

***In Vitro* anti-collagenase activity and total phenolic content of five selected herbs: a review**

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ABSTRACT

Etligeria elatior, *Persicaria odorata*, *Centella asiatica*, *Senna alata* and *Phyllanthus emblica* were reviewed for their *in vitro* anti-collagenase activity and its total phenolic contents. Plant herbs play a vital role to promote the production of collagen which is the component of the skin. Different solvents during plant extraction and various plant parts were used to determine anti-collagenase properties and total phenolic content. Generally, anti-collagenase assay and Folin-Ciocalteu method were used to determine the anti-collagenase activity and total phenolic content, respectively. The extracts of these herbs have the potential of inhibiting collagenase activity and they possess total phenolic content. The total phenolic was found to be important elements in collagenase inhibition activity other than flavonoid. The selected herbs are popular in Malaysia and this review can be a useful reference or information for future application in pharmaceutical, food and cosmetics fields.

Keywords: *Anti-collagenase activity, total phenolic contents; plant Extraction; flavonoids*

INTRODUCTION

Collagenase is an enzyme that can degrade collagen. Based on [1], depletion in collagen and elastin will promote the formation of wrinkles and initiate sagging of skin. The appearance of wrinkles, pigmentation, rough-textured, and loss of skin elasticity are the signs of skin aging. According to a previous study [2], the degeneration of Type I, II, and III collagens are induced by specific collagenase subunits of matrix metalloproteinases (MMPs). Collagenase is a part of the MMPs family that comprises a vast group of zinc-dependent endopeptidase [3]. Therefore, the metalloproteinase catalytic mechanism will be induced if zinc metal is present. Besides, it was also reported that intrinsic and extrinsic processes may affect the modification of skin structural integrity and physiological activity with an increase in age [4]. The UV rays are the main elements that help to trigger MMPs thus, inducing extrinsic aging. Connective tissue remodeling can be induced due to long-term revelation to UVA through lipid peroxides, cell materials, enzymes, and reactive oxygen species (ROS) production. The previous study [5] documented that the secondary messengers such as hydrogen peroxide, superoxide, singlet oxygen, and hydroxyl radicals are ROS which induces MAP-kinasep38, ERK (extracellular signal-regulated kinase), and JNK (c-Jun amino-terminal kinase). The production of MMPs is initiated by ROS indirectly through the MAP kinase pathway [5]. Besides, the initiation of MMPs is facilitated by the regulation of a transcription factor, the activator protein 1 (AP-1) [5]. The AP-1 also inhibits a major regulator for procollagen type I production activity which is transforming growth factor- β (TGF- β).

Nowadays, it is proven that herbs are beneficial as an important ingredient for cosmetics and skincare products. Besides, the World Health Organization (WHO) states that about 70-80% of the global population depends on herbal sources as their main remedies [6]. Many studies had proven that herbs contributed to pharmacological benefits such as anti-collagenase. According to a study [1], cosmetics containing herbal actives are currently emerging as a suitable solution to the skin aging issue. Therefore, the inhibition of collagenase activity by active compounds from herbs may lead to beneficial consequences such as delaying the collagen degeneration activity and other extracellular matrices (ECM) constituents. Five popular herbs namely *Etingera elatior*, *Persicaria odorata*, *Centella asiatica*, *Senna alata*, and *Phyllanthus emblica* were reviewed for their collagenase inhibition activity and total phenolic contents. This article reviewed the anti-collagenase properties and how the natural ingredients from these selected herbs can help in slowing down the skin aging process. It can be a useful reference and beneficial for future research on pharmaceutical and cosmeceutical studies.

Herb species

Herb is any plant that stems above ground does not become woody. Herbs have been used as an enhancement of flavor in cooking and medicinal purposes for a long time ago. For instance, *Etingera elatior*, which is a member of the Zingiberaceae family and *Etingera* genus. It is

infamously recognized as ‘Torch Ginger’, ‘Red Ginger Lily’ and ‘Bunga Kantan’ and has been extensively cultivated in South East Asia. It is taken as food by the natives especially the inflorescence bud part which is commonly being added in cooking as a flavor enhancer. The most notable biological activities of its flowers and leaves reported before are antioxidant, antibacterial, and anticancer [7]. According to a previous study [8], *E. elatior* has been commercialized for skincare products due to the presence of secondary metabolites which was proven to give whitening effect and anti-aging when tested on human volunteers.

