Volatile constituents, in vitro and in silico anti-hyaluronidase activity of the essential oil from Gardenia carinata Wall. ex Roxb. flowers

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ABSTRACT

Fresh flowers of Gardenia carinata Wall. ex Roxb. were collected from Nonthaburi, Thailand. These plant materials were extracted by steam distillation extraction. The results showed that the percentage of essential oil from steam distillation was 0.16. The extracts obtained were subjected to gas chromatography/mass spectrometry for the identification of volatile constituents. The essential oil extracted by steam distillation was characterized by the presence of trans-geraniol (1, 19.9 %) and farnesol (2, 13.2 %) as the main component. The essential oil from flowers of G. carinata and two main components were evaluated for in vitro hyaluronidase inhibitory activity using fluorometric method and compared to a reference hyaluronidase inhibitor (6-O-palmitoylascorbic acid). The results were indicated that essential oil gave the mild inhibitory activity on hyaluronidase with IC50 of 1200.4 ± 21.1 μg/mL as opposed to 6-O-palmitoylascorbic acid (IC50 =186.1 ± 3.9 μg/mL). In the case of two main components, trans-geraniol (1) and farnesol (2) displayed moderate hyaluronidase inhibition activity with IC50 value at 535.7 ± 42.2 and 292.9 ± 23.4 μg/mL, respectively. Additionally, in silico docking study of main component studies exhibited several important interactions between 1 and 2 and hyaluronidase binding site. Above finding confirmed the anti-hyaluronidase potential of G. carinata flowers.

Keywords: Gardenia carinata; essential oil; volatile constituents; anti-hyaluronidase; molecular docking;

1. INTRODUCTION

Nowadays, increased attention in the fight against aging skin has occurred in an increase in the number of cosmetics globally, and focused on anti-aging active ingredients such as antioxidants, vitamins, and bioactive compounds, restoring skin elasticity and protecting skin wrinkles [1]. Skin wrinkles are also associated with skin moisture loss [2]. Hyaluronic or hyaluronic acid (HA) is one of the crucial molecules involved in retaining skin moisture [3]. Hyaluronic acid (HA) is a glycosaminoglycan, a natural substance that is present in the body, and generally found in the area of the knee joint and, the skin cells [4]. This hardworking molecule plays an important role in lubricating our joints, and increasing the flexibility and moisture to the skin. However, the enzyme hyaluronidase (HYAL) can degrade HA. As a consequence, loss of hyaluronic acid leads to loss of elasticity and water retention capacity of skin which further leads to skin wrinkling and aging [5]. Thus, inhibition of HAYL is therefore obliged in the prevention of aging. Hyaluronidase inhibitor not only plays a role in maintaining the youthful appearance of the skin but also wound healing and tissue regenerating [6] anti-allergic [7], anti-inflammatory [8], prevent spreading of HIV and other illnesses that are sexually transmitted [9], and even anti-tumor agent [10]. Nowadays, plants are known to contain numerous bioactive compounds that could potentially hyaluronidase inhibition activity [11].

Plants of the genus Gardenia (Rubiaceae), which comprises more than 80 species, are widely distributed among the tropical forests of the world. Gardenia carinata Wall. ex Roxb is the member of Gardenia genus, commonly known as Pud nam bith in Thailand. G. carinata is a small deciduous ornamental tree. It is distributing in tropical and subtropical of Africa, Australia, Southern Asia, and Southeast Asian. G. carinata is a popular ornamental plant with yellow and sweetly odorous flowers. The flowers are about 12 cm. in diameter and open white then flowers slowly turn orange over a several days period before falling off [12]. The various pharmacological actions of these chemical constituents have been reported, including DNA topoisomerase IIα inhibitory activity, anti-HIV-1 activity [13] and larvicidal activity [14]. Notwithstanding, the anti-aging activity of essential oil from flower of this plant is still unknown. Considering the importance of the medicinal plant growth, the need of the day as to promote more and better-organized studies, in this research, the chemical components of essential oil from flowers of G. carinata are described. The essential oil was obtained by steam-distillation and analyzed using gas chromatography/mass spectrometry (GC/MS). The in vitro anti-hyaluronidase activity of the essential oil and main components were determined by hyaluronidase fluorometric inhibition assay. The anti-hyaluronidase potential of the oil was compared with known hyaluronidase inhibitor, 6-O-palmitoylascorbic acid (Asc6Plm). Molecular docking study was...
2. MATERIALS AND METHODS

2.1. Plant materials and chemicals.

Flowers of *G. carinata* were collected in from Nonthaburi province, located at the Central of Thailand (latitude: 13.8083923 longitude:100.3580321), in March 2019. trans-Geraniol and farnesol were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Hyaluronidase from bovine testes, Asc6Plm and *p*-dimethyl amino benzaldehyde (PDMAB) were supplied from Sigma-Aldrich Corp (USA). Hyaluronic acid sodium salt was purchased from Alfa Aesar (USA). All solvents and chemicals were of analytical grade.

