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Anti-collagenase, Anti-elastase and Antioxidant Activities of *Pueraria candollei* var. *mirifica* root Extract and *Coccinia grandis* Fruit Juice Extract: An *In vitro* study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TC and EO designed the study. Author TC carried out the entire work and wrote the first draft. Author EO supervised the study, proof read and corrected the final draft. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To determine the *in vitro* anti-elastase, anti-collagenase and antioxidant properties of two Asian herbs: *Pueraria candollei* Graham ex Benth. var. *mirifica* and *Coccinia grandis* (L.) Voigt that were selected for their anti-aging properties according to their ethnobotanical and chemotaxonomic information.
Place and Duration of Study: School of Agriculture, Food and Rural Development, Newcastle University, United Kingdom, between June and August 2014.
Methodology: The methanolic extract of the roots of *P. candollei*var. *mirifica* (PMM) and the fruit juice extract of *C. grandis* (CGJ) were investigated by *in vitro* enzymatic assays to mimic the breakdown of elastin and collagen fibres. Total phenolic content was determined using the Folin-

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Ciocalteu method (gallic acid equivalent, GAE), while antioxidant capacity was determined as Trolox equivalents (TE) by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assays.

Results: PMM significantly inhibited elastase activity with IC_{50} of 143.0±4.78 µg/mL, and its anti-collagenase activity was comparable with that of the positive control, epigallocatechin gallate (EGCG). Based on the same concentration, the elastase inhibitory activity of PMM was significantly higher compared with that of CGJ (P< .001), while the collagenase inhibitory activities of both extracts were comparable. Total phenolic content, DPPH and ABTS radical scavenging activities of 1.0 g equivalent of the dried roots of PMM were 0.6±0.04 mg of GAE, 16.4±6.20 and 9.3±1.38 µmole TE respectively. The antioxidant values of PMM were significantly higher compared with those of 1000 µg/mL of CGJ (GAE, P< .0001; DPPH radical scavenging activity, P< .05; ABTS radical scavenging activity, P< .001). The free radical scavenging activities of both plant extracts were positively correlated with their total GAE (P < .0001).

Conclusion: PMM may play a role in decelerating the skin ageing process and could be formulated for anti-wrinkle skincare products. Further research is required to examine anti-elastase, anti-collagenase and antioxidant activities of individual compounds contained in the roots of PMM and expand the investigation into such anti-ageing effects of the whole fruits of *C. grandis*, which might contain higher amounts of phytochemicals than the fruit juice.

Keywords: Skin ageing; Pueraria candollei var. mirifica; Coccinia grandis; anti-elastase; anti-collagenase; antioxidant.

1. INTRODUCTION

The human skin, like other organs, unavoidably undergoes chronological ageing or intrinsic ageing as a result of the passage of time, genetic influence and the changes of intrinsic factors such as telomere shortening, the imbalance between free radicals and antioxidant components and hormonal changes [1-7]. Additionally, solar ultraviolet (UV) radiation detrimentally contributes to photoageing or extrinsic ageing of the skin [1,5,7-10]. Both ageing processes contribute to the decline in the elasticity and strength of the skin, and the rise in wrinkle formation [5]. These structural alterations result from various pathomechanism including the enzymatic degradation and disintegration of dermal elastin fibres, which maintain resilience of the skin; the proteolytic breakdown and disorganisation of dermal collagen networks, which provide the skin with strength and resistance; and the continuous bombardments of free radicals against cellular components of the skin, especially mitochondrial DNA (mtDNA), [1,3-14].

Medicinal plants possessing inhibitory actions on elastase and collagenase, which degrade elastin and collagen fibres, and antioxidant efficacies might play significant roles in the deceleration of the skin ageing process and could potentially be developed for cosmetic purposes. In this project, the selection of plants was based on the integration between ethnobotanical and chemotaxonomic information of species commonly used in traditional Thai medicine. The studied plants include *Pueraria candollei* Graham ex Benth. var. *mirifica* (Airy Shaw et Suvatabandhu) Niyomdhamand *Coccinia grandis* (L.) Voigt which have been indigenously utilised for rejuvenation purpose and the treatment of various skin lesions [15-21].

