

# Anti-aging potential and phytochemicals of *Centella asiatica*, *Nelumbo nucifera*, and *Hibiscus sabdariffa* extracts

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## ABSTRACT

*Centella asiatica*, *Nelumbo nucifera* Gaertn, and *Hibiscus sabdariffa* have been used as medicinal plants in Thailand. They are sources of phytochemicals that applications for esthetic and healthcare. The aim of this research was to examine the phytochemical constituents and anti-aging potential of these plants. The phytochemical compounds were performed using gas chromatography-mass spectrometry. The anti-aging activities were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), anti-collagenase, and anti-elastase assays. The main interest phytochemical compounds of ethanolic extracts of *C. asiatica*, *N. nucifera*, *H. sabdariffa* were ethanol, 2-(Octadecenyloxy),  $\gamma$ -sitosterol and hexadecanoic acid, and ethyl ester, respectively. The DPPH half-maximal inhibitory concentration (IC<sub>50</sub>) results of *C. asiatica*, *N. nucifera*, and *H. sabdariffa* were  $0.32 \pm 0.01$ ,  $0.34 \pm 0.00$ , and  $0.35 \pm 0.01$  mg/mL, respectively. The ABTS result of *H. sabdariffa* extract showed high inhibitory activity at IC<sub>50</sub> of the extract was  $0.62 \pm 0.12$  mg/mL. The percentage of collagenase inhibition of *C. asiatica*, *N. nucifera*, and *H. sabdariffa* at 1.0 mg/mL was  $78.13 \pm 4.42$ ,  $85.94 \pm 2.21$ , and  $90.63 \pm 0.00$ , respectively. The *C. asiatica* extract had a high percentage of elastase inhibition. Consequently, these research results suggest that phytochemicals may also provide a range of esthetic and health benefits. The phytochemical constituent could be used as anti-aging active ingredient for cosmetic and pharmaceutical industrials.

**Key words:** Anti-aging, *Centella asiatica*, *Hibiscus sabdariffa*, *Nelumbo nucifera*, phytochemical

## INTRODUCTION

The world cosmetics market is estimated that in 2020 the cosmetics market grew to 335.6 billion US dollars<sup>[1]</sup>

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Nowadays, the data showed that the product has the ability to have antioxidant and anti-aging properties are growing trend.<sup>[2]</sup> The medicinal plants are enriched sources of antioxidants properties.<sup>[3,4]</sup> *Centella asiatica* L. is shown high polyphenol and triterpenes such as asiaticoside, madecassoside, and asiatic acid, it was also claimed to be useful in wound healing properties.<sup>[5-7]</sup> Many parts of *Nelumbo nucifera* has various biological components<sup>[8]</sup> It exhibits several pharmacological effects contain antioxidant and astringent properties.<sup>[9]</sup> *Hibiscus sabdariffa* L., the

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calyces extract, is the high content of anthocyanins,<sup>[10]</sup> which presents compounds with anti-inflammatory properties.<sup>[11]</sup> All of the above has shown that Thai edible plants are a potential source of therapeutic bioactive. This research aims to study the phytochemical compositions and bioactive activities of these plants. The results can provide a useful reference for use as anti-aging active ingredients for cosmetic and pharmaceutical industrials.

## MATERIALS AND METHODS

### Plant material

*C. asiatica* aerial part, *H. sabdariffa* flower, and *N. nucifera* petal were purchased from Thai Herbal Pharmacy Shop in Pathumthani, Thailand.

### Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ascorbic acid, *Clostridium histolyticum* collagenase type I, N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA), *porcine pancreas* elastase type I, N-Succinyl-Ala-Ala-Ala-p-nitroanilide (AAPVN), epigallocatechin gallate (EGCG), trizma® base, and potassium persulfate were purchased from Sigma-Aldrich; Merck for dimethyl sulfoxide.

### Extraction

All samples were washed with water, ground into powder, and then dehydrated by hot air oven at 50°C. The solvent extracts were prepared by adding 100 g of each sample powder to 1 L of 95% ethanol (1:10 w/v), applying ultrasound-assisted extraction as frequency 40 kHz at 40°C for 30 min then maceration until 7 days, therefore, using a rotary evaporator to remove the solvent and collected crude extract in -20°C consequently the percentage yield calculate.<sup>[12]</sup>

$$\% \text{ yield} = \frac{\text{weight of the plant extract}}{\text{weight of dried plant sample used}} \times 100$$

### Gas chromatography–mass spectrometry analysis

The phytochemical constituent present in the ethanolic extracts of *C. asiatica*, *N. nucifera* and *H. sabdariffa* were determined by gas chromatography–mass spectrometry equipped with HP5MS capillary column (30 m × 250 μm × 0.25 μm). The 2 μL of sample volume for injection. In the assay conditions, carrier gas was used ultra-high purity helium and flow rate 1.2 mL/min, split ratio 20:1, thus the inlet temperature at 250°C; consequently, MS programs scanned a quality range of 40–550 amu, solvent delay 3 min, transfer line 280°C.<sup>[13]</sup> Finally, the identification of the compounds by comparing them with the database of the US NIST mass spectra library.