2.2. Steam distillation.

The fresh flowering petal (30 g) was pulverized and placed in a round bottom flask with 500 mL of deionized water and then extracted by steam distillation using the Clevenger-type apparatus for 1.5 h [15]. At the end of the process water and oil were separated by extracted with dichloromethane (3×15 mL). The organic phase was separated. Anhydrous sodium sulfate was used to dehydrate the excess moisture. Next, the dried organic phase was filtered and then concentrated by a rotary evaporator Buchi® model R-114 under vacuum at 45°C for evaporating the solvent as much as possible. The pale-yellow oil was obtained and has a boiled *G. carinata* odor. The volatile oil was transferred to an amber vial with a Pasteur pipette. Oil yield was calculated by the weight of the solvent-free crude and dividing by the weight of the initial dry weight and multiplying by 100. The volatile oil was filled in a glass amber vial then capped under nitrogen and stored at 4°C for further investigation. The following equation was applied to determine the yield of essential oil.

\[
\text{Essential oil yield (\%) = } \frac{W_1}{W_2} \times 100
\]

Whereas: \( W_1 \) is the net weight of oil (grams), \( W_2 \) refer to the total weight of fresh petal (grams).

2.3. Gas chromatography–mass spectrometry (GC/MS).

The essential oil was dissolved in ethyl acetate before the chromatographic determination of their compositions. GC/MS analysis was carried out with an Agilent GC/MS (Agilent 7890A GC-7000 Mass Triple Quad) The one microliter of the extract was injected into a Agilent J&W DB-WAX (60 m × 0.25 mm i.d. × 0.25 μm film thickness) fused silica capillary column (J&W Scientific, Folsom, CA). Temperatures for injector was set at 250 °C, and split mode was applied to the ratio of 20:1. The flow rate of helium gas (carrier gas) was set to 1.0 mL/min. The follow oven temperature program was applied: column temperature was maintained at 30°C for 5 min, then increase the temperature with the rate of 5°C per min to 240 °C, and maintained at 240 °C for 20 min. The mass spectrometer was used in the electron ionization mode with the ion source temperature set at 250 °C, and the ionization energy set at 70 eV. The scan mode was used, and the scan range was 30 to 400 m/z. The data analysis was performed with MassHunter software. Identification of volatile compound was performed by comparing mass spectra with NIST mass spectral libraries (National Institute of Standards and Technology, 2011 version). The peak areas were used to calculate the content of volatile constituents.

2.4. Evaluation of in vitro anti-hyaluronidase activities.

The inhibition activity of the samples for hyaluronidase was analyzed by the previously published procedure [16] with slight modifications. 50 μL HYAL (400-1000 units/mg dissolved in 0.1 M acetate buffer, pH 3.5) was mixed with 50 μL of various concentrations of essential oil (concentration range of 500 to 2500 μg/mL) and commercially available, compound 1 and 2 (concentration range of 25 μg/mL to 1000 μg/mL). The mixture was then incubated at 37°C for 20 min. The assay was begun by adding 50 μL of 12.5 mM calcium chloride to the mixture and then incubated at 37 °C for 20 mins. Next, 250 μL of HA was added to the reaction mixture and then heated in the water bath at 100 °C, for 3 minutes. After the boiling period, allow the reaction mixture to cool at room temperature, then 1.5 mL of PDMAB was added to the reaction after that it was then incubated at 37 °C for 20 mins. The 200 μL of contents was transferred to respective a 96-well clear flat bottom microplate (Corning, Inc.). Fluorescence was detected using a VICTOR Nivo multimode microplate reader (PerkinElmer Inc.) at 545 nm excitation and 612 nm emission. Asc6Plm, the known hyaluronidase inhibitor [8] at a concentration range of 50 to 500 μg/mL was prepared and used as the positive control. The tested sample was analyzed in triplicate. The inhibition percentage was calculated using the hereinafter equation.

\[
\text{Inhibition (\%) = } \left( \frac{F_{\text{control}} - F_{\text{sample}}}{F_{\text{control}}} \right) \times 100
\]

whereas: \( F_{\text{control}} \) refers to the fluorescence intensity at 612 nm of buffer, hyaluronidase + solvent. \( F_{\text{sample}} \) means the fluorescence intensity at 612 nm of different concentrations of tested.

