| *Datura metel* L.       | 14.67 ± 1.10     | 14.85 ± 0.64     | 28.72 ± 0.67 | 2.00 ± 0.12     | 2.30 ± 0.06     | 5.83 ± 0.26   |
| *Garcinia mangostana* Linn. | 28.90 ± 0.99   | 80.87 ± 0.47     | 94.54 ± 0.15 | 6.18 ± 0.33     | 20.42 ± 0.20    | 24.28 ± 0.20  |
| *Gynura pseudochina* (L.) DC. | NA                | 11.90 ± 0.71     | 13.54 ± 0.67 | NA              | 1.49 ± 0.03     | 1.82 ± 0.13   |
| *Hibiscus mutabilis* L. | NA                | NA               | 26.28 ± 0.93 | NA              | NA             | 4.29 ± 0.21   |
| *Ipomoea pes-caprae* (L.) Rbr. | NA                | 16.27 ± 0.14     | 64.06 ± 1.23 | NA              | 2.49 ± 0.19     | 16.79 ± 0.41  |
| *Phyllanthus acidus* (L.) Skeels | NA                | 17.64 ± 1.05     | 94.17 ± 0.61 | NA              | 3.06 ± 0.28     | 23.84 ± 0.73  |
| *Rhinacanthus nasutus* (L.) Kurz | 7.38 ± 0.46    | 15.35 ± 1.27     | 29.63 ± 1.41 | 1.01 ± 0.08     | 2.32 ± 0.21     | 6.50 ± 0.16   |
| *Senna alata* (L.) Roxb. | 11.18 ± 0.99     | 17.99 ± 0.61     | 63.74 ± 0.54 | 1.48 ± 0.14     | 3.13 ± 0.31     | 15.35 ± 0.13  |
| *Stemona curtisii* Hook.f. | NA                | NA               | 93.63 ± 0.22 | NA              | NA             | 23.55 ± 0.55  |
| *Streblus asper* Lour.   | NA                | 14.65 ± 0.92     | 45.14 ± 0.67 | NA              | 1.94 ± 0.13     | 10.45 ± 0.30  |

Each value is mean ± S.D. of triplicate independent analyses. Calculations of values are described in the Materials and Methods section. VCEAC is Vitamin C Equivalent Antioxidant capacity, and NA denotes not available
in the petroleum ether fraction. No scavenging activity was detected in 7 petroleum ether extracts, and 2 dichloromethane extracts.

**ABTS radical scavenging activity in different extracts from 14 Thai plants**

Antioxidant activity of aqueous and lipid phases in the plants has also been evaluated by a decolorization assay using ABTS [13]. Again, ascorbic acid served as the standard antioxidant. As with the DPPH assay, scavenging activity in the ABTS assay varied greatly among the plant preparations with a similar broad range from 8.03 ± 0.54% to 99.84 ± 0.07% (Table 4). Furthermore, the next strongest scavenging activities (> 90%) were observed in the same 4 ethanol fractions as shown by the DPPH assay. In addition, no scavenging activity was found in the same 5 petroleum ether extracts. In general, the values obtained with the ABTS assay were higher than the DPPH values. Hence, activity in the ethanol extract from *Senna alata* (L.) Roxb. was now observed as >90%, and scavenger activity was detected in all dichloromethane extracts, and petroleum ether extracts from *Annona squamosa* L. and *Ipomoea pes-caprae* (L.) Rbr. which was not detected by the DPPH assay.

**Tyrosinase activity inhibition by plant extracts**
The ability of compounds from the Thai plants to inhibit mushroom tyrosinase activity was evaluated using an in vitro assay with L-DOPA as the substrate. Kojic acid served as a known inhibitor, and caused maximal enzymatic inhibition of 93.38 ± 1.63%. As shown in Table 5, only ethanol extracts significantly inhibited tyrosinase activity, with *Ardisia elliptica* Thunb. preparations being the exception. The petroleum ether and dichloromethane fractions of *Ardisia elliptica* Thunb. inhibited tyrosinase activity by approximately 20%. The ethanol fraction from *Rhinacanthus nasutus* (L.) Kurz (IC 50 value of 271.50 μg/ml) was the most potent tyrosinase inhibitor, followed by the ethanol extracts from *Ardisia elliptica* Thunb. and *Phyllanthus acidus* (L.) Skeels. Other ethanol fractions significantly decreased enzymatic activity by more than 20% (Table 5), whereas the remaining extracts did not have detectable inhibitory activity (data not shown).