P. candollei var. *Mirifica* (Fabaceae) or white kwaokrua is a medicinal plant which is indigenous to Thailand [22]. The tuber of this climbing legume has been used in traditional Thai medicine as a rejuvenating tonic and possesses oestrogenic activity [15,16]. Currently, the plant extract is also commercially present in various cosmetic formulations including topical skin moisturisers and breast creams, largely due to its aforementioned properties [23]. However, other underlying mechanisms against skin ageing such as anti-elastase and anti-collagenase activities of this plant have not been elucidated yet according to the search of MEDLINE and Embase.

C. grandis (Cucurbitaceae) or ivy gourd is a vegetable which is widely used in Thai cuisine; the young leaves, shoots and young fruits are its edible parts [19]. In Thai traditional medicine, the poultice prepared from its leaves is topically applied to affected areas for treating burning pain and insect bites [19,21]. The anti-elastase, anti-hyaluronidase and antioxidant activity of the fruit of *Cucumis sativus*, another plant in the family

Cucurbitaceae, have been investigated [24]. Thus, in addition to the medicinal properties of the leaf of *C. grandis*, the chemotaxonomic approach is taken to the fruit to explore its anti-ageing activities.

The primary objective of this project was therefore to screen the selected plant species for their anti-elastase, anti-collagenase and free radical scavenging activities which are considered to be therapeutic actions against skin ageing.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemical and assay kits

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), calcium chloride, collagenase derived from *Clostridium histolyticum*, 2,2-diphenyl-1-picrylhydrazyl (DPPH), disodium hydrogen phosphate, epigallocatechin gallate (EGCG), Folin-Ciocalteu reagent, gallic acid anhydrous, *N*-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA) *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES)), potassium persulfate, sodium carbonate, sodium chloride, sodium dihydrogen phosphate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich® UK. Sodium hydroxide (Fisher Bioreagents®, EnzChek®Elastase Assay kit (Molecular Probes®, hydrochloric acid (BDH®, UK), methanol (Fisher Chemicals®, UK).

2.1.2 Plant materials

The dried tuberous roots of *P. candollei* var. *mirifica*(Lot # A56072901) were purchased from Stherb Cosmetics International Co., Ltd., Pathumthani Province, Thailand. The fresh fruits of *C. grandis* were purchased from Indian Mart, Leicestershire, United Kingdom. Voucher specimens of all studied plant materials are deposited at School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, United Kingdom.

2.2 Methods

2.2.1 Extraction of plant materials

The methanolic extract of *P. Candollei* var. *mirifica* was prepared by adapting the procedure of Sookvannichsilp et al. [25]. The dried tuberous roots of *P. Candollei* var. *mirifica* were sliced and pulverised to yield approximately 60 g of powder that was subsequently macerated in 300 mL of 80% (v/v) methanol in a closed vessel for 72 hours. Shaking was done on a daily basis during this immersion period. Afterwards, this mixture underwent vacuum filtration, and the residue was then washed with 100 mL of 80% (v/v) methanol and discarded at the end of this stage. The obtained filtrate was collected, and the solvent was evaporated at 40°C using a rotary evaporator (Rotavapor® R-210, Buchi) to yield the dry methanolic extract of *P. candollei* var. *mirifica* (PMM). The weight of PMM was used for calculating the percentage extraction yield.

$$\text{Extraction yield } \left(\%\frac{\text{w}}{\text{w}}\right) = \left(\frac{\text{weight of PMM in gram}}{\text{60 g of the powder of the roots of } P.\ candollei \text{ var. } mirifica}\right) \times 100$$

The juice extract of the fresh fruits of *C. grandis* was studied according to the technique employed by the previous work with modification [24]. The fresh fruits were thoroughly washed by tap water and then sliced into small pieces that were subsequently homogenised in a blender for 5 mins. The derived mixture was instantaneously filtered under vacuum to separate the juice from the puree of the fruits. This crude juice (66 mL) was centrifuged at 10000 rpm for 10 mins using a microcentrifuge (Eppendorf Centrifuge 5417C) to obtain a clear supernatant that was subsequently lyophilised to obtain the dry fruit juice extract of *C. grandis* (CGJ). The weight of CGJ was used for calculating the extraction yield as the percentage of the weight of the extract to the volume of the crude juice.

