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Original Article

Bioactivities of selected herbs in relation to the body elements in Traditional Thai Medicine

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Abstract

Thai Traditional Medicine believes that human body is composed of four elements. It also mentions that skin problems related to each element can be alleviated by using suitable plants. This study aimed to determine the bioactivities related to skin effects of *Aloe vera*, *Cucumis sativus*, *Alpinia galanga* and *Phyllanthus emblica*. The plants were extracted with 50% ethanol prior to bioactivity evaluation. The highest anti-oxidant activity (IC_{50}, 14.01 µg/ml) was found for *P. emblica*. Tyrosinase inhibition was found with *P. emblica* (41.92% inhibition) and *A. vera* (26.67% inhibition) at 500 µg/ml concentrations. The cells treated with 500 µg/ml *A. vera* and 15.62 µg/ml *P. emblica* had after 48 h increased collagen type-1 production by around 14 and 4 -fold from those of untreated cells. Anti-glycation was found with *P. emblica*. *P. emblica*, *A. galanga* and *C. sativus* at 62.5 µg/ml exhibited anti-inflammatory activity. This information supports evidence based use of these plants as food and cosmetic ingredients.

Keywords: Thai Traditional Medicine, anti-aging, anti-oxidant, anti-glycation, collagen synthesis

1. Introduction

Traditional Thai Medicine (TTM) principles purport that the human body is composed of four elements (or 'tard' in the Thai language), namely earth, water, air and fire. If one element dominates in an individual, it can encourage certain personality and habitual traits to develop in that person. The body elements are correlated to emotions, temperament, direction, skin color, tastes, body type, illnesses, thinking style, and character. A person who knows which element dominates their body and what that imbalance causes can take care of their health to compensate for the imbalance (Laohapand, 2014).

In Thai scripture, the skin types were distinguished according to the body elements, and also suitable specific diets with some plants or vegetables were suggested accordingly. The care of health and skin was then managed by consuming appropriate plants or vegetables associated with the individual body elements. Those who are dominated by the fire element generally tend to have fairly dark skin, and suitable diets include cool or bitter flavors, such as watermelon and cucumber. Dominance of the air element promotes thin bodies and dry skin, and spicy flavored food with ginger, galangal and pepper is appropriate. Those with a large water element usually have healthy and sensitive skin, and preferable food ingredients include juicy and sour or bitter

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flavored food, such as orange, tomato, aloe or cucumber. The earth element generally induces fairly dark complexion and oily skin, and appropriate diet includes astringent, sweet or salty flavored foods, such as guava, banana, taro root, amla and potato (Akarayosapong, 2010; Laohapand, 2014).

Nowadays, botanical extracts play an increasingly important role in food and cosmetics due to the effectiveness of such extracts in improving skin properties, especially elasticity and moisture (Ribeiro *et al.et al.*, 2015). There is a large numbers of plant extracts that have chemical compounds suitable for use as food supplements or in cosmetic products.

However, the bioactivity of plants in relation to the body elements, according to Traditional Thai Medicine (TTM) principles, should be determined, and currently only few products are designated for individual body elements. Thus, the purpose of the current study was to investigate the bioactivities of plant extracts selected according to the body element principle. The plants included were *Aloe vera* (L.) Burm.f (Aloe), *Cucumis sativus* L. (Cucumber), *Alpinia galanga* (L.) Wild (Galangal) and *Phyllanthus emblica* L. (Amla), which were selected to match the body elements water, fire, air and earth in respective order. The selected plants are common in daily food, in Thailand. Moreover, some reports indicate that these plants possess many bioactive compounds associated with skin related benefits. Compounds such as 1-ácetoxycavichol acetate and catechin have been reported in *A. galanga* (Mahae & Chaiseri, 2009), while flavonoids, polyphenols, glycosides and tannins are mainly found in *C. sativus* (Narra, Nisha, & Nagesh, 2015). The fruit of *P. embrica* contain flavonoids and proanthocyanidins (Liu, Zhao, Wang, Yang, & Jiang, 2008) and *A. vera* has aloesin and aloe sterols (Takahashi *et al.*, 2009; Tanaka *et al.*, 2015). Thus the results from this study may be useful for further development of food supplements and skincare products containing suitable plant extracts related to each body element of TTM.

