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Artemisia Vulgaris: Biological Properties of a Potential Source for Cosmetics

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Abstract

Artemisia vulgaris, commonly known as mugwort, has been traditionally used for the treatment of gastrointestinal and gynecological disorders. This plant has been scientifically reported to posess various biological activities such as antimicrobial, antioxidant and anti-inflammatory. This study aimed to investigate various biological activities including antioxidant, anti-tyrosinase, anti-collagenase and cytotoxicity. The antioxidant of this plant was evaluated using DPPH assay. Tyrosinase inhibitory assay was performed with the presence of DOPA as oxidase activity while the assessment of collagenase inhibitory activity was carried out based on spectrophotometric methods. MTT assay was conducted for the investigation of cytotoxicity against L-929 fibroblast cell lines. The IC_{50} of DPPH free-radical scavenging activity was recorded to be 38.42 ± 6.3 µg/mL compared to that of vitamin C (2.71 ± 0.8 µg/mL). The IC_{50} of tyrosinase inhibitory activity was 18.1 ± 2.4 compared to that of kojic acid (51.34 ± 2.8 µg/mL). Meanwhile, the ethanolic *Artemisia vulgaris* leaf extracts were capable of inhibiting collagenase activity with IC_{50} value of 10.38 ± 4.32 µg/mL compared to that of quercetin (8.68 ± 0.84 µg/mL). The extracts exhibited a cytotoxic effect on L-929 fibroblast cells in a dose-dependent manner; however, a significant cytotoxic effect was only documented at a dose of larger than 250 µg/mL. These promising findings partially elucidated the potential of *Artemisia vulgaris* as a source of cosmetics but need to be further extensively investigated.

Keywords: *Artemisia vulgaris*, Antioxidant, Collagenase, Cosmetics, Cytotoxicity, Mugwort, Tyrosinase

Introduction

Plant-based beauty products have currently gained popularity worldwide as recent years have seen the spectacular growth of global herbal cosmetic markets. There is a common belief that herbal formulations are preferred over chemical based-cosmetics, owing to their pleasant fragrance, purported therapeutic properties, and widespread belief that these products are safer than synthetic counterparts [1,2]. Indeed, various active ingredients derived from plants have proven to be capable of nourishing skin and hair, delaying aging and enhancing human beauty and attractiveness, and are thus marketed as being developed with environmentally friendly practices, or as being gently effective and safe, and particularly free from synthetic chemicals [3]. Herbal cosmetics are formulated using different bioactive ingredients extracted from a variety of botanical sources. These ingredients can be natural nutrients like protein, vitamins and minerals or phytoconstituents such as alkaloids, flavonoids, phenolics or essential oils. These bioactive components present in plants have been scientifically proven to protect and improve human beauty, owing to their valuable properties such as UV light blocking, moisturizing, cleansing, exfoliating or free radical scavenging activity [4-9]. Plant extracts are now commonly incorporated into a variety of cosmetic preparations. Plant-based cosmetics, practically, consist of a wide range of formulations that can be labeled as skin care, hair care and body care, which are now widely available over the counter in drug stores or chemists' shops [10].

Artemisia vulgaris L. (*A. vulgaris*), commonly known as mugwort or wormwood, is a member of the family Asteraceae [11]. This rhizomatous perennial species is well-known for its distinctive odor and unique medicinal and food flavoring applications. The plant has

been traditionally utilized as an alternative and complementary medicine to control gastrointestinal functions and cure a variety of gynecological disorders. Contemporary scientific reports have indicated the significant pharmacological properties of *A. vulgaris* extracts, including hepatoprotective, cytotoxic, anti-spasmolytic, antinociceptive, antioxidant and antimicrobial activity which are attributed primarily to major phytoconstituents present in the extracts including coumarins, flavonoids, sesquiterpenoids lactones and essential oil [12]. Furthermore, this plant has been currently targeted as a valuable source in the production of cosmetics and the food industry [13]. Some active ingredients present in mugwort show benefits in maintaining and enhancing human beauty such as astringent, skin lighting, exfoliating or reducing dark spots. Si-Hyang *et al.* [14] suggested that mugwort extract was even more effective than that of ascorbic acid in protecting hairless mouse skin from photo-irradiation, and can be used as a potential anti-aging cosmetic ingredient. Essential oil derived from this plant possesses various biological activities including pain relief, anti-melanogenic, anti-inflammatory and antioxidant properties [15-17].

Materials and methods

Plant materials and extraction

Fresh *A. vulgaris* leaves were collected from a local garden and washed several times with running water to remove any impurities. The plant leaves were immediately air-dried and cut into small pieces. Plant materials were then extracted with 70 % ethanol using a Soxhlet apparatus for 7 h. Once the distillation had been complete, the crude extract was concentrated by a rotary evaporator under a vacuum at 50 °C. The extract was then stored in sealed amber vials for further analysis.