### 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay

The DPPH assay of the extract was modified method as previously described.<sup>[14]</sup> The 0.2 mM DPPH solution was

prepared in absolute ethanol. The solution was added with 100 μL of the tested samples and DPPH solutions. The reaction was determined at wavelength 520 nm after incubated in the darkroom for 30 min; the percent inhibition of radical scavenging activity was calculated as follows Eq. (1):

$$\% \text{ inhibition} = \frac{A_{(\text{control})} - A_{(\text{sample})}}{A_{(\text{control})}} \times 100 \quad (1)$$

Moreover, the result was expressed as IC<sub>50</sub> value.

### 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging assay

The ABTS scavenging assay was measured following the modified method by Kim *et al.*<sup>[15]</sup> The ABTS reagent was mixed with 7 mM ABTS and 2.45 mM potassium persulfate (8:12 v/v ratio) and incubated in the darkroom temperature for 16 h. The working solution was diluted to 0.70 ± 0.085 OD at 750 nm with water. In addition of 180 μL of the working ABTS solution was mixed with 20 μL of the test sample, the mixture was incubated at room temperature for 30 min, and then absorbance was measurement at 750 nm. The percentage of ABTS radical scavenging was calculated as: (1). Moreover, the result was expressed as an IC<sub>50</sub> value.

### Anti-collagenase activity

The collagenase activity was modified method by Liyanaarachchi *et al.*<sup>[16]</sup> The reaction mixture contained 20 μL samples and 20 μL enzyme solution (0.8 units/mL dissolved in 50 mM Tricine buffer solution, pH 7.5 which was incubated at 37°C for 10 min before use) was incubated for 10 min after adding 200 μL FALGPA substrate (0.5 mM dissolved in buffer) the absorbance was immediately determined at 340 nm at the time interval of 0 and 1 min. Finally, enzymatic activity was assessed by decreased kinetic absorbance which the percentage of collagenase inhibition was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Activity}_{(\text{control})} - \text{Activity}_{(\text{inhibitor})}}{\text{Activity}_{(\text{control})}} \times 100 \quad (2)$$

### Anti-elastase activity

The elastase inhibition assay was a method modified by Panjapa and Bungorn.<sup>[17]</sup> Elastase 2 Units/mL dissolved in 1 M Tris-HCL buffer (pH 8) then incubated at 37°C for 10 min and the substrate AAPVN was also dissolved in buffer at 1.6 mM. Briefly, the mixture of 40 μL test extracts (mg/mL) and 20 μL enzyme solutions was incubated for 10 min; therefore, add substrate solution 540 μL. Using EGCG as standard, the absorbance was immediately determined at 410 nm at time interval of 0 and 5 min. In the end, the elastase inhibitory effect of test extracts was expressed by the following Eq. (2).

### Statistical analysis

The data were showed in triplicate as means ± standard deviation. The statistically significant was analyzed using

one-way ANOVA at  $P < 0.05$ . Moreover, statistical analysis was performed with GraphPad Prism version 5.01 (San Diego, CA, USA).

## RESULTS AND DISCUSSION

### Extract yield

The percentage ethanolic extract yield of *C. asiatica*, *N. nucifera*, and *H. sabdariffa* were presented 11.53, 13.28, and 27.50, respectively. The differences in percent yield from the sample plants in the present analysis may be determined the availability of different extracted components, which is a result of the chemical composition of various plants.<sup>[18]</sup>