2.5. Statistical analysis.

IC<sub>50</sub> was expressed as mean±standard deviation of separate groups for determinations in triplicates.

2.6. In silico Molecular Docking Studies.

Investigation of the mechanism of action and molecular interaction of two main components, trans-geraniol (1) and farnesol (2) with HYAL were performed by molecular docking simulation using AutoDock software (ver. 4.2, USA). Molecular docking studies explained interactions between enzymes and ligands by a computer program that determined the interaction from the three-dimensional structure. The 2D chemical structure of compounds 1, 2 and Asc6Plm was created using ChemSketch program (ver. 11.0, Canada) followed by 3D structure conversion and the molecular geometry was further optimized by Gaussian09 (USA) using Hartree Fork 3-21G basis set. The crystal structure of Human Hyaluronidase 1 (HYAL, code ID: 2PE4) was achieved from protein data bank [17]. The grid boxes at the center of the binding site of the enzyme were defined. The center of the grid box was assigned at \( x = 55.813, y = -28.162 \) and \( z = 6.854 \). The dimensions of the active site box were set at 100 Å × 100 Å × 100 Å. The number of runs for each docking experiment was set to 100. The subsequent parameters were adopted for docking simulation experiment as follows: population size of 150 individuals, 2.5 million energy evaluations, while other docking parameters were set to default values. The docked complexes were

performed by Autodock 4.2 to investigate the binding mode of interaction.
3. RESULTS

3.1. Chemical composition of the G. carinata essential oil.

The essential oil of the flower of G. carinata obtained from steam distillation was pale yellow colorless oil and possessed an aromatic odor. It gave an average yield of 0.16% on a dry weight basis. The essential oil compositions of G. carinata were determined by GC-MS. By comparing the retention time and mass spectra of authentic samples with standard library NIST (2011), the volatile constituents were interpreted. The chromatogram from the fresh flower of G. carinata is shown in Figure 1. The percentage of chemical components of the flower of G. carinata is shown in Table 1. Nineteen volatiles were identified in the sample of G. carinata flower by GC/MS. The main components of the essential oil from the steam distillation were monoterpene alcohol trans-geraniol (1, 19.9%) and sesquiterpene alcohol, farnesol (2, 13.2%). trans-Geraniol (1) is clear to a pale-yellow oil. trans-Geraniol (1) is produced from several species’ flowers and is found in many herbal vegetative tissues and often coexists with geraniol and nerol [19]. Farnesol (2) is classified as acyclic sesquiterpene alcohol. It is a colorless liquid. A chemo-prevention, anti-tumor [20] and also an anti-bacterial agent of farnesol (2) have been reported [21]. Farnesol (2) is applied as a deodorant in personal care products due to its anti-bacterial activity [22].

![Figure 1. GC-MS spectrum of essential oil obtained from G. carinata and Structure of trans-geraniol (1) and farnesol (2)](image)

Table 1. Chemical composition of G. carinata flowers essential oil

| Peak no. *a | Retention time, (min) | Peak area (%) | Possible compounds | Molecular formula |
|-------------|----------------------|--------------|--------------------|-------------------|
| 1           | 26.93                | 2.6          | acetic acid        | C₂H₄O₂            |
| 2           | 29.37                | 1.6          | β-linalool         | C₀H₁₀O₂           |
| 3           | 29.43                | 0.6          | cis-α,α,4,5-tetramethyl-1-cyclopentene-1-methanol | C₀H₁₈O₂ |
| 4           | 32.47                | 5.5          | 2-chlorocyclohexanol | C₆H₁₃ClO        |
| 5           | 33.81                | 6.9          | 2,6,6-trimethyl-2-cyclohexene-1-methanol | C₁₀H₁₆O₂ |
| 6           | 36.47                | 19.9         | trans-geraniol    | C₁₀H₁₈O₂ |
| 7           | 38.06                | 1.0          | phenylethyl alcohol | C₆H₁₀O₂       |
| 8           | 40.273               | 1.7          | myristaldehyde    | C₁₃H₂₆O₂         |
| 9           | 41.48                | 10.8         | Heneicosane       | C₁₉H₃₈O₂         |
| 10          | 45.08                | 8.0          | jasmine lactone   | C₁₉H₃₂O₂         |
| 11          | 45.73                | 2.2          | (Z)-9,17-octadecadienal | C₁₉H₃₀O₂ |
| 12          | 46.08                | 2.1          | 4-(2-propenyl)phenol | C₁₉H₂₀O₂ |
| 13          | 46.31                | 13.2         | farnesol          | C₁₉H₂₆O₂         |
| 14          | 46.95                | 8.0          | methyl linolenate | C₁₉H₂₆O₃         |
| 15          | 47.99                | 1.5          | trans-p-mentha-2,8-dienol | C₁₀H₁₆O₂ |
| 16          | 49.40                | 8.2          | 2-allylphenol      | C₁₀H₁₈O     |
| 17          | 50.63                | 1.8          | vanillin          | C₆H₁₀O₃         |
| 18          | 58.64                | 0.6          | geranylgeraniol   | C₂₀H₃₂O₂         |
| 19          | 60.26                | 3.6          | palmitic acid     | C₁₆H₃₂O₂         |
| total       |                      | 100          |                    |                   |