2. Materials and Methods

2.1 Preparation of plant extracts

2.1.1 *A. vera* extract

The preparation of *A. vera* extract followed the method reported by Lee *et al.* (2012). The *A. vera* leaves were peeled, then the gel was collected and freeze-dried. The *A. vera* powder was then extracted using 50 %(v/v) ethanol at a solid (g): solvent (ml) ratio of 2: 400 for 24 h. The mixture was then centrifuged for 5 minutes at 10,000 rpm. The supernatant was collected and concentrated in a rotary evaporator at 40°C.

2.1.2 *C. sativus* extract

The *C. sativus* extract was prepared according to the method presented by Narra, Nisha, and Nagesh (2015). Whole fruit of *C. sativus* were cut into small pieces and dried in shade at room temperature. The dry material was mechanically crushed into coarse powder. The powder was subsequently extracted with 50 %(v/v) ethanol at a solid (g): solvent (ml) ratio of 1: 20 for 24 h. The extract was filtered through a No.1 sinter glass funnel and concentrated using a rotary evaporator at 40°C.

2.1.3 *A. galanga* extract

Extraction of *A. galanga* was done according to the method reported by Mahae and Chaiseri (2009). Fresh *A. galanga* rhizomes were cleaned, washed with water, cut into small pieces and dried in a tray dryer at 50°C. The dried sample was ground to a fine powder using a blender. The powder was extracted using 50 %(v/v) ethanol at a solid (g): solvent (ml) ratio of 1:10 for 24 h. The extract was filtered through a No.1 sinter glass funnel and concentrated using a rotary evaporator at 40°C.

2.1.4 *P. emblica* extract

The *P. emblica* extract was prepared according to the method presented by Mayachiew & Devahastin (2008). *P. emblica* fresh fruit were cleaned, washed with water, and dried in a tray dryer at 50°C. The dried materials were pulverized into coarse powder. *P. emblica* powder was extracted using 50 %(v/v) ethanol at a solid (g): solvent (ml) ratio of 1:10 for 24 h. The extract was filtered through a No.1 sinter glass funnel and concentrated using a rotary evaporator at 40°C.

2.2 Biological activity

2.2.1 Anti-oxidant activity

The anti-oxidant activity was determined with a 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay. The extracts and the positive control, L-ascorbic acid, were dissolved in 2%v/v DMSO in PBS buffer at the concentration of 156.25-40,000 µg/ml (*A. vera*), 24.41-100,000 µg/ml (*C. sativus*), 62.50-10,000 µg/ml (*A. galanga*), 0.49-1,000 µg/ml (*P. emblica*) and 0.98-250 µg/ml (L-ascorbic acid). Briefly, 75 µl of each extracted solution were mixed with 150 µl of 0.2 mM DPPH. The blends were allowed to react in dark for 30 minutes. Then, the absorbance at 517 nm was measured and the inhibition of radical was calculated using the following equation.

$$\% \text{ Inhibition of DPPH radical} = \frac{(A_{517}\ \text{control} - A_{517}\ \text{sample})}{A_{517}\ \text{sample}} \times 100$$

The IC_{50} was determined by log-probit analysis using GraphPadPrism software version 7.0 (GraphPad Software, USA).

2.2.2 Tyrosinase inhibition assay

B16-F1 mouse melanoma cell line (ATCC® CRL-6323 ™) was injected into a 96-well plate at 10^5 cells/well in complete low glucose DMEM containing 10% Fetal Bovine Serum and 1% 10 µg/ml penicillin, and 10 µg/ml streptomycin, at 37 °C with 5% CO_2 in a humidified atmosphere. One-hundred ml of an extract solution (or kojic acid as positive control) in the concentration range 0-500 µg/ml was added, and then 20 mM L-DOPA (100 µl) was added as the substrate in order to induce the reaction. The plate was left in dark for 1 h at room temperature. Conversion of L-DOPA to dopachrome was measured spectrophotometrically at 450 nm

by a microplate reader. The inhibition of tyrosinase activity was calculated as follows.

$$\text{Inhibition \%} = \frac{(A_{450} \text{ of control} - A_{450} \text{ of sample})}{A_{450} \text{ of control}} \times 100$$

Here, A_{450} of control is the absorbance at 450 nm of a case without the plant extracts or kojic acid, while A_{450} of sample is the absorbance at 450 nm of a case treated with a plant extracts or with kojic acid.