$$\text{Extraction yield } \left(\%\frac{\text{w}}{\text{v}}\right) = \left(\frac{\text{weight of CGJ in gram}}{\text{66 mL of the crude juice of the fruits of C. grandis}}\right) \times 100$$

2.2.2 Preparing plant samples

PMM (100 mg) and CGJ (100 mg) were dissolved in a final volume of 100 mL of 18.2 MΩ water to make 100 mL of 1000.0 µg/mL PMM and CGJ stock solution respectively. The stock solutions underwent serial dilution in 18.2 MΩ.com water to make 500.0, 250.0, 125.0 and

62.5 µg/mL PMM and CGJ samples for both enzymatic and antioxidant analyses.

2.2.3 Enzymatic assays

2.2.3.1 In vitro elastase inhibition assay

This assay was performed according to the descriptive instructions supplied with the EnzChek® Elastase Assay Kit and the previous procedure with minor modification [26,33]. The principles of this assay are that the elastase directly breaks down the elastin substrate to yield the fluorescently labelled fragments that are subsequently measured using a fluorescence microplate reader (excitation wavelength at 485±10 nm and emission wavelength at 530±15 nm), and this enzymatic hydrolysis is disrupted in the presence of elastase inhibitors contained in those samples. The assay was performed in 1X Reaction Buffer. Porcine pancreatic elastase (100 µL) was allowed to react with each plant sample (50 µL) in a 96-well microtitre plate in the dark at 25°C for 30 minutes. Controls (no inhibitor) and positive controls were performed with 50 µL of 18.2 MΩ.cm water and 50 µL of the elastase inhibitor working solution (*N*-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone or MAAPVC as positive control) respectively. Blanks contained 50 µL of 18.2 MΩ.cm water and 100 µL of the buffer working solution. After the preincubation, an amount of 50 µL of the elastin working solution was added to each well to make a final reaction volume of 200 µL, and the microplate was further incubated in the dark at 25°C for 120 minutes. The final concentrations of reaction mixture included 25 µg/mL elastin, 0.25 units/mL elastase and 0.01 mM MAAPVC. All experiments were performed in triplicate. The fluorescence intensity of the solution in each assay well was determined using a fluorescence microplate reader (FLUOstar Omega, BMG LABTECH) at the excitation wavelength of 485 nm and the emission wavelength of 520 nm. To correct background fluorescence of plant samples, control and positive control, subtracting the fluorescence intensity value of blank or no-enzyme control was performed. The percentage of elastase inhibitory activity was calculated from the following equation.

$$\text{The percentage of elastase inhibition (\%)} = \left(1 - \frac{S}{C}\right) \times 100$$

Where '**S**' is the corrected fluorescence intensity of the samples containing elastase inhibitor (*the enzyme activity in the presence of the samples*), and '**C**' is the corrected fluorescence intensity of controls (*the enzyme activity in the absence of the plant extracts*).

2.2.3.2 In vitro collagenase inhibition assay

The previous procedure was modified and subsequently employed to determine the anti-collagenase activity of the plant extracts [27]. The principles of this assay are based on the enzyme-substrate interaction between bacterial collagenase derived from *Clostridium histolyticum* and the synthetic *N*-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA), which its proteolytic degradation results in the reduction in the absorbance between 324 and 345 nm, and the limited breakdown of FALGPA in the presence of collagenase inhibitors, which is preincubated with the enzyme before adding the substrate. The assay was performed in 50 mM TES buffer with 0.36 mM calcium chloride (pH 7.4 at 37°C) [34]. Collagenase derived from *Clostridium histolyticum* (20 µL) was allowed to react with each plant extract (20 µL) in a 96-well microtitre plate containing 20 µL of the buffer in the dark at 37°C for 20 minutes. Controls and positive controls were performed with 20 µL of 18.2 MΩ water and 20 µL of epigallocatechin gallate (EGCG) working solution respectively. Blanks contained 20 µL of 18.2 MΩ water and 40 µL of the buffer working solution. After the preincubation, an amount of 40 µL of the FALGPA working solution was added to each well to make a final reaction volume of 100 µL, and the microplate was further incubated in the dark at 37°C for 30 minutes. The final concentrations of reaction mixture included 0.8 mM FALGPA, 0.16 units/mL collagenase and 0.023 mg/mL EGCG as positive control. All experiments were performed in duplicate. The absorbance of the solution in each assay well was determined using a microplate reader (SpectraMax Plus^{384}, Molecular Device Corporation) at the wavelength of 335 nm. To correct background absorbance of plant samples, control and positive control, subtracting the absorbance value of blank was performed. The percentage of collagenase inhibitory activity was calculated from the following equation.