Another famous herb is *Persicaria odorata* or *Polygonum minus* which was previously known as *Polygonum odoratum*. This culinary herb belongs to the *Persicaria* genus and is classified under the *Polygonaceae* family. In Malaysia, it is usually recognized as ‘Vietnamese coriander’, ‘Daun Kesum’ or ‘Laksa plant’ among the locals. This herb can be found in tropical and subtropical regions, which are warm and damp zones [9]. The leaves have been utilized for medicinal and cosmetics purposes. The natural compounds contributed to their useful properties also include antimicrobial, anti-inflammatory, antitumor, and antioxidative activities [10].

This herb is recognized as ‘Gotu Kola’, ‘Pennywort’ and ‘Pokok Pegaga’ in Malaysia, is a common species in the parsley family. It is grouped under the genus of the *Centella* and *Apiaceae* family. It can be found in swampy areas in the Indian subcontinent, Southeast Asia, and wetland zones of the Southeastern United States. *Centella asiatica* was commonly used in Ayurvedic medicine for the past 3000 years ago. This herb contains a great amount of pentacyclic triterpenoid saponins, also known as centelloids [11]. The key active constituents which contribute to wide therapeutic actions found in Gotu Kola are asiaticoside and madecassoside which lead to antioxidant and anti-inflammatory activities [12].

Next, *Senna alata* is also known as ‘Ketepeng Badak’, ‘Christmas candles’ and ‘Emperor’s candlesticks’, is a widely harvested plant under the *Fabaceae* family and *Senna* genus. Previously known as *Cassia alata*, this plant can be found in Southeast Asia, Northern Australia, Africa, and Latin America [13]. *Senna alata* is used as a remedy to cure infections such as ringworm and parasitic skin diseases in Nigeria [14]. The ethanolic extract of stem bark has the antifungal property which prevents the growth of fungi [15]. Additionally, antimicrobial, anti-inflammatory, anti-tumor, and analgesic are among the other pharmacological benefits of the plant.

Lastly, *Phyllanthus emblica* is one of the most important Ayurvedic herbs. Generally, it is known as ‘Indian Gooseberry’, ‘Amla’ or ‘Malacca tree’ and belongs to the *Phyllanthaceae* family. This herb is extensively dispersed in subtropical and tropical regions of India, China, and Peninsular Malaysia [16]. *P. emblica* shows a wide range of pharmacological activities due to its natural bioactive compounds. The benefits include antioxidant, anticancer, anti-inflammatory, and cytoprotective properties.

Anti-collagenase Assay

A procedure of the collagenase inhibition activity was done according to Van-Wart and Steinbrink method [17] with some changes for use in 96 well microtiter plates. The test was carried out in a TES or Tricine buffer. Then, collagenase enzyme from *Clostridium histolyticum* (ChC – EC.3.4.23.3) and synthetic substrate N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA) were prepared by dissolving in a buffer to produce a concentration of 0.8 units/mL and 2mM respectively [18]. The final mixture contained a buffer, FALGPA, collagenase enzyme, and herbs extract. Next, collagenase enzymes in the buffer were incubated at room temperature [18]. It was done to initiate the chemical reaction. Water or buffer acts as negative control while epigallocatechin gallate (EGCG) as a positive control. By using a spectrophotometer, the absorbance at 335-340 nm was read quickly after the substrate was added and then continuously for 20 minutes [18, 19]. Lastly, the calculation was made to determine the inhibition activity for collagenase. This method was used in [18] and [19] to study the anti-collagenase activity for *P. odorata* and *C. asiatica*.