*a Peak number correspond to those indicated in Figure 1.*
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3.2. In vitro anti-hyaluronidase activity evaluation.

In order to know the anti-aging activity of the essential oil and commercially available two main components (compound 1 and 2) of G. carinata, in vitro HYAL inhibition test was performed. The biological activity profiles were assayed in comparison with Asc6Plm as the reference compound using fluorometric method. The IC₅₀ value of each test was determined by using the calibration curve, which gave a linear regression equation (R² > 0.9000), and the results are tabulated in Table 2. The study revealed that the essential oil was mild inhibitors of
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hyaluronidase with the IC\textsubscript{50} value of 1200.4 ± 19.1 μg/mL. Compounds 1 and 2 possess appreciable anti-hyaluronidase activity with IC\textsubscript{50} values of 535.7 ± 39.2 and 292.9 ± 13.4 μg/mL, respectively. In the case of the standard positive control, Asc6Plm showed an IC\textsubscript{50} of 186.1 ± 3.9 μg/mL. However, essential oil, compound 1 and 2 showed inhibitory activity but still lower than that of Asc6Plm. Several studies also reported the essential oil from plant exhibited high anti-hyaluronidase activity [23, 24]. To our knowledge, the rejuvenation effect essential oil of *G. carinata* on anti-hyaluronidase activity is reported here for the first time.

Table 2. Anti-hyaluronidase activity of essential oil, 1 and 2.

| Sample      | IC\textsubscript{50}SD, μg/mL |
|-------------|-----------------------------|
| Essential oil | 1200.4 ± 21.1               |
| trans-Geraniol (1) | 535.7 ± 42.2               |
| Farnesol (2)     | 292.9 ± 23.4               |
| Asc6Plm\*     | 186.1 ± 3.9                |

Values are mean±SD (n=3): SD: Standard deviation, *standard positive control.*

3.3. In silico Molecular Docking Study.

To explain the interactions of the 2 main constituents (trans-geraniol (1) and farnesol (2)) of essential oil obtained from *G. carinata* in the active site of HYAL (2PE4) in comparison with Asc6Plm as the reference compound, molecular modeling studies were performed using Autodock (4.2). The details of docking study were listed in Table 3. Figure 2 gave the views of the interaction between hyaluronidase and major compositions. Molecular docking of ligands against the binding site of the enzyme will elucidate the interactions between them. This paves the way for the discovery and develops of new phytomedicines. The enzyme’s binding site was docked with selected compounds from *G. carinata*. Visual inspection of binding modes of tested molecules within the binding site of HYAL was done to identify the common binding orientation and possible interactions. The result reveals that the conformation of Asc6Plm in HYAL is similar to the docking pose of farnesol (2) in HYAL. Whereas, the different orientation of trans-geraniol (1) in the binding site of HYAL was observed, as presented in Figure 2. The binding interactions Asc6Plm into the binding site of HYA (2PE4) modeled protein resulted in an energy conformation of −8.70 kcal/mol. Compound 1 revealed the binding free energy of -4.71 kcal/mol while compound 2 exhibited -5.40 kcal/mol. The interactions of the HYAL and Asc6Plm proposed in this study were useful to understand the potential mechanisms of the interaction between HYAL and Asc6Plm. The residues of ASN350, LEU379, LEU381, LEU382, ASN383 and GLU415 were important for strong hydrogen bonding interaction with Asc6Plm. The hydrophobic interaction analyses revealed that Asc6Plm had hydrophobic interaction with LEU300, PRO346, PRO384 and ILE389. In the complex of farnesol (2) and HYAL, compound 2 stretched to the space surrounding with several hydrophobic residues including LEU300, PRO346 and LEU381. Moreover, compounds 2 had two hydrogen bonds with the HYAL amino acid residues with include ASN350 and LEU382. For trans-geraniol (1), show two hydrogen bond interaction with residues including LEU383 and ASN383. In addition, the hydrophobic interaction between compound 1 with LEU380, LEU382, LEU404, GLN411, MET412 and PHE416 were observed (Table 3). These common binding orientation and possible interactions were reasonably in good agreement with the experimentally derived IC\textsubscript{50} values.