Chemicals and reagents

All chemicals were provided by the Pharmaceutical Chemistry Laboratory and Applied Biochemistry Laboratory of the Applied Biochemistry Department, International University, Vietnam National University - Ho Chi Minh City. 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin Ciocalteu reagent, quercetin, L-DOPA, tyrosinase, kojic acid, dimethyl sulfoxide (DMSO) and reference standards (apigenin, artemisinin, kaempferol, luteolin, naringenin, pinocembrin, pinostrobin and rutin) were purchased from Sigma-Aldrich. High glucose Dulbecco's modified eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco-BRL Life Technologies. All chemicals and reagents were stored in accordance with the most stringent regulations and freshly prepared with distilled water to the desired concentrations for experiment purposes.

Determination of total phenolic and flavonoid content

The total phenolic content (TPC) was determined using Folin-Ciocalteu method [18]. Gallic acid was used as a standard for the calibration curve. The assay was prepared by mixing 25 µL of plant extract with 100 µL of Folin-Ciocalteu reagent, followed by the addition of 75 µL of 7.5 % Na_2CO_3. The mixture was then vortexed for 5 min and kept in the dark at room temperature for 30 min. The absorbance of the sample was measured against the blank at 750 nm using a spectrophotometer and Biotek Synergy HT 96-well plate. Meanwhile, a calibration curve of the known concentration of gallic acid was made using the linear regression equation $y = bx + a$. TPC was expressed in terms of gallic acid equivalent (GAE) per gram of sample (mg GAE/g of sample).

The total flavonoid content (TFC) was determined using aluminum chloride method [19]. Quercetin was used as a standard for the calibration curve. The assay was prepared by mixing 25 µL of plant extract with 150 µL of absolute methanol, followed by the addition of 10 µL of 10 % $AlCl_3$ and 10 µL of 1 M CH_3COOK. The mixture was then vortexed for 5 min and kept in the dark at room temperature for 40 min. The absorbance of samples was measured against the blank at 415 nm using a spectrophotometer and Biotek Synergy HT 96-well plate. Meanwhile, a calibration curve of known concentration of quercetin was made using the linear regression equation $y = bx + a$. TFC was expressed in terms of quercetin equivalent (QE) per gram of sample (mg QE/g of sample).

HPLC Analysis

The high-performance liquid chromatography (HPLC) was employed using the Agilent series 1200 system with variable wavelength. Chromatographic separations were done on a Zorbax extend C18 column

(250×4.6 mm^2, 5.0 µm, 2.1×50 mm^2) at 30 °C under a gradient program using a mobile phase consisting of 1 % acetic acid and methanol. The injection volume was 20 µL, and the flow rate was set at 0.7 mL/min. The UV detector at wavelength of 290 nm was applied to monitor the presence of apigenin, kaempferol, naringenin and pinostrobin while quercetin was at 360 nm.

Analysis of 1,3-dicaffeoylquinic acid, rutin, and artemisinin was performed using a HPLC hp Hewlett Packard 1050 equipped with Phenomenex Ultracard ODS (30) (150×4.6 mm^2; 5 µm). The mobile phase consisted of 0.1 % (v/v) formic acid and acetonitrile with different gradient programs for each analytical component with a flow rate of 1 mL/min and an injection volume of 5 µL. The monitoring wavelengths for quantifications were set at 320 nm (1,3-dicaffeoylquinic acid), 254 nm (rutin) and 203 nm (artemisinin). The luteolin component was analyzed with UPLC Thermo scientific system using the column Thermo Scientific Hypersil GOLD (150×2.1 mm^2). The mobile phase consisted of 0.1 % (v/v) formic acid and acetonitrile following the gradient program: beginning with 85 of 0.1 % formic acid and 15 % of acetonitrile with a flow rate of 0.4 mL/min and an injection volume of 2 µL. The UV detector at a wavelength of 254 nm was applied.

DPPH free-radical scavenging assay

The antioxidant activity of the plant extracts was quantitatively assessed using DPPH assay [20]. Ascorbic acid was used as a reference standard with 8 different concentrations ranging from 5 to 0.039 µg/mL. The assay was prepared by mixing 50 µL of sample with 150 µL of 0.1 mM DPPH and then vortexed vigorously. The mixture was subsequently incubated for 30 min in the dark at room temperature. The absorbance of the mixture was spectrophotometrically measured against the blank at 517 nm using Biotek Synergy HT 96-well plate. The DPPH free-radical scavenging activity was expressed as IC_{50} which was the concentration of the sample required to inhibit 50 % of DPPH free radicals.