2.2.3 Procollagen type-1 assay

The procollagen type-1 assay was performed with primary human dermal fibroblast (HDF) cells isolated from neonate foreskin. The extracts were dissolved in 2%v/v DMSO in PBS buffer at the following concentrations: 250 and 500 µg/ml (*A. vera*), 7.81 and 15.62 µg/ml (*P. emblica*), and 100 µM (17.6 µg/ml) (L-ascorbic acid, a positive control). The HDF cells were seeded onto 24-well plates at $5x10^4$ cells/well in complete high glucose Dulbecco's Modified Eagle's Medium (DMEM) containing 10% Fetal Bovine Serum, 1% Gluta max, 1% 10 µg/ml penicillin, and 10 µg/ml streptomycin at 37 ℃ with 5% CO_2 in a humidified atmosphere. After that, the cells were exposed to 1 ml of a selected concentrations of extracts or L-ascorbic acid for 48 h. Then, the cell-free supernatant was collected to determine the procollagen type I content. At the same time, 1 ml of new medium was added for replacement prior to further incubation for 24 h without the extracts or positive control. The cell-free supernatant was collected and the amount of procollagen type I was determined by using a commercial human procollagen type-I C-peptide EIA kit (Abcam, USA).

2.2.4 Anti-inflammatory activity assay

The anti-inflammatory activity was determined with RAW 246.7 macrophage cells (ATCC® TIB-71) in a nitric oxide assay. The extracts and methylarginine (positive control) were dissolved in 2%v/v DMSO in PBS buffer at concentrations of 0-62.50 µg/ml. The cells were seeded at approximately 2×10^4 cells/well in 96-well culture plates in complete low glucose DMEM containing 10% Fetal Bovine Serum, 1% 10 µg/ml penicillin, and 10 µg/ml streptomycin at 37 ℃ with 5% CO_2 in a humidified atmosphere. Then, the wells were supplemented with 90 µl of selected concentration of an extract or positive control and 10 µl of LPS (10 µg/ml), and incubated for 24 h. The nitric oxide production was determined using a Griess reagent system (Promega, USA) according to the protocol descripted by the manufacturer.

2.2.5 Determination of anti-glycation activity

The extracts were dissolved in 2%v/v DMSO in PBS buffer at concentrations of 125-10,000 µg/ml (*A. vera*), 3.91-125 µg/ml (*P. emblica*) and 15.62-1,000 µg/ml (rutin hydrated, positive control). Bovine serum albumin (BSA), 10 mg/ml in 50 mM phosphate buffer at pH 7.4 containing 0.02% sodium azide, was pre-incubated with the 50 µl extract solution or rutin hydrate. In control, the extracts were replaced by the buffer in the same volume. After that, 100 µl glucose solution was added to the reaction mixture and incubated at 37ºC for 3 days. The fluorescence intensity (FI) was measured at an excitation wavelength of 350 nm and an emission wavelength of 450 nm by a microplate reader. The results are expressed as 'percentage inhibition' of the advanced glycation end products (AGEs) formed.

$$\text{Inhibition of AGEs formation (\%)} = \frac{(F_c - F_b) - (F_s - F_{sb})}{(F_c - F_b)} \times 100$$

Here, Fc is the fluorescence intensity of incubated BSA, glucose, and 2%v/v DMSO in PBS buffer (control), Fb is the fluorescence intensity of incubated BSA alone (blank), Fs is the fluorescence intensity of the incubated BSA, glucose, and an extract or rutin hydrate (positive control), and Fsb is the fluorescence intensity of incubated BSA with an extract or rutin hydrate (positive control).