Table 3. Molecular docking experiments: binding of interactions of the compounds 1, 2 and Asc6Plm with HYAL (2PE4).

| Entry                  | Binding Energy (kcal/mol) | Interaction (Amino-ligand) | Distance (Å) | Interaction (Amino-ligand) | Distance (Å) | Interaction (Amino-ligand) | Distance (Å) |
|------------------------|--------------------------|---------------------------|--------------|---------------------------|--------------|---------------------------|--------------|
| Residual               | ASC6PLM                   | C···C                     | 3.770        | C···C                     | 3.669        | -                         | -            |
|                        | FARNESOL (2)              | O···H-C                   | 3.154        | O···H-C                   | 3.408        | -                         | -            |
|                        | trans-Geraniol (1)        | O···H-C                   | 3.030        | C···C                     | 3.566        | -                         | -            |
|                        | LEU300                    | H-bond                    | 3.035        | H-bond                    | 3.132        | -                         | -            |
|                        | LEU379                    | H-bond                    | 2.590        | O···H-C                   | 3.862        | -                         | -            |
|                        | LEU380                    | O···H-O                   | 3.917        | C···C                     | 3.155        | -                         | -            |
|                        | LEU381                    | H-bond                    | 3.082        | C···C                     | 3.174        | H-bond                    | 2.835        |
|                        | LEU382                    | H-bond                    | 3.065        | H-bond                    | 2.944        | C···C                     | 3.401        |
|                        | ASN383                    | H-bond                    | 3.835        | -                         | -            | H-bond                    | 2.963        |
|                        | PRO384                    | C···C                     | 3.707        | -                         | -            | -                         | -            |
|                        | PHE387                    | O···H-C                   | 3.222        | O···H-C                   | 3.793        | -                         | -            |
|                        | SER388                    | N···H-C                   | 3.793        | C···C                     | 3.147        | -                         | -            |
|                        | ILE389                    | C···C                     | 3.696        | N···H-C                   | 3.221        | -                         | -            |
|                        | LEU404                    | -                         | -            | -                         | -            | C···C                     | 3.894        |
|                        | ASP408                    | -                         | -            | -                         | -            | O···H-C                   | 3.495        |
|                        | GLN411                    | -                         | -            | -                         | -            | C···C                     | 3.579        |
|                        | MET412                    | -                         | -            | -                         | -            | C···C                     | 3.439        |
|                        | GLU415                    | H-bond                    | 3.481        | -                         | -            | O···H-C                   | 3.001        |
|                        | PHE416                    | -                         | -            | -                         | -            | C···C                     | 3.373        |

4. CONCLUSIONS

*G. carinata* essential oil was isolated from flower that was chemically analyzed by GC/MS and evaluated for in vitro and in silico anti-hyaluronidase activity. The yields of *G. carinata* flower oil from steam distillation was 0.16%, GC/MS analysis was carried out on the essential oil and was found to contain nineteen compounds. The principal constituents are trans-geraniol (1, 19.9%) and farnesol (2, 13.2%), anti-hyaluronidase activity test of the oil and two main components, trans-geraniol and farnesol were carried out to determine the anti-aging property. The oil showed mild hyaluronidase inhibitory activity (IC\textsubscript{50} = 1200.4 ± 21.1 μg/mL). In case of two main component, farnesol (2) possessed the high anti-hyaluronidase activity (IC\textsubscript{50} = 292.9 ± 23.4 μg/mL). The molecular docking simulation results explain the binding of selected compounds 1, 2, and the binding sites of the corresponding target imply the feasibility for the mode of action of the compounds in the anti-hyaluronidase activity. Overall, the data showed the importance of preliminary bioassays as a screening of the bioactive from plant products. In this study an essential oil
from *G. carinata* established its importance as potential source of anti-aging.

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