Tyrosinase inhibitory activity assay

The tyrosinase inhibitory activity of the ethanolic *A. vulgaris* leaf extracts was evaluated using L-tyrosine as a substrate. The L-tyrosine test included a total of 4 wells, which were labeled as follows: A (control), B (blank control), C (sample) and D (blank sample). Samples consisting of 9 different concentrations of the extracts were dissolved with 5 % DMSO in phosphate buffer of pH 6.5 (**Table 1**)

Table 1 Preparation of tyrosinase inhibitory assay.

	Control (A)	**Blank control (B)**	**Sample (C)**	**Blank sample (D)**
Phosphate buffer (pH 6.5) (µL)	90	120	80	110
Sample (µL)	-	-	10	10
Tyrosinase 100 U/mL (µL)	30	-	30	-
		Incubated for 5 min at 25 °C		
L-DOPA 10 mM (µL)	80	80	80	80

After 40 min of incubation at 25 °C, the absorbance at 475 nm was applied to measure the enzyme activity, and the percent tyrosinase inhibition was computed using the following formula:

$$\text{Tyrosinase inhibition (\%)} = \frac{(A-B)-(C-D)}{A-B} \times 100$$

where, A: The absorbance of the control with the enzyme, B: The absorbance of the control without the enzyme, C: The absorbance of the test sample with the enzyme and D: The absorbance of the test sample without the enzyme.

Collagenase inhibitory activity assay

The collagenase inhibitory assay was performed based on spectrophotometric methods reported in the literature [21] with slight modification. Collagenase was prepared in 50 mM Tricine buffer (with 10 mM $CaCl_2$ and 400 mM NaCl), adjusted pH 7.5, achieving the final concentration of enzyme of 0.8 U/mL. The substrate collagen (1 mg/mL) was dissolved in the same buffer.

Ethanolic *A. vulgaris* leaf extract (2 mg/mL) was considered as an inhibitor compound of the tests. Distilled water was used as a negative control and quercetin as a positive control. The preparation of the assay is briefly presented in **Table 2.**

Table 2 Preparation of collagenase inhibitory assay.

	Positive control	Background	Sample	Negative control
50 mM Tricine buffer, pH 7.5 (µL)	25	75	25	25
Sample (µL)	-	-	25	-
Collagenase (0.8 U/mL) (µL)	25	-	25	25
Quercetin	25	-	-	-
Distilled water	-	-	-	25
		Pre-incubated in 15 min		
Collagen (625 µg/mL) (µL)	50	50	50	50

The collagenase inhibitory activity was calculated using the following formula:

$$\text{Collagenase Inhibitory Activity } \left(\frac{\text{U}}{\text{mL}}\right) = \frac{(\Delta\text{OD}/\Delta\text{T})\times\text{V}\times\text{D}}{0.53\times\text{v}}$$

where, ΔOD: The changes in absorbance that were obtained between T2 and T1, corrected for background and ΔT: Linear phase reaction time T2 − T1 (min), V: Reaction volume (mL), D: Sample dilution factor, 0.53: Millimolar extinction coefficient of collagen substrate and v: Sample volume added into the reaction well (mL).

The percentage of collagenase inhibition was calculated using the following formula:

$$\text{Collagenase inhibition (\%)} = \frac{\text{Activity (Enzyme)} - \text{Activity (Inhibitor)}}{\text{Activity (Enzyme)}} \times 100$$

Cytotoxicity assay

Cell line

The L-929 fibroblast cells of the mouse were thawed in a warm medium (50 °C) and removed frozen medium by centrifugation. The cells were then resuspended in a culture medium containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 5 % fetal calf serum, 100 U/mL penicillin and 100 mg/mL streptomycin at 37 °C in a humidified atmosphere of 5 % CO_2 and 95 % air. Fibroblast cells were then cultured in a 96-well plate with a density of 1×10^4 cells/mL.

MTT assay

The cytotoxicity of investigated treatments was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. This method is a colorimetric assay for assessing the cell metabolic activity which is based on the catalyzing capacity of NADPH-dependent cellular oxidoreductase enzymes for the reduction reaction from tetrazolium dye MTT to its water-insoluble formazan with purple color.