3. Results and Discussion

3.1 Extraction

The final output from extracting each plant was sticky resin with a distinct color. The *A. vera* extract was clear yellow, while *A. galanga* and *P. emblica* extracts were brown and dark brown, respectively. These differed from the *C. sativus* extract that was dark green in color. The extraction yields are presented in Table 1. With the chosen extraction methods, the highest yield was obtained from *P. emblica* at 64.89%, followed by *C. sativus* at 36.31%, *A. galanga* at 18.24%, and the lowest yield was from *A. vera* at 10.36%. The extraction yields are related to the polarity of compounds in the plants, since hydrophilic compounds tend to be extracted (dissolved) by highly polar solvents. High yields were mainly obtained on extracting fruits, as was done with *P. emblica* and *C. sativus*.

Table 1. The extraction yields of crude ethanolic extracts from *A. vera*, *C. sativus*, *A. galanga* and *P. Emblica*.

Plant	Part used	Extraction yield (%)
Aloe vera	Leaf (gel)	10.36±2.85
Cucumis sativus	Fruit	36.31±0.34
Alpinia galanga	Rhizome	18.24±1.94
Phyllanthus emblica	Fruit	64.89±2.79

3.2 Anti-oxidant activity

The DPPH anti-oxidant activities of the extracts are expressed as IC_{50} values, and are shown in Table 2. *P. emblica* extract showed the highest antioxidant activity with IC_{50} of 14.10 ± 2.66 µg/ml, followed by the *A. galanga* extract with IC_{50} = 826.00 ± 60.73 µg/ml, *C. sativus* extract with IC_{50} = 4,339.00 ± 1537.96 µg/ml, and *A. vera* extract with IC_{50} = 5,333.00 ± 252.95 µg/ml. As regards the positive control, L-ascorbic acid, its IC_{50} was around 7.47 ± 1.15 µg/ml. The *P. emblica* extract had very strong anti-oxidant activity in comparison to the other extracts. The strong anti-oxidant potential of an extract may be due to tannins, phenolic compounds, and L-ascorbic acid that have been reported in *P. emblica* (Tasduq *et al.*, 2015; Yokozawa, Kim & Kim, 2007). L-ascorbic acid,

Table 2. The antioxidant activities (IC_{50}) of *A. vera*, *C. sativus*, *A. galangal*, and *P. emblica* extracts, and L-ascorbic acid (positive control) in a DPPH assay.

Plant or substance	Antioxidant activity IC_{50} (µg/ml)
Aloe vera	5,333.00 ± 252.95
Cucumis sativus	4,339.00 ± 1537.96
Alpinia galanga	826.00 ± 60.73
Phyllanthus emblica	14.01± 2.66
L-ascorbic acid	7.47 ± 1.15

an essential anti-oxidant, was also previously reported in *P. emblica* at around 3.25-4.50% w/w (Khopde *et al.*, 2001), while the total phenol content was approximately 81-120 mg gallic acid equivalent/g (Liu, Zhao, Wang, Yang, & Jiang, 2008). Due to its powerful anti-oxidant potential, this extract can act as a natural functional ingredient in food supplements and cosmetic products.

3.3 Tyrosinase inhibition assay

The tyrosinase enzyme induces the production of melanin, which leads to hyperpigmentation (Thangboonjit, Limsaeng-u-rai, Pluemsamran & Panich, 2014). In this study, the inhibition of melanin production was determined by anti-tyrosinase activity in B16F1 cells, when treated with the extracts and the positive control, kojic acid. The results reveal that *A. vera* extract and *P. emblica* extract possessed tyrosinase inhibitory activity, whereas *C. sativus* and *A. galanga* extracts did not show such activity in this study. The *A. vera* extract and *P. emblica* extract exhibited dose-dependent inhibition of tyrosinase activity in the cells. Considering the tyrosinase inhibition at the concentration of 500 µg/ml, *A. vera* and *P. emblica* extracts exhibited 26.67±2.39% and 41.92±1.88% respective inhibitions. Kojic acid at the same concentration showed anti-tyrosinase activity of around 94.48±1.06%. This tyrosinase inhibition activity of *A. vera* extract is in agreement with a prior report, in which tyrosinase inhibition increased with dose of the extract (Gupta & Masakapalli, 2013). However, the result for *P. emblica* extract with a prior report of Homklob, Winitchai, Rimkeeree, Luangprasert & Haruthaithanasan (2012), who reported high tyrosinase inhibition of crude ethyl acetate extract of *P. emblica*. This might be due to the different extraction solvents, as well as the assay protocols, since they used a mushroom tyrosinase assay to determine tyrosinase inhibition.