Another procedure was similar to the previous method with some modifications. The concentration of collagenase from *C. histolyticum* in the 50mM Tricine buffer was set at 0.25 units/mL. Next, the enzymatic activity was measured by reduced absorbance throughout the time interval [20]. This method was used in a study on *S. alata* [20]. In the meantime, the anti-collagenase assay extract was also carried out by using spectrophotometric methods as described by Weingarten and Feder [21] for *E. elatior*. The assay was performed in duplicate and the inhibitory activity was expressed as a half-maximal inhibitory concentration (IC₅₀) [22].

On the contrary, the EnzChek Collagenase/Gelatinase kit was used to determine collagenase inhibition activity specifically for MMP-1 and MMP-2. Some members of MMPs induce the degeneration of type I, II, and III fibrillar collagens [2]. For example, collagen type I can be degraded by MMP-1, type II by MMP-8, and type III by MMP-13. The DQTM collagen 1 and DQTM gelatin worked as substrates for MMP-1 and MMP-2 inhibition assays respectively [23]. Collagenase, DQTM substrate, and different concentrations of the sample extract were dissolved in the buffer separately. Then, the diluted extract, DQTM gelatin, and collagenase were combined in a 96-well plate. A fluorescent microplate reader was used to measure the excitation and emission wavelength after incubation and protected from light at room temperature [23]. This method was reported [23] in studying *P. emblica*.

All the methods carried out used collagenase enzyme from *C. histolyticum* and EGCG as a positive control. EGCG is a polyphenol in green tea and EGCG-treated collagen showed 95% inhibition against collagenolytic hydrolysis by collagenase. Meanwhile, the interaction between EGCG with collagenase showed 88% inhibition of collagenase activity against collagen [24]. The EGCG could be a dominant collagenase inhibitor as documented in many studies of anti-collagenase activity.

Plant Extraction

The plant herbs were extracted before proceeding with the determination of anti-collagenase activity and total phenolic content. The extraction process is crucial as it may affect the quality and constituents of herbs [25]. Different species underwent different extraction processes such as using methanol and ethanol as solvents. Both methanol and ethanol are alcohols which are known as universal solvents in solvent extraction. The herb's constituents are polar or nonpolar. It was reported that ethanolic extraction was used for *P. emblica*, *P. odorata*, and *S. alata*. In addition, other methods were used such as hydroglycolic extraction, aqueous extraction, and decoction. The decoction method was used to extract *C. asiatica* leaf and stems. The polarity value of solvents which is close to the solute polarity is expected to work better and the other way round [26].

Various parts of the plant were used such as leaf, stems, and fruits for an anti-collagenase test. Only leaf parts of *E. elatior*, *P. odorata*, and *S. alata* were studied while for *C. asiatica*, both leaf and stems parts were used. In another study, the fruit of *P. emblica* was utilized. Other parts of the plant such as flowers and inflorescence were also utilized. An inflorescence is a group of flowers arranged on a stem that comprises the main branch. For instance, a study was conducted on *E. elatior* inflorescence [22].

Anti-collagenase activity

Table 1 shows the collagenase inhibition activity which was expressed in IC₅₀ or percentage of collagenase inhibition. The half-maximal inhibitory concentration (IC₅₀) is a measure of the effectiveness of a substance in restricting a particular biological or biochemical activity. The IC₅₀ value for hydroglycolic extract of *E. elatior* inflorescence was 0.22 ± 0.03 mg/mL [22]. Furthermore, [7] reported that the different concentrations (2, 3, and 4 mg/mL) of flowers and leaf extracts of *E. elatior* suppress collagenase activity in a dose-dependent manner. At 4 mg/mL, the collagenase inhibition activities were 51.73 ± 0.20 % and 41.54 ± 0.75 % respectively [7]. Thus, a higher concentration of extracts resulted in higher inhibition activity.

The *P. emblica* fruit extract expressed low IC₅₀ values which were 95.97 ± 3.28 µg/mL and 89.32 ± 0.88 µg/mL for MMP-1 and MMP-2 respectively [23]. The results showed that the number of extracts needed to inhibit MMP-2 was less compared to MMP-1. Inhibitor of MMPs activity is useful as it prevents the collagenase from cleaving the X-gly bond of collagen and –Pro-X-Gly-Pro chain of synthetic peptides thus, inhibiting collagen degeneration [19]. X is any amino acid in which the amino terminus is obstructed.