After 24-hour incubation from the first day, the culture medium was aspirated and 100 µL of plant extract at various concentrations (1,000, 500, 250, 125 and 62.5 µg/mL) was added to a 96-well plate. A culture medium supplemented with 20 % DMSO served as a positive control. The culture medium should be measured as negative control. After 24 h of incubation period, cell culture was carefully removed to ensure that reductive chemicals in plant extract were not able to reduce the MTT. Shortly afterward, 50 µL of MTT solution was added to each tested well and the 96-well plate was further incubated for 2 h at 37 °C. Thereafter, the MTT solution was descanted, followed by the addition of 100 µL of isopropanol to each well. Absorbance was spectrophotometrically measured against the blank at a wavelength of 570 nm for determination of the relative degree of proliferation (RGR).

$$\text{RGR (\%)} = \frac{\text{OD treated cell}}{\text{OD negative control}} \times 100$$

where, OD treated cell: The mean value of the measured optical density of the sample cell with different concentration and OD negative control: The mean value of the measured optical density of the negative control.

Statistical analysis

All experiments were conducted in triplicate, and the results were expressed in terms of mean values ± standard deviation (SD). Analysis of variance and significant differences among means were determined by 1-way ANOVA using OriginPro (Version 2024). Significant differences were declared at $p < 0.05$.

Results and discussion

Determination of total phenolic and flavonoid contents

TPC of ethanolic *A. vugaris* leaf extract was illustrated in **Table 3**. The TPC value was calculated using the gallic acid standard curve ($y = 0.0905x + 0.0228$, $R^2 = 0.9898$) and expressed in milligram gallic acid equivalents (GAE) per gram of extract. Accordingly, the content of phenolic compounds (mg/g) in ethanolic extract was found to be 121.95 ± 13.49 mg/g plant extract (GAE) which was approximately 6 and a half times greater than TPC of the aqueous extracts by maceration process (19 ± 0.16 mg/g) [22]. There are considerable differences between the results which can be explained by using various solvents of different solubility, particularly affinity sites for the uptake of phenolic compounds [23].

Table 3 shows the value of TFC of ethanolic *A. vulgaris* leaf extract. The results were derived from the calibration curve ($y = 0.0595x + 0.0187$, $R^2 = 0.9992$) of quercetin and expressed in milligram quercetin equivalents (QE) per gram of dried extract. The total flavonoid contents of ethanolic *A. vulgaris* leaf extracts were recorded at 51.42 mg QE/g which was approximately equal to half the value of methanolic *A. vulgaris* leaf extracts (101.17 mg QE/g) conducted by Kunal [2].

Table 3 Total phenolic and flavonoid contents of ethanolic *Artemisia vulgaris* leaf extract.

	Total phenolic content (mg (GAE)/g)	**Total flavonoid content (mg (QE)/g)**
Artemisia vulgaris extracts	121.95 ± 13.49	51.42 ± 0.31

HPLC analysis

Ten polyphenolic compounds were subjected to the investigation of the major phytoconstituents in ethanolic *A. vulgaris* leaf extracts. However, not all target compounds were detected as HPLC analysis revealed the absence of 3 components, particularly the void of artemisinin. Rutin, on the other hand, was found to be the highest quantity among the 7 constituents detected with a value of 2.4 mg/g ethanolic *A. vulgaris* leaf extracts (**Table 4**). As reported in previous literature, rutin, also called rutinose, possesses a number of significant pharmacological properties, including antioxidant, anti-inflammatory, anti-carcinogenic, neuroprotective and vasoprotective effects [24]. Since plant extracts consist of a mixture of natural components, their different biological activities result from not only the distinct actions of single components but also their synergistic effects. The biological activity of ethanolic *A. vulgaris* leaf extracts thus would be likely variable chiefly depending on chemotypes and quantity of various constituents present in the extracts

Table 4 Quantity of major compounds present in ethanolic *Artemisia vulgaris* leaf extracts.

Composition	Quantity (mg/g)
Apigenin	0.13
Artemisinin	-ND-
Cynarine	-ND-
Kaempferol	0.21
Luteolin	0.30
Naringenin	0.04
Pinocembrin	-ND-
Pinostrobin	0.58
Quercetin	0.06
Rutin	2.40

-ND-: Not detected.

DPPH free-radical scavenging assay

The DPPH free-radical scavenging capacity of ethanolic *A. vulgaris* leaf extracts was found to be in a concentration-dependent manner (**Table 5**). Indeed, the percentage of DPPH free-radical scavenging activities by ethanolic *A. vulgaris* leaf extracts were recorded to be 10.4, 16.9, 25.8, 44.1 and 75.7 % corresponding to the extract concentration of 3.9, 7.81, 15.63, 31.25 and 62.5 µg/mL, respectively. The IC_{50} of DPPH free-radical scavenging activity was documented to be 38.42 ± 6.30 µg/mL which was approximately 14 times greater than that of ascorbic acid (2.71 ± 0.80 µg/mL). Previous studies by Karabegović *et al.* [25] reported the IC_{50} value of 28.1 µg/mL for ethanolic extract while Ahmed *et al.* [26] recorded 63.34 ± 1.1 µg/mL for methanolic extract, reflecting the effects of extracting solvents. As scientifically reviewed elsewhere, in general, the antioxidant activity might be likely varied owing to not only the presence of flavonoid and phenolic compounds but also some other phytoconstituents such as peptides and polysaccharides [27].