### Gas chromatography–mass spectrometry analysis

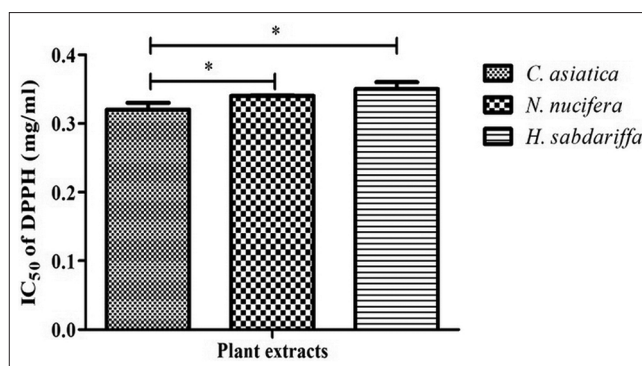
The chromatographic ion spectrum of *C. asiatica* contained 10 peaks indicating the presence of 7 chemical constituents. Nevertheless, Ethanol, 2-(9-octadecenyloxy)-, (Z)-giving the highest peak. *N. nucifera* were classified 30 peaks signify of 25 components by means of the results exhibited that the capacity of  $\gamma$ -Sitosterol in *N. nucifera* was the maximum while the substance Ethanol 2-(9 Octadecenyloxy)-(Z)-is found similarly in *C. asiatica*, the spectrum was described 18 peaks and 15 bioactive constituents, in the present investigation, a variety of compounds have been detected in *H. sabdariffa* that Hexadecanoic acid shows the maximum peak. Three different extracts show that Ethanol, 2-(Octadecenyloxy),  $\gamma$ -Sitosterol and Hexadecanoic acid, ethyl ether are representative of the main interest. Especially, Ethanol, 2-(Octadecenyloxy) is biochemical compounds because it has a good application for pharmacological actions as anti-cancer.<sup>[19]</sup> The  $\gamma$ -Sitosterol has been reported from soya which have the benefit of anti-diabetic activity.<sup>[20]</sup> And finally, the extracts of *H. sabdariffa* showed the presence of Hexadecanoic acid, ethyl ester which has the antioxidant and hemolytic 5-alpha reductase inhibitor feature.<sup>[21]</sup> Consequently, these compositions may be use as ingredients for the cosmetic and pharmaceutical industrials.

### 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity

The scavenging activity of *C. asiatica*, *N. nucifera* and *H. sabdariffa* extracts against DPPH [Figure 1]. The results exhibited that  $IC_{50}$  values are  $0.32 \pm 0.01$ ,  $0.34 \pm 0.00$  and  $0.35 \pm 0.01$  mg/mL, respectively, and standard ascorbic acid in this assay was  $0.005 \pm 0.00$  when comparing the antioxidant activity of all plants, the result shown that the extract of *C. asiatica* is high significantly effect ( $P < 0.05$ ). Corresponding to this result, reported the ethanolic extract of *C. asiatica* contains the highest phenolic content, which presented the great value of antioxidant more than hexane and chloroform extracts.<sup>[5]</sup>

### 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity

The ABST scavenging activity of *C. asiatica*, *N. nucifera* and



**Figure 1:** Antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl method, the tested samples show a significant difference in *Centella asiatica* and *Hibiscus sabdariffa* similar to *Centella asiatica* and *Nelumbo nucifera* at  $P < 0.05$  (\*)

*H. sabdariffa* extracts inhibit ABTS [Figure 2]. The results showed that  $IC_{50}$  values are  $0.71 \pm 0.02$ ,  $0.93 \pm 0.06$  and  $0.62 \pm 0.12$  mg/mL, respectively, and ascorbic acid were  $0.033 \pm 0.001$ , after assessing the antioxidant activity of all plants, the result shown that the extract of *H. sabdariffa* significantly ( $P < 0.05$ ) is the finest effect. This result agrees with this reported that ethanolic extract of *H. sabdariffa* had great antioxidant activity.<sup>[22,23]</sup> Although the effect of antioxidant tests on the DPPH and ABTS methods of the three plants will provide different results. In order that it may be due to the rapidity electron transfer during reactions and the phytochemical composition of plants with different antioxidant effects or from other factors, for example, light protection, reaction quality, cleaning, etc.

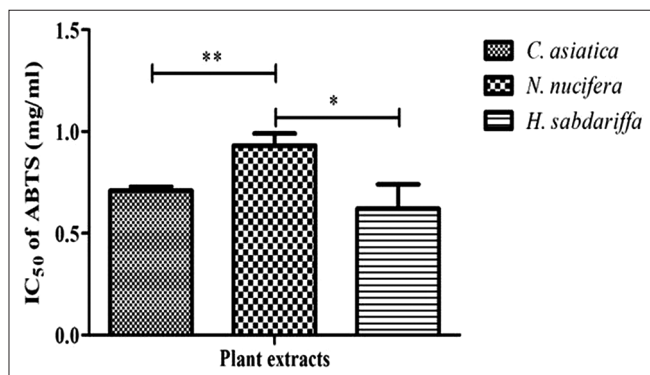
### Anti-collagenase activity

The ethanolic extract of *H. sabdariffa* has an excellent collagenase inhibition with a percentage of  $90.63 \pm 0.00$ . *N. nucifera* and *C. asiatica* also showed anti-collagenase activity with the percentage of  $85.94 \pm 2.21$  and  $78.13 \pm 4.42$ , respectively. The results are presented in Figure 3. The EGCG was used as standard, and it showed lower activity than all plants at  $71.88 \pm 0.00$ . Nevertheless, the effect of collagenase inhibition of ethanolic extracts might involve to different mechanisms. It has been reported that phytochemical compositions are mainly responsible for collagenase inhibition. Besides, collagen is an ample structural protein and extracellular matrix components, which are degraded by collagenase that is a zinc-containing multi-domain enzyme.<sup>[24]</sup> In conclusion, collagen fibers will decrease with age and damage from UV rays that cause wrinkles and sagging. Consequently, all of these extracts have possible uses as anti-aging ingredients in health products like dietary supplements or cosmetics.