3.4 Effects on the synthesis of procollagen type 1

The effects of the extracts on procollagen type 1 synthesis were studied in a fibroblast cell model. The results are expressed as percentages of pro-collagen type 1 in the test groups versus that in the control group. The result indicate that only *A. vera* and *P. emblica* extracts stimulated procollagen type 1 synthesis. At 48 h, the *A. vera* extract showed concentration dependent activation of procollagen type 1 synthesis since increasing the concentration from 250 to 500 µg/ml increased pro collagen type 1 contents by about 7.3 to 14.4 -fold from that in the control. This differs from *P. emblica* extract, which increased procollagen type 1 content approximately by 9.1 and 7.4 -fold with treatment concentrations 7.81 to 15.62 µg/ml, respectively, without significant mutual difference. Regarding the test concentration, *P. emblica* was more efficient in improving procollagen type 1 synthesis as a low concentration, 7.81 µg/ml elevated the pro-collagen type 1 contents by 9.1 fold. However, this effect is still lesser than that found with L-ascorbic acid at 100 µM (17.6 µg/ml) that can raise the procollagen type 1 content by around 12.3 fold within 48 h (Figure1).

After 48 h, the cells were incubated with fresh medium without an extract for 24h. In this period the pro-collagen type 1 content decreased. In the groups treated with 250 and 500 µg/ml of *A. vera* extract, the amount of pro-collagen type 1 was measured as around 2.9 and 2.1 fold, respectively, whereas using 7.81 and 15.62 µg/ml of *P. emblica* extract, the procollagen type 1 was detected at around 3.3 and 3.6 fold, respectively. On increasing incubation time to 72 h, the procollagen type 1 content decreased due to shortage of the extracts. This was clearly different from the observations at 48 h, in that the amount of procollagen type 1 in the extract treated cells was higher than that found when treated with L-ascorbic acid by 1.1% (Figure 1).

There are reports indicating that aloesin in *A. vera* gel can induce human fibroblast proliferation, and that both aloesin and aloe sterols could promote type 1 collagen synthesis (Takahashi *et al.*, 2009; Tanaka *et al.*, 2015). Moreover, our results also agree with the report of Fujii, Wakaizumi, Ikami & Saito (2008), who suggested that *P. emblica* extract induces prolonged production of procollagen in a concentration and time-dependent manner. The time-dependent production of carboxy-terminal propeptide of procollagen type 1 (PIP) showed that PIP was significantly increased at 48 h although not significantly at 24 h.

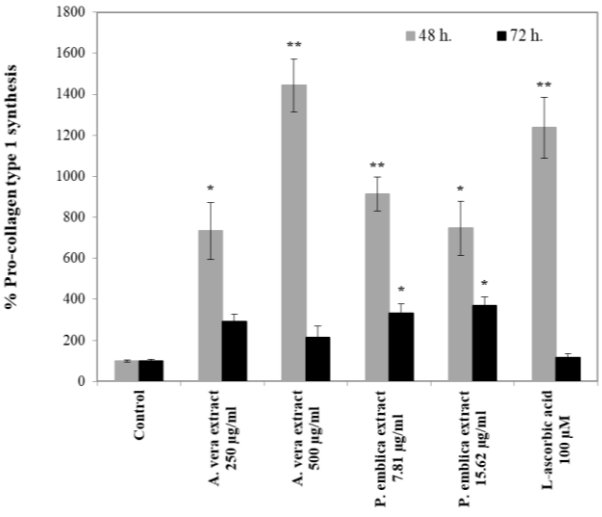

Figure 1. Pro-collagen type 1 synthesis by human dermal fibroblast cells after treatments with *A. vera* extracts at concentrations of 250 µg/ml and 500 µg/ml, *P. emblica* extracts at concentrations of 7.81 µg/ml and 15.62 µg/ml and L-ascorbic acid at a concentration of 100 µM for 48 h, and then in fresh medium without an extract before determination at 72 h. Each bar represents the mean value ± SE of three replicates (n = 3). *p < 0.05, **p< 0.01 when compared with untreated cells (Student's t-test).