$$\text{The percentage of collagenase inhibition (\%)} = \left(1 - \frac{S}{C}\right) \times 100$$

Where '**S**' is the corrected absorbance of the samples containing collagenase inhibitor (*the enzyme activity in the presence of the samples*), and '**C**' is the corrected absorbance of controls (*the enzyme activity in the absence of the samples*).

2.2.4 Antioxidant assays

2.2.4.1 Determination of total phenolic content (Folin-Ciocalteu method)

The total phenolic content of plant samples was determined using Folin-Ciocalteu method in a 96-well microplate according to the previous procedure with minor modification [28]. In the assay, either gallic acid standard solutions (20 µL), plant samples (20 µL) or 18.2 MΩ water (20 µL) for blanks were thoroughly mixed with the 10% (v/v) Folin-Ciocalteu working solution (100 µL) at room temperature and then left for 5 minutes. Subsequently, 7.5% (w/v) sodium carbonate solution (80 µL) was added to each assay well, and the microplate was incubated in the dark at 25°C for 60 minutes. All experiments were performed in triplicate. The absorbance of the solution in each assay well was determined using a microplate reader (SpectraMax Plus^{384}, Molecular Device Corporation) at the wavelength of 750 nm. To correct background absorbance of standard solutions and plant samples, subtracting the absorbance value of blank was performed. The total phenolic content was expressed as gallic acid equivalent (GAE) through a calibration curve (62.5, 125.0, 250.0, 500.0 and 1000.0 µg/mL of gallic acid). Additionally, the total phenolic content was expressed as GAE in 1 g of the dried root extracts of *P. candollei*var. *mirifica* or 1 mL of the crude fruit juice extract of *C. grandis*

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

The DPPH radical scavenging activity assay was performed by adapting the previous procedure [29]. DPPH stock solution (0.24 mg/mL), which was prepared in methanol and kept in the dark at 4°C overnight, was mixed with 80 mL of methanol to prepare the working solution. Trolox (6.3 mg) was thoroughly dissolved in a final volume of 50 mL of 50% methanol to prepare 1000.0 µM Trolox stock solution, which underwent serial dilution using 50% methanol as a solvent to make 500.0, 250.0, 125.0 and 62.5 µM Trolox standard solutions. In the assay, either Trolox standard solutions (15 µL), plant samples (15 µL) or 50% (v/v) methanol (15 µL) for blanks were thoroughly mixed with the DPPH working solution (285 µL) in assay wells. The microplate was subsequently incubated in the dark at 30°C for 30 minutes. All experiments were performed in triplicate. The absorbance of the solution in each assay well was determined using a microplate reader (SpectraMax Plus^{384}, Molecular Device Corporation) at the wavelength of 517 nm. To correct background absorbance of standard solutions and plant samples, subtracting the absorbance value of blank was performed. The DPPH radical scavenging activity expressed as Trolox equivalent (TE) of 1 g of the dried tuberous roots of *P. candollei*var. *mirifica* and 1 mL of the crude fruit juice of *C. grandis.* The percentage of such inhibition was calculated from the following equation.

$$\text{The percentage of DPPH radical scavenging activity (\%)} = \left(1 - \frac{\mathrm{S}}{\mathrm{C}}\right) \times 100$$

Where '**S**' is the mean corrected absorbance of the samples, and '**C**' is the mean corrected absorbance of controls.