Table 5 IC_{50} of DPPH Free-radical scavenging activity of the extracts.

	***Artemisia vulgaris* extracts**	**Ascorbic acid**
IC_{50} (µg/mL)	38.42 ± 6.3	2.71 ± 0.8

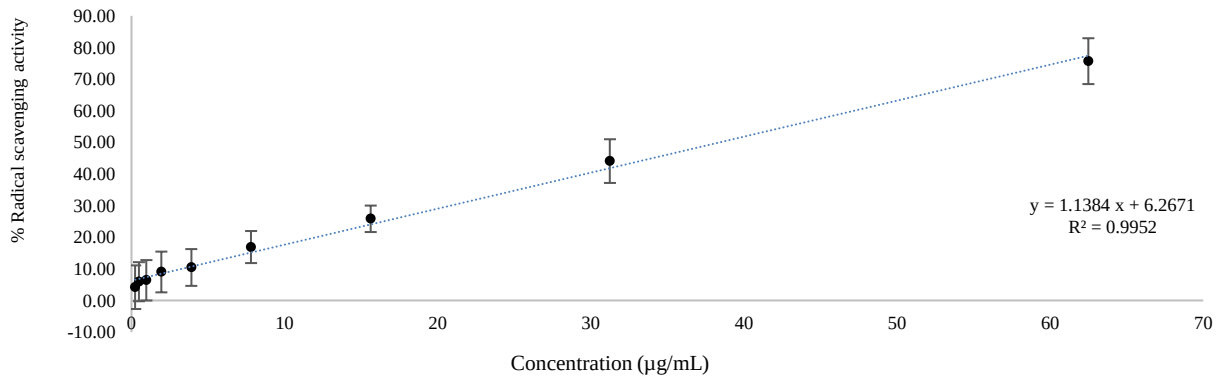

Figure 1 DPPH free-radical scavenging activity of ethanolic *Artemisia vulgaris* leaf extracts.

Tyrosinase inhibitory activity assay

Figures 2 and **6** show a dose-dependent effect of ethanolic *A. vulgaris* leaf extracts on inhibiting tyrosinase, beginning at a dose of 6.25 µg/mL inducing the tyrosinase inhibition with a value of 38.91 %. The percentage of tyrosinase inhibitory activity was then considerably increased to 70.1 % corresponding to the dose of 50 µg/mL. At a dose of 100 µg/mL, the ethanolic *A. vulgaris* leaf extracts exerted powerful inhibition of tyrosinase as the percentage of tyrosinase inhibition was recorded to be 73.1 %. However, there was no significant variation and even dropped down when reaching the dose of 125 µg/mL or higher. The IC_{50} value of kojic acid, which served as a positive control, was documented to be 51.34 µg/mL which was approximately 3 times greater than IC_{50} of ethanolic *A. vulgaris* leaf extracts (18.1 µg/mL), denoting the potential of ethanolic *A. vulgaris* leaf extracts as a powerful tyrosinase inhibitory agent which can be exploited towards the development of cosmetics (**Table 7**).

Tyrosinase is well-known as an enzyme participating in the rate-limiting step for controlling melanin production. The structure of the enzyme tyrosinase has 3 domains, of which the central domain contains 2 Cu binding sites. These coppers belong to the active site of the enzyme tyrosinase that interacts with both molecular oxygen and its phenolic substrate [28]. Therefore, any substance which reduces this metal ion was considered as an effective tyrosinase inhibitor [29]. The tyrosinase inhibition ability of plant extract can be explained in terms of the similarity between the dihydroxyphenyl group in L-DOPA and the a-keto group in flavonoids [34-36]. Tyrosinase inhibitors may thus be used as a treatment for some skin disorders associated with melanin hyperpigmentation and can be incorporated into cosmetic formulas for skin-whitening effects [30-32].