**Collagenase activity inhibition by 14 plants**

Extracts were tested for anti-collagenase activity using *Clostridium histolyticum* collagenase, and N-\([3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala\) (FALGPA) as the substrate. Epigallocatechin gallate is a known collagenase inhibitor, and decreased enzymatic activity by 90.51 ± 2.79%. As shown in Table 5, only 4 ethanol extracts contained detectable collagenase inhibitory activity. Of those causing inhibition, *Ardisia elliptica* Thunb. (IC 50 value of 157.78 μg/ml) exhibited the highest level of collagenase inhibition, followed by *Annona squamosa* L. (IC 50 value of 426.67 μg/ml), *Senna alata* (L.) Roxb., and *Croton sublyratus* Kurz in rank order. Other plant extracts did not significantly inhibit collagenase activity under the reaction conditions utilized in this study (data not shown).

**Discussion**

Solar radiation is a significant environmental factor in skin damage and can induce skin cancer [14]. UV radiation causes a pro-inflammatory response, extracellular matrix degradation and antioxidant depletion [15, 16]. UV causes

### Table 4 Scavenging activity by ABTS assay

| Extract                              | %Scavenging activity (%SC) Petroleum ether | Dichloromethane | Ethanol | mg VCEAC/g dry weight Petroleum ether | Dichloromethane | Ethanol |
|--------------------------------------|-------------------------------------------|-----------------|--------|---------------------------------------|-----------------|--------|
| *Annona squamosa* L.                 | 29.89 ± 0.79                              | 36.31 ± 0.60    | 99.13 ± 0.29    | 25.76 ± 0.76                        | 34.40 ± 0.32    | 96.15 ± 0.38 |
| *Ardisia elliptica* Thunb.           | 73.08 ± 1.48                              | 90.01 ± 0.54    | 99.84 ± 0.07    | 65.28 ± 1.86                        | 86.34 ± 1.07    | 96.97 ± 0.34 |
| *Croton roxburghii* N.P.Balakr       | 12.19 ± 0.91                              | 33.54 ± 0.65    | 73.86 ± 1.22    | 9.14 ± 0.45                         | 33.22 ± 0.46    | 69.62 ± 0.90 |
| *Croton sublyratus* Kurz             | 16.34 ± 0.69                              | 18.47 ± 0.73    | 60.36 ± 0.17    | 13.07 ± 1.29                        | 17.81 ± 1.20    | 58.62 ± 0.68 |
| *Datura metel* L.                    | 20.17 ± 0.68                              | 43.79 ± 0.89    | 61.94 ± 0.50    | 16.42 ± 0.65                        | 42.36 ± 1.11    | 60.06 ± 0.16 |
| *Garcinia mangostana* Linn.          | 47.01 ± 2.73                              | 97.96 ± 0.85    | 99.66 ± 0.05    | 45.10 ± 2.45                        | 95.19 ± 1.43    | 96.52 ± 0.34 |
| *Gymnura pseudochina* (L.) DC.       | NA                                        | 28.88 ± 0.45    | 27.52 ± 1.66    | NA                                   | 28.00 ± 0.36    | 25.37 ± 1.99 |
| *Hibiscus mutabilis* L.              | NA                                        | 21.36 ± 0.51    | 44.74 ± 0.23    | NA                                   | 20.34 ± 0.42    | 43.29 ± 1.26 |
| *Ipomoea pes-caprae* (L.) Rbr.       | 8.03 ± 0.54                               | 32.30 ± 1.93    | 77.43 ± 1.74    | 4.53 ± 0.34                         | 31.41 ± 2.73    | 75.16 ± 2.37 |
| *Phyllanthus acidus* (L.) Skeels     | NA                                        | 40.72 ± 1.25    | 99.43 ± 0.17    | NA                                   | 40.06 ± 1.90    | 96.27 ± 0.10 |
| *Rhinacanthus nasutus* (L.) Kurz     | 13.55 ± 0.49                              | 28.90 ± 0.57    | 56.26 ± 0.90    | 10.60 ± 0.24                        | 28.29 ± 1.77    | 53.26 ± 1.95 |
| *Senna alata* (L.) Roxb.             | 12.51 ± 0.41                              | 29.21 ± 1.35    | 99.45 ± 0.11    | 8.79 ± 0.17                         | 28.97 ± 0.56    | 96.28 ± 0.12 |
| *Sterculia curtisii* Hook.f.         | NA                                        | 27.65 ± 1.30    | 95.49 ± 0.25    | NA                                   | 27.48 ± 0.11    | 91.91 ± 0.63 |
| *Streblus asper* Lour.               | NA                                        | 33.22 ± 0.77    | 87.32 ± 0.39    | NA                                   | 32.92 ± 1.57    | 83.78 ± 0.90 |