2.2.4.3 Trolox equivalent antioxidant capacity (TEAC) assay or ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging assay

For the TEAC assay, the procedure followed the previous method with slight modification [30]. The ABTS working solution was derived from the chemical reaction between 7 mM ABTS stock solution and 2.45 mM potassium persulfate in 5 mM phosphate buffer solution (pH 7.4). Trolox (6.3 mg) was thoroughly dissolved in a final volume of 50 mL of 50% methanol to prepare 1000.0 µM Trolox stock solution, which underwent serial dilution using 50% methanol as a solvent to make 500.0, 250.0, 125.0 and 62.5 µM Trolox standard solutions. In the assay, either Trolox standard solutions (10 µL), plant samples (10 µL) or 50% methanol (10 µL) for blanks were thoroughly mixed with ABTS working solution (290 µL) in assay wells. The microplate was subsequently incubated in the dark at 37°C for 6 minutes. All experiments were performed in triplicate. The absorbance of the solution in each assay well was determined using a microplate reader (SpectraMax Plus^{384}, Molecular Device Corporation) at the wavelength of 734 nm. To correct background absorbance of standard solutions and plant samples, subtracting the absorbance value of blank was performed. The ABTS radical scavenging activity

expressed was expressed as Trolox equivalent (TE) of 1 g of the dried tuberous roots of *P. candollei*var. *mirifica* and 1 mL of the crude fruit juice of *C. grandis*. The percentage of inhibition of ABTS radical of plant samples was compared with that of Trolox standard solutions. The percentage of such inhibition was calculated from the following equation.

$$\text{The percentage of ABTS radical scavenging activity (\%)} = \left(1 - \frac{S}{C}\right) \times 100$$

Where '**S**' is the corrected absorbance of the samples, and '**C**' is the corrected absorbance of controls.

2.2.5 Statistical analysis

The results are presented as the mean with the standard deviation (mean ± SD) of individual experiments. Regression analysis was carried out to examine the relation between explanatory and response variables, and determine the half maximal inhibitory concentration (IC_{50}) of each plant extract. Either independent samples *t*-test (unpaired *t*-test) or one-way analysis of variance (ANOVA) in conjunction with a post hoc Tukey HSD test was used for estimating statistical significance. Pearson's correlational analysis was performed to estimate the correlation between two variables. The statistical analysis was performed using R 3.0.2 for Windows.

3. RESULTS AND DISCUSSION

3.1 Extraction Yields of PMM and CGJ

The extraction yield of PMM was found to be 13.76% (w/w). The obtained extraction yield, which accounts for approximately 14% by weight of the dried root, is three times higher than that of the ethanolic maceration (80% (v/v)) employed by previous studies, despite the fact that both extraction solvents are fairly similar in physicochemical properties such as polarity index, and the extraction period in this experiment was shorter [25,31]. This discrepancy could be attributed to the plant age, the genetic, seasonal and geographical variations of phytochemical composition of this species [32,35]. The extraction yield of CGJ accounted for 3.33% (w/v). As the fruits of *C. grandis* and those of *Cucumis sativus* or cucumber, which belongs to the same family, are similar in appearance, the extraction technique of the previous study was adopted [24]. Contrary to the expectation, the extraction yield of the fruit juice extract of *C. grandis* or CGJ in this experiment was approximately 3% weight by volume of the crude fruit juice, and this yield is twelve times lower than that of *C. sativus*. This discrepancy may be due to the difference in phytochemical composition and fruit juice content between both species.

3.2 Comparison of Elastase Inhibitory Activity between PMM and CGJ

Based on the similar concentrations, elastase inhibitory activity of PMM was significantly superior to that of CGJ ($P < .001$) Fig. 1. PMM at the lowest concentration (62.5 µg/mL) and CGJ at the concentrations in the range 250.0 to 1000.0 µg/mL similarly inhibited elastase activity. The calculated IC_{50} value of PMM was 143.0 µg/mL. As the highest response for CGJ was under 30%, the IC_{50} value could not be extrapolated.

3.3 Comparison of Collagenase Inhibitory Activity between PMM and CGJ

The comparison of collagenase inhibitory activity between PMM and CGJ is illustrated in Fig. 2. Based on the similar concentration, collagenase inhibitory activity of CGJ was not significantly higher than that of PMM.