3.5 Anti-inflammatory activity

Nitric oxide (NO) in macrophages is an important signaling and effector molecule in inflammation and immunity (MacMicking *et al.*, 1997). Thus, inhibiting excessive production of NO could serve as a criterion to evaluate potential anti-inflammatory compounds (Sarkar *et al.*, 2005). This study investigated inhibition of the production of NO in RAW 246.7 macrophage activated by the endotoxin LPS. The results indicate that the extracts of *C. sativus, A. galanga* and *P. emblica* exhibited nitric oxide inhibition activity. The inhibitory effect increased consistently with the concentration from 3.91 to 62.5 µg/ml for all these extracts (Figure 2). The *P. emblica* extract showed the highest nitric oxide inhibition of 87.99±1.93 % at 62.5 µg/ml. This inhibition level was almost similar to the positive control, methylarginine, while it was higher than those of *A. galanga* and *C. sativus* extracts that exhibited nitric oxide inhibitions of 57.62±0.28% and 36.50±0.79%, respectively.

The methanolic extract of *P. emblica* fruit was previously reported for nitric oxide scavenging activity of around 12.94-70.16% and for anti-inflammatory activity in carrageenan induced paw edema model (Middha *et al.* 2015). Furthermore, nitric oxide scavenging activity of aqueous *C. sativus* fruit extract was concentration-dependent (Kumar *et al.*, 2010), whereas 1-S-1-acetoxychavicol acetate from *A. galanga* was reported to inhibit NO production strongly in a macrophage-like cell line with IC_{50} = 2.3 µM (Morikawa *et al.*, 2005). These are in agreement with our findings, except for the information on *A.vera* extract. In our study, the *A. vera* extract did not show any inhibition of nitric oxide production. The leafy exudate of *A. vera* L. (AVL) has been reported to reduce NO production in macrophages and to inhibit the release of inflammatory inhibitors such as prostaglandins, resulting in suppression of inflammation (Sarkar *et al.*, 2005).

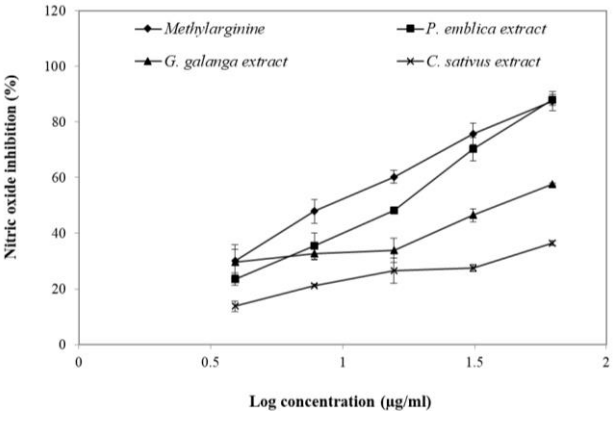

Figure 2. The nitric oxide inhibition by various concentrations of *C. sativus* extract, *A. galanga* extract, *P. emblica* extract, and methyl arginine (positive control). The control group was untreated cells. Each dot represents the mean value ± SD of three replicate determinations (n = 3).

3.6 Anti-glycation activity

Glycation is the covalent bonding of a protein or lipid molecule with a reducing sugar molecule without the controlling action of an enzyme. The final products of this non-enzymatic reaction are advanced glycation end products (AGEs). The presence and accumulation of AGEs have been etiologically implicated in aging (Tanaviyutpakdee, 2015). *In vitro* anti-glycation activity of the selected plant extracts using BSA-Glucose assay showed that only two extracts, of *A. vera* and *P. emblica*, possessed anti-glycation activity. The *A. vera* extract had concentration dependent activity in the range of 125-10,000 µg/ml (Figure 3A).