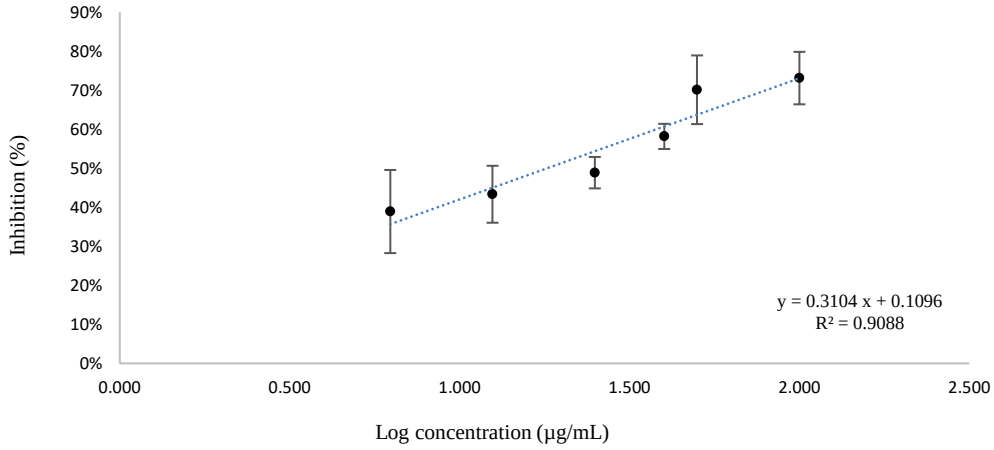

Figure 2 Tyrosinase inhibitory activity of ethanolic *Artemisia vulgaris* leaf extracts of various concentrations.

Table 6 Enzymatic tyrosinase inhibitory activity of the *Artemisia vulgaris* extracts.

Concentration (µg/mL)	Percentage of Inhibition (%)
400	27.09 ± 3.16
200	47.41 ± 4.31
125	53.69 ± 3.45
100	73.11 ± 7.76
50	70.12 ± 10.16
40	58.17 ± 3.72
25	48.87 ± 4.65
12.5	43.34 ± 8.43
6.25	38.91 ± 12.30

Table 7 IC_{50} of tyrosinase inhibitory activity of the *Artemisia vulgaris* leaf extracts.

	***Artemisia vulgaris* leaf extracts**	**Kojic acid**
IC_{50} (µg/mL)	18.1 ± 2.4	51.34 ± 2.8

Collagenase inhibitory activity assay

Figure 3 and **Table 9** illustrate a dose-dependent effect of collagenase inhibition by the ethanolic *A. vulgaris* leaf extracts. Accordingly, ethanolic *A. vulgaris* leaf extracts could effectively inhibit 75.86 % of enzyme activity at a low dose of 25 µg/mL. The remaining collagenase was continuously inhibited at 97.37 %. In this study, the IC_{50} of collagenase inhibitory activity by the ethanolic *A. vulgaris* leaf extracts was noticed to be 10.38 ± 4.32 µg/mL, which was approximately equal to that of quercetin (8.68 ± 0.84 µg/mL) (**Table 8**). As scientifically reviewed from previous study, the flavonoid compounds present in plant extract could act as a metal chelator capable of bonding to the Zn^{2+} ion in the collagenase structure, thereby preventing the enzyme from binding to the substrate-collagen [37]. It is worth knowing that the more inhibitor molecules are, the faster the reaction happens, until the saturation point is reached and then the collagenase inhibition percentage may stay constant. Collagen is well-known as a major extracellular matrix protein in the dermis layer of the skin. However, collagen can be biochemically degraded by a protease, namely collagenase, resulting in photoaging and wrinkling of the skin [38].

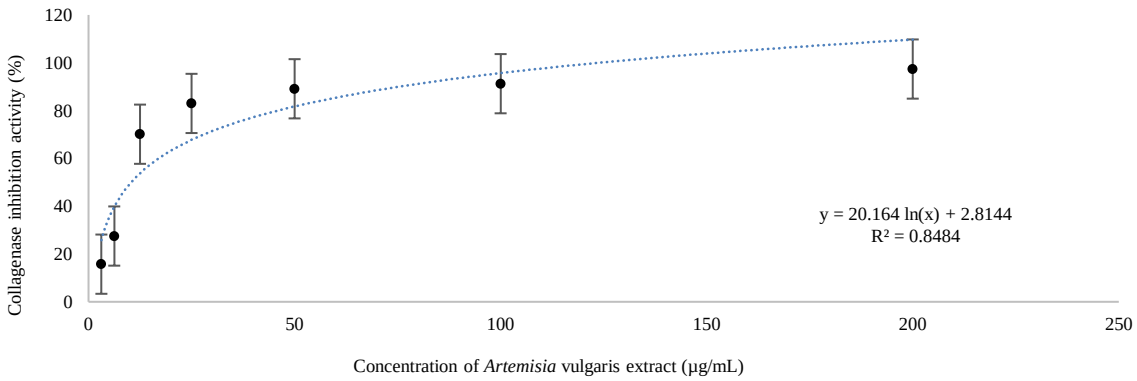

Figure 3 Collagenase inhibitory activity of ethanolic *Artemisia vulgaris* leaf extracts at various concentrations.