The inhibition percentage of MMP-1 was proportionate (98, 101, 106 and 110 %) to the concentrations (5, 10, 30 and 50 mg/mL) for *P. minus* extracts [27]. Similarly, the collagenase inhibition of MMP-13 (100, 101 and 102 %) was proportionate to the concentration (5, 10 and 30 mg/mL) [27]. Thus, the concentration of the extract was effective at 5 mg/mL for *P. minus*. The

highest collagenase inhibition percentage was *P. odorata* leaves (71.00 ± 1.08 %) followed by *S. alata* leaves (41.49 ± 2.63 %) and *C. asiatica* leaves (<10 %).

Table 1: Species of herbs and its collagenase inhibition activity

Species of herbs	Parts of plant	IC ₅₀ or Collagenase inhibition (%)	References
<i>E. elatior</i>	Inflorescence	IC ₅₀ = 0.22 ± 0.03 mg/mL	[22]
	Flowers	IC ₅₀ = 3.89 ± 0.17 mg/mL	
	Leaves	IC ₅₀ = 5.02 ± 0.47 mg/mL	[7]
<i>P. emblica</i>	Fruits	IC ₅₀ = 95.97 ± 3.28 µg/mL (MMP-1)	[23]
		IC ₅₀ = 89.32 ± 0.88 µg/mL (MMP-2)	
<i>P. odorata</i>	Leaves	Collagenase inhibition (%) = 71.00 ± 1.08	[18]
<i>S. alata</i>	Leaves	Collagenase inhibition (%) = 41.49 ± 2.63	[20]
<i>C. asiatica</i>	Leaves, stems	Collagenase inhibition (%) <10	[19]

Total phenolic content (TPC)

TPC activity is the process to figure out the amount of phenolic content in the extracts [28]. Based on [18, 20, 23, 34, 35, 36], stem, leaf, and flower contain phenolic compounds to help boost anti-collagenase, antioxidant, and anti-elastase activity. Phenolic compounds are the major class of secondary metabolites present in plants and can be further categorized into phenolic acids and polyphenols. These compounds can also be categorized into other subgroups such as flavonoids, tannins, lignans, and stilbene [29]. Polyphenols exhibit potent antioxidant activities that allow them to scavenge a broad range of ROS such as hydroxyl radicals and superoxide radicals [30]. Furthermore, it may inhibit the action of proteolytic enzymes *in vitro* by functioning as a complexing or precipitating agent [30]. A high level of radical scavenging potential is associated with the comparatively higher accumulation of different secondary metabolites.

Different types of solvents were used for the extraction. Methanol was used as solvents to extract aerial parts of *C. asiatica* [31]. Ethanolic extraction was carried out to extract the stems and leaves of *P. odorata* [32]. The existence of hydroxyl group side chains caused it to be more

soluble in polar organic solvents, therefore ethanol, methanol, and water were selected as the extracting solvent.

Based on the accumulation of studies, the TPC was discovered using the Folin-Ciocalteu (FC) method for all of the herbs [7, 20, 22, 23, 31, 32]. Folin-Ciocalteu reagent is a combination of phosphomolybdate and phosphotungstate, which oxidizes phenolates and reduces heteropoly acids [23]. A blue-colored complex was formed in which the intensity of the color is corresponding to the amount of reactive phenolic content [33]. The herbs extracts were diluted in distilled water and added with FC reagent. Then, sodium carbonate (Na_2CO_3) solution was added to end the activity [32], followed by incubation in dark at room temperature. The sample solution absorbance was measured at 750-765 nm. The gallic acid worked as a standard in determining TPC and was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). Table 2 shows the results of the total phenolic content of all herbs. The results of TPC were expressed in mg GAE/g. The ethanolic extracts of *P. emblica* fruit TPC was 362.43 ± 11.22 mg GAE/g [23]. Another study found that the TPC of ethyl acetate extract of *P. emblica* stem bark was 12.818 g GAE/100 g [34].