P. emblica extract was distinctive having activity at the lowest concentration, 3.91 µg/ml, of 20.3% while the 95.5% maximum activity was found at 125 µg/ml (Figure 3B). Comparing the 125 µg/ml concentrations of all extracts and the positive control, the highest activity was found for *P. emblica* extract, 95.5%, followed by rutin hydrate, 47.9%, (Figure 3C) and *A. vera* extract with 15.2%.

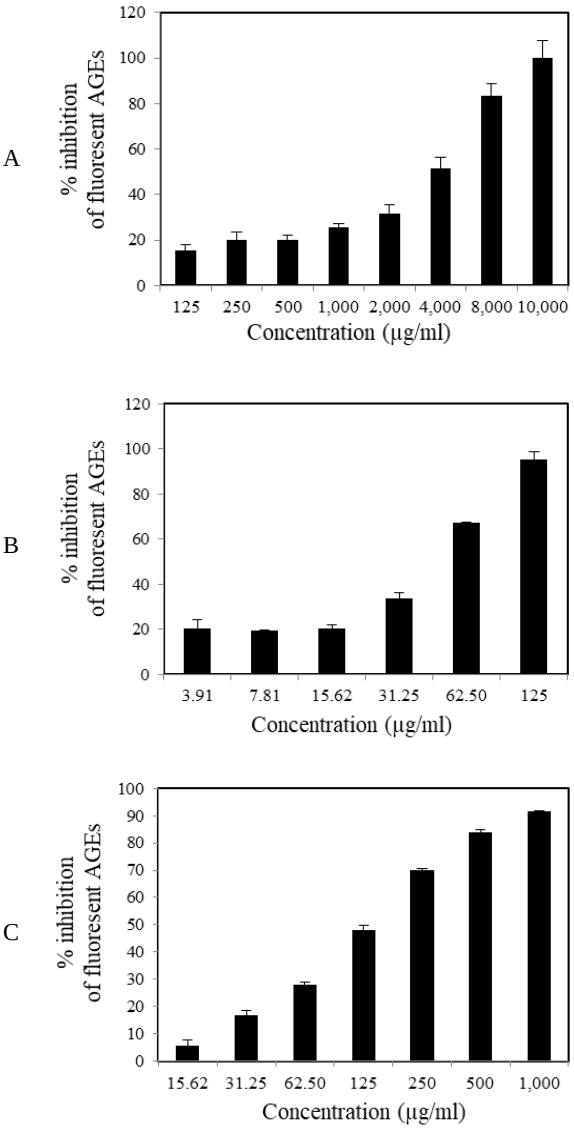

Figure 3. The inhibition of AGEs formation by (A) *A. vera* extract, 125-10,000 µg/ml, (B) *P. emblica* extract, 3.91-125 µg/ml, and (C) rutin hydrate, 15.625-1,000 µg/ml. Each bar represents the mean value ± SD of three replicate determinations (n = 3).

The high activity of *P. emblica* extract might be influenced by compounds such as rutin, gallate, pyrogallate and catechin persisting in the extracts. From previous reports, the anti-glycation activity of *P. emblica* extract was positively correlated with the total content of phenolics and flavonoids (Gkogkolou, 2012; Han *et al.*, 2015, Kusirisin *et al.*, 2009). Also, amino acids in *A. vera*, especially lysine, may participate in anti-glycation activity (Sulochana *et al.*, 2003).

4. Conclusions

The present work indicates that each tested plant that was recommended for an individual body element of Traditional Thai Medicine possessed some biological activities that might affect skin properties. *C. sativus* (for fire element -type people) and *A. galanga* extracts (for air element -type people) had anti-inflammatory and anti-oxidant activities. *A. vera* extract (for water element -type people) was abundant in anti-oxidant, anti-tyrosinase, and anti-glycation activities, and increased collagen synthesis. *P. emblica* extract (for earth element -type people) showed anti-oxidant, anti-tyrosinase, anti-inflammatory, and anti-glycation activities, and collagen synthesis enhancement. This information might be used in food or skin care product development, such that relates to specify body elements in traditional Thai wisdom.

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