3.4 Comparison of Total Antioxidant Capacities between PMM and CGJ

To compare the antioxidant capacities of PMM at the highest concentration of 1000 µg/mL with that of CGJ at the same concentration, independent samples *t*-test was used. From the data in Table 1, it can be seen that free radical scavenging activity of 1000 µg/mL PMM was significantly higher compared to that of 1000 µg/mL CGJ ($P< .05$). Data from this table can be compared with those of Table 2 which shows that 1.0 g of the dried tuberous roots of *P. candollei* var. *mirifica* had significantly higher DPPH ($P< .05$) and ABTS ($P< .001$) radical scavenging activity compared with 1000 µg/mL of the crude fruit juice of *C. grandis*. A similar trend was also observed in the total phenolic content expressed as GAE of the starting plant materials. Such findings are also consistent with the weak radical scavenging activity of soya bean extracts rich in isoflavones and total phenolics [36]. This result fairly agrees with the finding of the previous work that revealed the significant link between DPPH radical scavenging activity and puerarin, an isoflavone found in the roots of *P. candollei* var. *mirifica* [37].

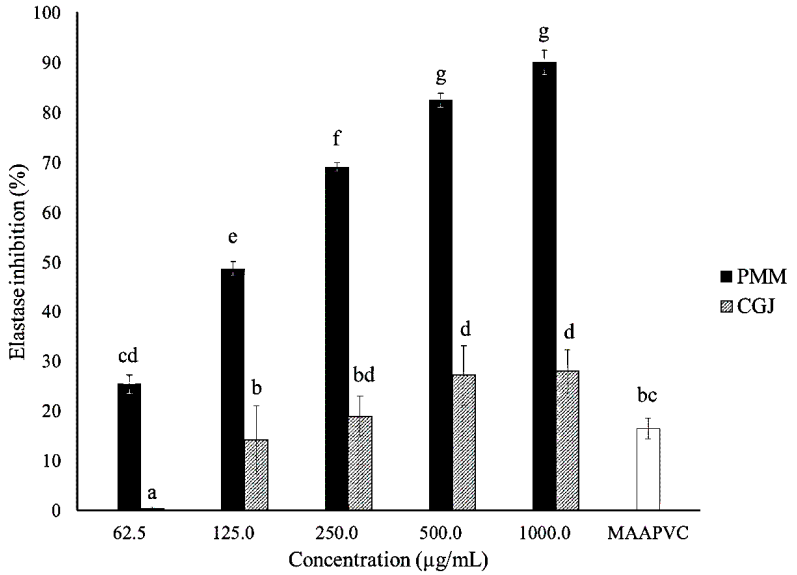

Fig. 1. Comparison of elastase inhibitory activity between PMM and CGJ*

**The values having different letters are significantly different at P < .001 from each other, except, differences in the values having a versus b, b versus d and f versus g are significant at P < .01, and differences in the values having b versus c and c versus d are significant at P < .05*

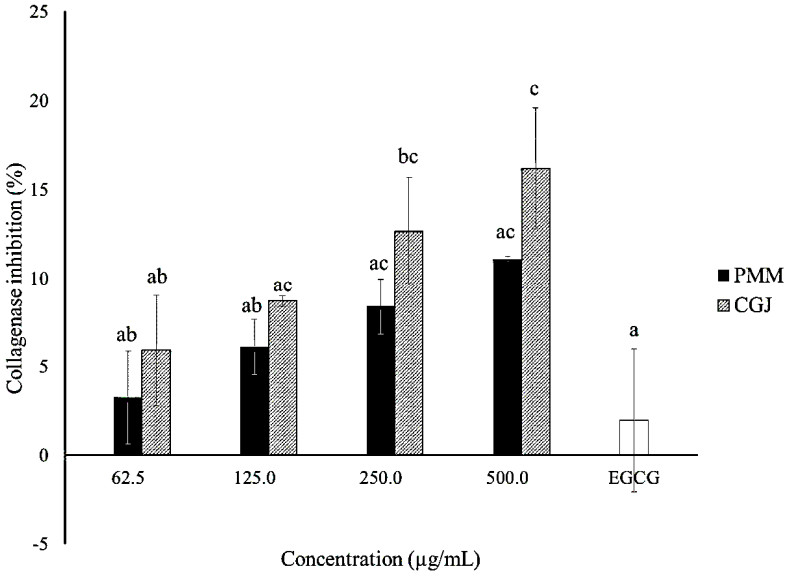

Fig. 2. Comparison of collagenase inhibitory activity between PMM and CGJ*

**The values having different letters are significantly different at P < .05) from each other, except, difference in the values having a versus c are significant at P < .01*