Each value is mean ± S.D. of triplicate independent analyses. Calculations of values are described in the Materials and Methods section. VCEAC Vitamin C Equivalent Antioxidant capacity, NA not available.
formation of reactive oxygen species (ROS) that induce hyperpigmentation and collagenase expression [17, 18]. Our study investigated 14 Thai plants extracted with three different solvents for their potential as anti-wrinkle and skin whitening ingredients. In this study, we used petrol-

| Plant                          | Extract      | Tyrosinase inhibition (%) | Collagenase inhibition(%) |
|-------------------------------|--------------|---------------------------|---------------------------|
| Annona squamosa Linn.         | Ethanol      | 21.92 ± 1.45**            | 55.12 ± 3.18***           |
| Ardisia elliptica Thunb.      | Petroleum ether | 19.42 ± 1.21**            | NA                        |
| Ardisia elliptica Thunb.      | Dichloromethane | 21.40 ± 1.61**            | NA                        |
| Ardisia elliptica Thunb.      | Ethanol      | 49.54 ± 1.23***           | 94.88 ± 6.93***           |
| Croton sublyratus Kurz        | Ethanol      | NA                        | 24.14 ± 0.98**            |
| Datura metel L.               | Ethanol      | 20.91 ± 1.70**            | NA                        |
| Ipomoea pes-caprae (L.) R.br. | Ethanol      | 23.01 ± 1.65**            | NA                        |
| Phyllanthus acidus (L.) Skeels.| Ethanol      | 42.92 ± 3.85***           | NA                        |
| Rhinacanthus nasutus (L.) Kurz| Ethanol      | 64.68 ± 5.46***           | NA                        |
| Senna alata (L.) Roxb.        | Ethanol      | 23.49 ± 1.09**            | 41.49 ± 2.63***           |
| Kojid acid (tyrosinase inhibitor) | –            | 93.38 ± 1.63***           | NA                        |
| Epigallatecathechin gallate (collagenase inhibitor) | – | 90.51 ± 2.79***          |                            |

Each value is mean ± S.D. of triplicate independent analyses. Significantly different from the control group (*P < 0.05, **P < 0.01, ***P < 0.001). NA not available

plants and are widely distributed among plant species [22]. Next, the flavonoid content within the Thai plants was evaluated using the aluminium chloride colorimetric assay. Our results showed that the highest flavonoid quantity was found in the ethanol extract from *Senna alata* (L.) Roxb leaves. In a previous study, high flavonoid content was found in water (4.25 mg QE per 100 g) and methanol fractions (3.97 mg QE per 100 g) of *Senna alata* (L.) Roxb. [23]. Thus, *Senna alata* (L.) Roxb preparations have a high flavonoid content when extracted with high polarity solvents including ethanol, methanol and water. *Ardisia elliptica* Thunb. had the richest flavonoid content in the dichloromethane fraction. Fruit of this plant also has a high flavonoid content 36.91 ± 2.37 mg QE per g extract [24]. Hence, fruit and leaves of *Ardisia elliptica* Thunb. are rich in flavonoids. Total flavonoid content did not correlate with total phenolic content. However, flavonoids have many biological activities such as UVB protection [25], anti inflammatory [26], anti-hepatotoxicity [27] and anti cancer [28].