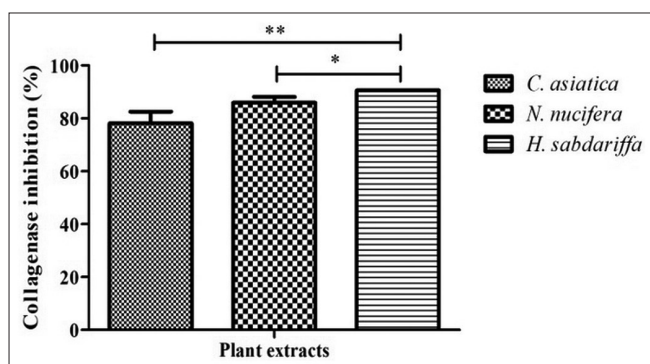
### Anti-elastase activity

The anti-elastase activity assay performed by taking EGCG as a standard, showed that *C. asiatica* with the great elastase inhibition of these plants that percentage

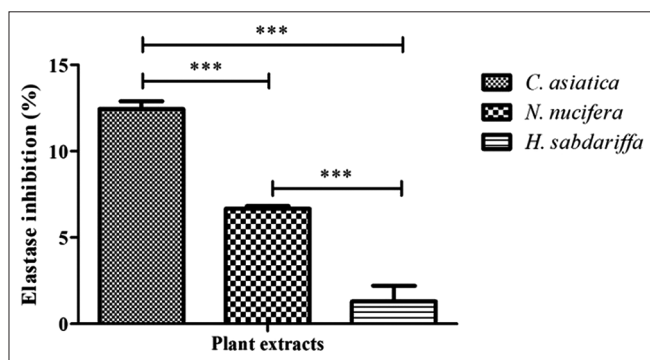
of  $12.45 \pm 0.44$ . *N. nucifera* and *H. sabdariffa* also presented anti-elastase activity with a percentage of  $6.67 \pm 0.15$  and  $1.29 \pm 0.91$ , respectively. The results are presented in Figure 4. However, their inhibitions were lower than the standard EGCG, with the percentage of  $95.89 \pm 0.22$ . Elastin is a 2%–4% insoluble fiber protein found in the dermis



**Figure 2:** Antioxidant activity determined by 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) method, the tested samples show a significant difference in *Centella asiatica* and *Nelumbo nucifera* at  $P < 0.01$  (\*\*) during *Nelumbo nucifera* and *Hibiscus sabdariffa* at  $P < 0.05$  (\*)



**Figure 3:** Anti-collagenase of three extracts, the tested-samples show a significant difference in *Centella asiatica* and *Hibiscus sabdariffa* at  $P < 0.01$  (\*\*) while *Nelumbo nucifera* and *Hibiscus sabdariffa* at  $P < 0.05$  (\*)



**Figure 4:** Anti-elastase of three extracts, the test sample shows a significant difference in *Centella asiatica* and *Nelumbo nucifera* at  $P < 0.001$  (\*\*\*) while *Nelumbo nucifera* and *Hibiscus sabdariffa* at  $P < 0.001$  (\*\*\*)

matrix, which plays an important role in skin elasticity. In contrast, it is often degraded by elastase enzyme, which can be hydrolyzed on the peripheral proteins and structures in connective tissues.<sup>[13]</sup> Although, the experimental results show a low elastase inhibition rate ( $12.45 \pm 0.44$  mg/ml) when compared to the standard. However, there are still observations that medicinal plants are lower price than synthetic chemicals sold in the market. It also gives a feeling of safety for customers because it is a natural ingredient. Therefore, the results suggested that *C. asiatica* can be used as a raw material to resist the enzyme elastase but may need to be used at higher concentrations for improved qualifications.

## CONCLUSION

Our results indicate the various bioactive contents of *C. asiatica*, *N. nucifera*, and *H. sabdariffa* ethanolic extracts have an interest function for pharmacological actions such as antioxidant capability due to its good ability to resist DPPH and ABTS free radical scavenging activities ( $IC_{50} < 0.93$ ) and total extract are great capable of inhibiting collagenase (inhibiting collagenase more than 78.13%), but elastase is poorly inhibited especially from *H. sabdariffa*. Consequently, these edible plants can use as an anti-aging active ingredient for cosmetic and pharmaceutical industrials.

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## Conflicts of interest

There are no conflicts of interest.

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