Table 1. Comparison of total antioxidant capacities between PMM and CGJ

Antioxidant capacity	PMM (1000 µg/mL)	CGJ (1000 µg/mL)	Significant level
Inhibition of DPPH radical (%)	8.9±1.31	3.6±2.72	*P*< .05
Inhibition of ABTS radical (%)	8.6±1.27	5.3±1.48	*P*< .05

Table 2. Comparison of total antioxidant capacities between the dried tuberous roots of *P. candollei* var. *mirifica* and the crude juice derived from the fresh fruits of *C. grandis*

Antioxidant capacity*	Dried tuberous roots of *P. candollei* var. *mirifica* (per g)	Crude juice derived from the fresh fruits of *C. grandis* (per mL)	Significant level
GAE (mg)	0.6±0.04	0.2±0.01	*P*< .0001
DPPH radical scavenging activity expressed as TE (μmole)	16.4±6.20	1.8±1.70	*P*< .05
ABTS radical scavenging activity expressed as TE (μmole)	9.3±1.38	1.4±0.40	*P*< .001

**Calculated from the concentration of 1000.0 μg/mL*

In terms of the comparison with PMM, it is apparent that all anti-ageing properties, but for anti-collagenase activity, of PMM were significantly superior to those of CGJ. Therefore, one question that needs to be addressed is about the anti-ageing potency of the fruits of *C. grandis.* In this research project, the juice of the green fruits was only studied. It is likely that a significant amount of phytochemicals that possess anti-collagenase, anti-elastase and antioxidant activities are contained in other fruit structures such as pericarp and seeds. As a result, one suggestion that can be drawn from the present study is that the extract obtained from the solvent extraction of the whole fruits might exhibit more potent anti-ageing activity. It is worth mentioning that the extraction ability varies according to extraction solvents [40]. This is an important issue for future investigations to examine the optimum solvent system that is able to extract a variety of hydrophobic phytochemicals including carotenoids and triterpenoids contained in the fruits of *C. grandis*.

4. CONCLUSION

This is the first research project examining the elastase and collagenase inhibitory activities of the plant extract obtained from the tuberous roots of *P. candollei*var. *mirifica* and the fruit juice of *C. grandis.* The results of this investigation indicate that the methanolic extract of the roots of *P. candollei* var. *mirifica* exhibited potent elastase inhibitory activity but exhibited weak anti-collagenase and antioxidant activities. In terms of the comparison with the fruit juice extract of *C. grandis*, it is apparent that all anti-ageing properties, but for collagenase inhibitory activity, of the extract of *P. candollei* var. *mirifica* were significantly superior to those of the extract of *C. grandis.* Additionally, the free radical scavenging activities of both plant extracts were positively correlated with their total phenolic content. The evidence from this study, therefore, suggests that the extract of the roots of *P. candollei* var. *mirifica* may play a role in decelerating the ageing process of the skin and could be formulated for anti-wrinkle skin care products. The most principal phytochemicals found in the tuberous roots of this plant are oestrogenic phenolic compounds such as daidzein, genistein, deoxymiroestrol, miroestrol and coumestrol, where genistein has been found to inhibit neutrophil elastase activity [38].Therefore further research is required to examine anti-elastase, anti-collagenase and antioxidant activities of individual compounds contained in the roots of *P. candollei* var. *mirifica,* screening out potentially undesirable compounds [39] and expand the investigation into such anti-ageing effects of the whole fruits of *C. grandis*, which might contain higher amount of phytochemicals than the fruit juice and although high in carotenoids, they possess low DPPH scavenging activities [41].

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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