Free radical scavenging activity using DPPH and ABTS assay. In the DPPH assay, DPPH receives a hydrogen atom from an antioxidant [29]. We found that *Ardisia elliptica* Thunb ethanol extract had the highest scavenging activity. Other investigators have also reported that dichloromethane leaf extracts of *Ardisia elliptica* Thunb. have a phenolic content of 101 ± 1.3 mg GAE per g dry plant material, which is more than the content in a twig extract [20]. Moreover, a methanol extract of ripe *Ardisia* fruit contained 5.64 ± 0.37 g GAE per 100 g extract [21]. Hence, leaves and fruits of *Ardisia elliptica* Thunb. have a high phenolic content that can be easily extracted with methanol, dichloromethane and ethanol.

Flavonoids are pigments in flowers, leaves, fruits and seeds. These compounds are secondary metabolites of phenolics are the largest group of phytochemicals found in plants and they have various biological activities in animals, including humans [19]. Total phenolic content in the plants was determined by the Folin-Ciocalteu method. Overall, the ethanol fraction had the richest phenolic content, followed by dichloromethane, while petroleum ether with low polarity had the lowest phenolic content compared to the other solvents. In this study, *Ardisia elliptica* Thunb. had the highest phenolic content in all three types of extracts. In previous studies, dichloromethane leaf extracts of *Ardisia elliptica* Thunb. have a phenolic content of 101 ± 1.3 mg GAE per g dry plant material, which is more than the content in a twig extract [20]. Moreover, a methanol extract of ripe *Ardisia* fruit contained 5.64 ± 0.37 g GAE per 100 g extract [21]. Hence, leaves and fruits of *Ardisia elliptica* Thunb. have a high phenolic content that can be easily extracted with methanol, dichloromethane and ethanol.

Flavonoids are pigments in flowers, leaves, fruits and seeds. These compounds are secondary metabolites of
active. In the ABTS assay, ABTS is converted to its radical
cation by the addition of potassium persulfate. In the pres-
ence of an antioxidant, the reactive ABTS cation (or ABTS· *)
is converted to its colorless natural form [9]. In agree-
ment with the DPPH assay, ethanol extracts contained the
highest levels of scavenger activity as compared with the
other extracts. Again, the highest scavenging activities in
ethanol, dichloromethane and petroleum ether extracts
were from the same plants as shown by the DPPH assay.
The results of the DPPH and ABTS assays were highly cor-
related as expected (Fig. 1f).

However, total flavonoid content of the plant extracts
did not correlate with free radical scavenger activity as
detected by the DPPH assay (Fig. 1c) or by the ABTS
assay (Fig. 1e). Our findings of no significant relationship
between flavonoid content and scavenger activity using
the ABTS assay is consistent with other investigators’ re-
sults [30]. By contrast, total phenolic content of the
plant preparation positively correlated with scavenger
activity measured by both assays (Fig. 1b and d) in agree-
ment with a previous study [31]. Noticeably, the
scavenging activity depended on total phenolic content
and solvents with high polarity, such as ethanol and di-
chloromethane. These results suggest that the phenolic
content is the major constituent with antioxidant activity
in the 14 Thai plants.