Table 8 IC_{50} of collagenase inhibitory activity of the *Artemisia vulgaris* leaf extracts.

	***Artemisia vulgaris* leaf extracts**	**Quercetin**
IC_{50} (µg/mL)	10.38 ± 4.32	8.68 ± 0.84

Table 9 Enzymatic collagenase inhibitory activity of the *Artemisia vulgaris* leaf extracts.

Concentration (µg/mL)	**Percentage of Inhibition (%)**
200	97.37 ± 1.76
100	91.24 ± 5.20
50	89.10 ± 6.11
25	82.98 ± 6.16
12.5	70.10 ± 3.73
6.25	27.50 ± 6.90
3.125	15.74 ± 5.61

MTT cytotoxicity assay

Morphological assessment of cytotoxicity before treatment is illustrated in **Figures 5I(A-G)**. The percentage of cellular vitality was confirmed to be larger than 90 % for all samples tested, including positive and negative controls and 5 ethanolic *A. vulgaris* leaf extracts at various concentrations ranging from 1,000 to 62.5 µg/mL. It was also shown that the normal spindle-shaped L-929 mouse fibroblast cells were spread homogenously on the surface of the flask and ground as a confluent monolayer.

After 24-hour exposure to ethanolic *A. vulgaris* leaf extracts at various concentrations; however, there were significant changes in cellular morphology, reflecting the cytotoxic effects of components present in the samples tested (**Figures 5II(A-G)**). Cells of negative control groups cultivated in DMEM media exhibited a normal cellular morphology, having spindle or irregular triangle shape, exponential growth, and well-attached in the flask (**Figure 5II(F)**). Meanwhile, the 20 % DMSO-treated positive controls could induce the disturbance of the phospholipid membrane resulting in pores formation, and decrease in cell selectivity, and an increase in cell permeability. Besides, a change in cellular morphology could be visually observed as the loss of spindle shape and the largest number of detached cells from the bottom was confirmed (**Figure 5II(G)**).

There were significant differences in cytotoxic properties among 5 ethanolic *A. vulgaris* leaf extracts at various concentrations (**Figures 5II(A-E)**). The ethanolic *A. vulgaris* leaf extract of 1,000 µg/mL could induce the change of fibroblast cell appearance with a smooth surface and ruffled border, which also detached from the bottom of the flask and rounded up. The morphology of L-929 fibroblast cells became abnormal, thereby damaging the structure of organelles, resulting in the inhibition of cell function (**Figure 5II(A)**). Notably, decreasing concentrations of ethanolic *A. vulgaris* leaf extracts caused a barely change in morphological characteristics of the L-929 cell lines as the level of cell debris and morphological changes was approximately proportional to the applied concentrations, typically identified from the concentration of 250 to 62.5 µg/mL (**Figures 5II(C-E)**). Besides, the lower concentrations of ethanolic *A. vulgaris* leaf extracts, the smaller number of cells with round shrinking and decreasing abnormal floating was observed, indicating that the ethanolic *A. vulgaris* leaf extracts were not significantly toxic to L-929 fibroblast cells at a concentration of less than 250 µg/mL.

It is noteworthy to know that the MTT-formazan production rates are directly proportional to the number of vitality cells, which can thus be used as an indicator for the assessment of cytotoxic capacity (**Figures 5III(A-G)**). In this study, practically, the ethanolic *A. vulgaris* leaf extracts of 1,000 and 500 µg/mL concentration showed significant cytotoxic effects against L-929 fibroblast cells. The treatment by ethanolic *A. vulgaris* leaf extracts of 1,000 µg/mL concentration could considerably decrease the vitality of cells up to 15.67 % compared to 10.2 % of the positive control. The ethanolic *A. vulgaris* leaf extracts of the 250 µg/mL concentration-treated group, on the other hand, were noted as mildly toxic to L-929 fibroblast cells whereas the groups received ethanolic *A. vulgaris* leaf extracts of 125 and 62.5 µg/mL concentration were not significantly toxic to the L-929 fibroblast cells tested (**Figure 4** and **Table 10**).