Table 2: Species of herbs and their total phenolic content

Species of herbs	Extraction	Parts of plant	Total phenolic content	References
<i>E. elatior</i>	Hydroglycolic	Inflorescence	13.85 mg GAE/g crude extract	[22]
	Aqueous	Flowers	38.68 ± 0.45 mg GAE/g	[7]
Leaves		246.52 ± 0.26 mg GAE/g		
<i>P. emblica</i>	Ethanolic	Fruits	362.43 ± 11.22 mg GAE/g dry basis	[23]
<i>P. odorata</i>	Ethanolic	Stem, leaves	85.31 ± 4.52 mg GAE/g dry basis (Fresh)	[32]
			8.60 ± 1.54 mg GAE/g dry basis (Dried)	
<i>S. alata</i>	Ethanolic	Leaves	36.83 ± 2.30 mg GAE/g	[20]
<i>C. asiatica</i>	Methanolic	Aerial parts	23.1 ± 0.2 mg GAE/g dry basis	[31]

The TPC result of *E. elatior* inflorescence extract was 13.85 mg GAE/g crude extract [22]. Meanwhile, the aqueous extract of the flowers and leaves was 38.68 ± 0.45 mg GAE/g and 246.52 ± 0.26 mg GAE/g respectively [7]. A high amount of total phenolic and flavonoids were discovered in the leaf, stems, flower, and rhizomes of *E. elatior* [35]. TPC for ethanolic extract of stem and leaves of *P. odorata* study has also been reported [32]. TPC in fresh plant extracts (85.31 ± 4.52 mg GAE/ g dry basis) was higher than dried plant extracts (8.60 ± 1.54 mg GAE/ g dry basis) [32]. Meanwhile, the TPC value for ethanolic extract of *S. alata* leaves was 36.83 ± 2.30 mg GAE/g [20]. Lastly, the TPC for a methanolic extract of *C. asiatica* aerial parts 23.1 ± 0.2 mg GAE/g on a dry basis was reported [31].

Besides, based on previous studies [13, 18, 20, 23, 34, 35, 36], all of these herbs contained flavonoids. Flavonoids possess a large number of biological activities including UVB defense, anti-inflammatory, anti-hepatotoxicity, and anti-cancer [20]. Flavonoid is a crucial element as its 3-hydroxyflavon structure chelated Zn metal [23], especially in MMPs preventing the metalloproteinase catalytic mechanism. Thus, collagenase activity cannot function normally. Besides, tannins can cause protein precipitation which leads to tissue refinement [13]. *E. elatior* and *S. alata* were found to exhibit tannins. Other notable natural compounds that might contribute to anti-collagenase properties are quercetin, quercetin-3-O-rhamnoside, catechin, and kaempferol [18]. These natural compounds were expressed by *P. odorata*.

The results showed that the inhibition effect of herbs extract involves certain mechanisms. As collagenase is a member of the MMPs family, the Zn ion active site is involved in assisting the reaction with an inhibitor. The presence of metal chelators from herbs extracted such as polyphenols, catechin, and tannin may attach to the active site and inhibit the substrate from the digestion of the enzyme. For instance, the polyphenol is attached by its hydroxyl groups. There could be an interaction between the hydroxyl group and collagenase side chains [23]. There could be enzyme dysfunction due to the conformational changes when there is a hydrophobic interaction between rings of polyphenol and collagenase [24]. The existence of phenolic compounds largely contributed to the anti-collagenase activity. It was demonstrated in another study that *Hemerocallis* cultivars contain significant amounts of phenolic compounds with good skin-related activities [37].

CONCLUSION

In conclusion, all five herbs reviewed in this study have the potential of inhibiting collagenase activity and possess TPC. The inhibition of collagenase activity can occur in a dose-dependent manner such as for *E. elatior* and *P. minus* extracts. Furthermore, different solvents used in the extraction method and different parts of plants vary the results of anti-collagenase activity and total phenolic content. The phenolic content and other compounds such as flavonoid, act as a crucial element in anti-collagenase activity. Further study should be done by using the same method and

same solvents to ensure the results of all herbs can be standardized and more reliable. The natural compounds have other pharmacological benefits such as antioxidant, anti-tyrosinase, or anti-elastase which also helps to reduce skin aging.

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