Melanin, the major pigment of skin and hair color, is syn-
thesized by melanocytes within melanosomes. Overproduc-
tion and accumulation of melanin in skin may lead to
pigmentary disorders and aesthetic problems. Hyperpig-
mentation occurs in sun-exposed areas of the skin [32]. In
the melanogenesis, tyrosinase is the key enzyme in the rate-
limiting step in which L-tyrosine is hydroxylated to L-
DOPA, which is further oxidized into DOPAquinone. After
that, it is converted into DOPAchrome that is a substrate
for melanin synthesis [3]. Downregulation of tyrosinase ac-
tivity has been proposed to be responsible for decreased
melanin production. The development of novel whitening
phytochemical compounds from natural products has re-
cently become a growing trend. Our finding showed that
the ethanol fraction from Rhinacanthus nasutus (L.) Kurz
was the most potent tyrosinase inhibitor, followed by the
ethanol extracts from Ardisia elliptica Thunb. and Phyl-
lanthus acidanus (L.) Skeels. Obviously, 7 plants from 14
plants had the high phenolic content, especially Ardisia
elli p t i ca Thunb. and Annona squamosa L. Moreover, Senna alata (L.) Roxb. had the richest flavonoid content
which can inhibit tyrosinase activity. Active compounds
from the plants such as arbutin, aloesin, gentisic acid, flavo-
oids, hesperdin, licorice, niacinamide, yeast derivatives,
and polyphenols, can inhibit melanogenesis without cyto-
toxicity to melanocytes [6].

Collagenase is a transmembrane zinc peptidase that
cleaves the X-Gly bond of collagen. Collagen is an abundant
structural protein and extracellular matrix component [33].
Decreased collagen and elastin fibers increases with age
and damage from UV radiation inducing wrinkled skin
[34]. Collagenase inhibition has been proposed to prevent
skin aging. Of those causing inhibition in our study, Ardisia
elliptica Thunb. exhibited the highest level of collagenase
inhibition, followed by Annona squamosa L., Senna alata
(L.) Roxb., and Croton sublyratus Kurz in rank order. In a
previous study, cocoa pod extract had phenolic acid and fla-
ovoids that inhibited elastase and collagenase activity [35].
Notably, three ethanol extracts (Ardisia elliptica Thunb.,
Annona squamosa L. and Senna alata (L.) Roxb. inhibited
both tyrosinase and collagenase. These plants also had high
phenolic and flavonoid levels, and antioxidant activity.
Interestingly, these extracts have possible uses as ingredi-
ents for cosmetic products.

**Conclusion**

Our results demonstrate that extracts of 14 Thai plants
had varying degrees of total phenolic and flavonoid con-
tent as well as free radical scavenging activities, depend-
ning on the extraction solvents. There was a high
correlation between total phenolic content and free rad-
ical scavenging activity as assessed by the DPPH and
ABTS assays. The ethanol fraction of Ardisia elliptica
Thunb. had the highest phenolic content, followed by
Annona squamosa L. Both plants significantly inhibited
tyrosinase and collagenase activities, while Rhinacanthus
nasutus (L.) Kurz showed the highest tyrosinase inhib-
ition. Moreover, Senna alata (L.) Roxb. was richest in
flavonoid content, and also exhibited tyrosinase and col-
lagenase inhibitory behavior. The ethanol fraction of
three plants, namely Annona squamosa L., Ardisia ellipti-
ca Thunb and Senna alata (L.) Roxb., have the poten-
tial to be ingredients in cosmetic products for anti-
wrinkling as well as skin whitening. Further studies are
necessary to investigate the active components and
safety of these extracts.

**Abbreviations**

ABTS: 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); DHI: 5,6-
dihydroxyindole (DHI); DHICA: 5,6-dihydroxyindole-2-carboxylic acid;
DMSO: Dimethyl sulfoxide; DPPH: 2,2-diphenyl-1-picrylhydrazyl;
EGCG: Epigallocatechin gallate; FALGPA: N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala;
GAE: Gallic acid equivalents; KA: Kojic acid; L-DOPA: 3,4-dihydroxyphenyl alanine;
Na2CO3: Sodium carbonate; QE: Quercetin equivalents; ROS: Reactive oxygen
species; SC: Scavenging activity; TPC: Total flavonoid content; TPC: Total
phenolic content; UVR: Ultraviolet radiation; VC: Vitamin C equivalent
antioxidant capacity

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Availability of data and materials
Not applicable since all the data supporting the results reported are in this manuscript.

Authors’ contributions
MC and AC designed hypothesis and supervised experiments. MC performed the experiments in this study, analyzed the data and drafted the manuscript. AC assisted with analyzing the data, drafting and revising the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
This information is not relevant since our study does involve neither animals nor humans.

Consent for publication
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