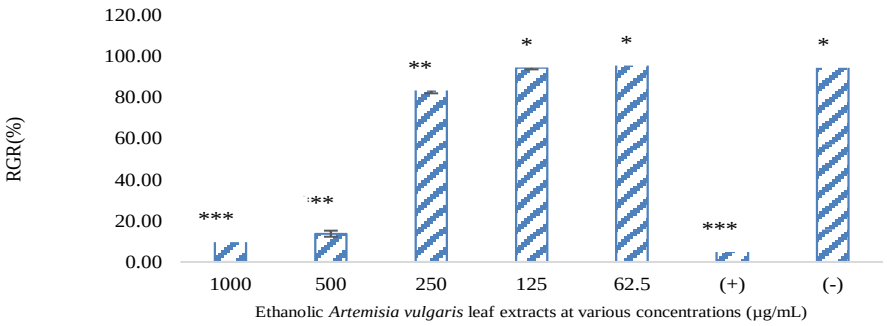

Figure 4 Percentage of cell viability following the exposure to ethanolic *Artemisia vulgaris* leaf extracts at various concentrations. The data are presented as mean ± SD, n = 3. Note: Asterisk (*) denotes the cytotoxicity grade: ***toxic, **mild toxic and *non-toxic.

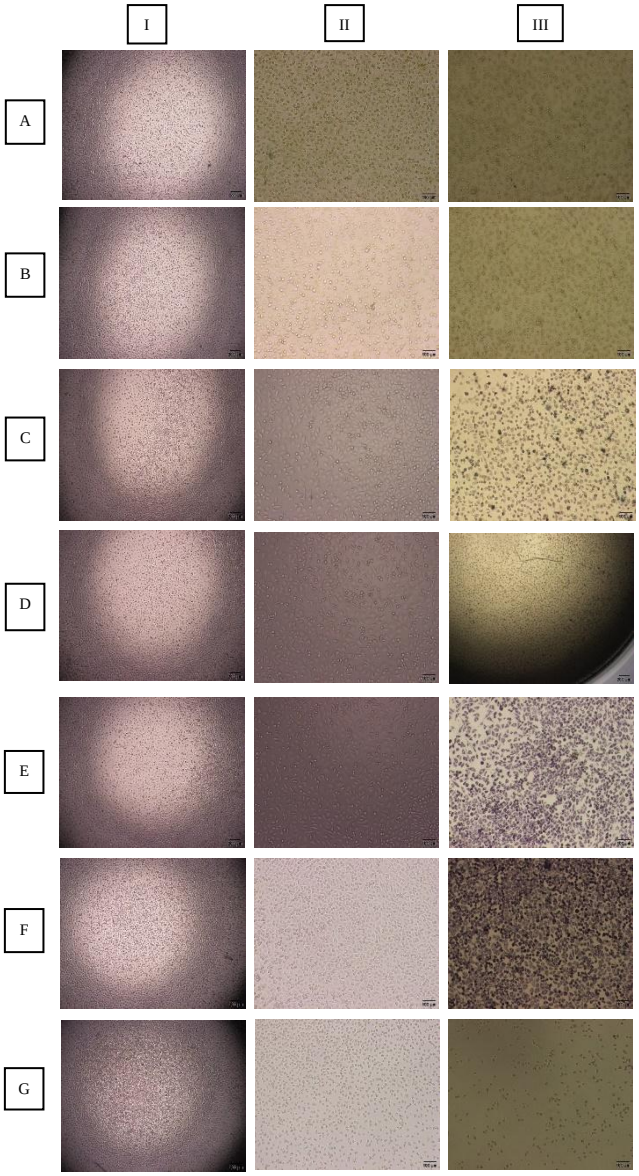

Figure 5 Assessment of morphological changes in L-929 fibroblast cells treated with ethanolic *Artemisia vulgaris* leaf extracts. From left to right in order: Cells before treatment (I), cells after 24 h treatment (II), cells exposed to MTT (III). Row (A) to (G) in order: 1,000, 500, 250, 125 and 62.5 µg/mL, negative control and positive control.

Table 10 The proliferation of the cells after being treated with *Artemisia vulgaris* leaf extract.

Group	RGR (%)	Cytotoxicity grade
1,000 µg/mL	15.63 ± 0.38	Toxic
500 µg/mL	13.63 ± 2.63	Toxic
250 µg/mL	84.98 ± 5.48	Mild toxicity
125 µg/mL	94.23 ± 1.47	Non-toxic
62.5 µg/mL	97.41 ± 1.65	Non-toxic
Positive control	10.20 ± 0.82	Toxic
Negative control	98.06 ± 2.51	Non-toxic

Conclusions

Overall, this study demonstrates the power of ethanolic *Artemisia vulgaris* leaf extracts, capable of inhibiting tyrosinase and collagenase. Besides, the extracts of a dose less than 250 µg/mL were not significantly toxic to L-929 fibroblast cell lines. These promising findings could be favorable for the development of plant-based cosmetics but need to be further extensively investigated.

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