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Research Article

In Silico Evaluation of Anti-Aging Potential of *Centella asiatica* L. Urb. Extract via MMP-1 and Elastase Inhibition and Its Microneedle Formulation

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Abstract. Ultraviolet radiation accelerates premature skin aging by increasing the production of matrix metalloproteinases (MMPs) and elastase, leading to degradation of the extracellular matrix (ECM). Centella asiatica (L.) Urb. is known to contain bioactive compounds with anti-aging potential; however, conventional topical formulations such as gels and suspensions often show limited skin penetration. This study aimed to identify bioactive compounds from C. asiatica herb extract, evaluate their anti-aging activity through in silico inhibition of MMP-1 and elastase, and analyze the effect of different extract concentrations on the physical characteristics of microneedles (MNs) formulations. The extract was obtained using ultrasonic-assisted extraction (UAE) with 70% ethanol and analyzed by GC–MS. Molecular docking was performed using AutoDock Tools against MMP-1 (PDB ID: 966C) and elastase (PDB ID: 1BRU). Drug-likeness and safety profiles were evaluated using Lipinski's rule of five and ADMET prediction via pkCSM. MN formulations were prepared with extract concentrations of 1%, 3%, and 5% and evaluated for physical characteristics. The results indicated that 9,12-Octadecadienoic acid (Z,Z)-, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester and 2-Carbamyl-9-[β-D-ribofuranosyl] hypoxanthine showed strong binding affinity toward both enzymes and were predicted to be safe. Higher extract concentrations significantly affected pH, moisture content, and average weight ($P<0.05$). All MN formulations exhibited folding endurance above 350 cycles. The needle morphology test showed sharp needle tips. Overall, the evaluated parameters results complied with microneedle quality standards.

Keywords: Elastase; Herba Pegagan; In Silico; Microneedles**;** MMP-1.

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1. Introduction

Premature skin aging is a common dermatological concern among women and has a significant impact on self-confidence. A survey conducted by Jakpat in 2021 reported that approximately 60% of female respondents aged around 20 years exhibited signs of premature aging, including wrinkles, dark spots, enlarged pores, fine lines, dull and dry skin, as well as decreased skin elasticity [1]. The primary contributing factor to skin aging is ultraviolet (UV) radiation exposure. UV radiation induces the expression of matrix metalloproteinases (MMP-1) and elastase, enzymes that play a crucial role in the degradation of extracellular matrix (ECM) components. Increased degradation of collagen and elastin accelerates the premature aging process of the skin [2].

Centella asiatica (L.) Urb., has been reported to possess anti-aging activity attributed to its triterpenoid compounds, particularly asiaticoside (AD). An in silico study conducted by (Budiman et al., 2025) demonstrated that asiaticoside exhibits inhibitory activity against several aging-related enzymes, including collagenase (PDB ID: 4JP4), hyaluronidase (2PE4), tyrosinase (1WX2), and elastase (1HAZ), with binding affinity values of −16.7, −12.7, −16.3, and −11.3 kcal/mol, respectively

These findings indicate a strong inhibitory potential of AD against elastase and collagenase, reflected by its favorable binding affinity values. However, the effectiveness of *C. asiatica* bioactive compounds in conventional topical formulations is often limited by inadequate skin penetration. Microneedles (MNs) have emerged as a promising alternative delivery system due to their ability to by pass the stratum corneum barrier.

2. Method

The materials used were *Centella asiatica* (L.) Urb. herb simplicia. The technical-grade materials included PVP, PVA, glycerin, distilled water (aquadest), and 70% ethanol. The equipment used in this study were an ultrasonic bath (Benchmark Scientific), a light microscope, a moisture analyzer (Mettler Toledo), a centrifuge (Gemmy PLC-05), AutoDock Tools software, BIOVIA Discovery Studio software, and a 3D printer (Anycubic Mono 4 10K).

Centella asiatica (L.) Urb. herb simplicia was obtained and taxonomically identified at the UPF Yankestrad Laboratory, Tawangmangu. The *C. asiatica* herb was extracted using the Ultrasound-Assisted Extraction (UAE) method at a ratio of 1:50 (simplisia: 70% ethanol). The UAE process was conducted at 37°C for 32 minutes with a frequency of 38 kHz. The extract was then concentrated, and the extraction yield was calculated.

$$Yield\ (\%) = \frac{Weight\ of\ concentrated\ extract\ obtained}{Weight\ of\ simplicia\ used} \times 100\%$$

Compound identification was performed using an Agilent GC–MS system equipped with an HP-5MS column (30 m × 250 µm × 0.25 µm). The oven temperature was programmed from 70°C to 330°C. The operating pressure was set at 8.8085 psi, with a column flow rate of 1 mL/min. Helium (He) was used as the carrier gas, and detection was carried out using a mass selective detector (MSD).

The compound structures were obtained from PubChem. The compounds were converted into 3D structures and energy-minimized using ChemDraw. Hydrogen atoms and charges were added, torsion angles were assigned, and redocking and molecular docking were performed using AutoDock Tools. The collagenase enzyme (PDB ID: 966C) was prepared with a grid box size of 40 × 40 × 40 Å at coordinates X:Y:Z (9.166: −10.353: 38.398), and the elastase enzyme (PDB ID: 1BRU) was prepared with a grid box size of 25 × 25 × 25 Å at coordinates X:Y:Z (23.204: 44.660: 17.090) for redocking. The redocking procedure was considered valid if the root mean square deviation (RMSD) was < 2.0 Å with 10 conformations. Molecular docking was subsequently performed with 100 conformations (Arda et al., 2024). Molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors, and partition coefficient were evaluated according to Lipinski's Rule of Five (Roskoski, 2024). ADMET properties, including skin absorption, logP, and membrane permeability, were predicted using pkCSM [4].

Table 1. Formulation of MNs Extract Centella asiatica L. Urb.

Materials	Concentration		
	F1	F2	F3
Centella asiatica L. Urb. Extract	1%	3%	5%
Base (ad)	100%	100%	100%
		Base Ratio	
PVA 30%	7	7	7
PVP 10%	2,5	2,5	2,5
Glycerine	0,5	0,5	0,5

Microneedle formulations were prepared using PVA 30%, PVP 10%, and glycerin in a ratio of 7:2.5:0.5. PVA was dissolved and developed at 90°C, and all components were mixed until homogeneous. The extract was added according to the designated concentration. The mixture was then poured into microneedle molds and centrifuged at 3500 rpm, followed by drying at room temperature for 5 days.

Characteristic of Microneedle (MNs) Formulations

Organoleptic Test

The MNs were visually observed for physical characteristics, including shape, color, and odor.

Homogeneity Test

The MNs were examined for the presence or absence of aggregates and for color uniformity within the formulation.

pH Test

The pH of the formulation was measured to determine its suitability for skin application. The physiological pH range of the skin is 4–8 [5].

Moisture Content Test

Moisture content analysis was conducted to determine the water content in the MNs [6].

Needle Morphology Test

The needle morphology was observed to evaluate the structural characteristics of the MNs. The MNs were sectioned vertically, and the cross-sectional morphology was examined [7].

Weight Uniformity Test

The test was performed by individually weighing 20 MNs using an analytical balance, and the average weight was calculated [8].

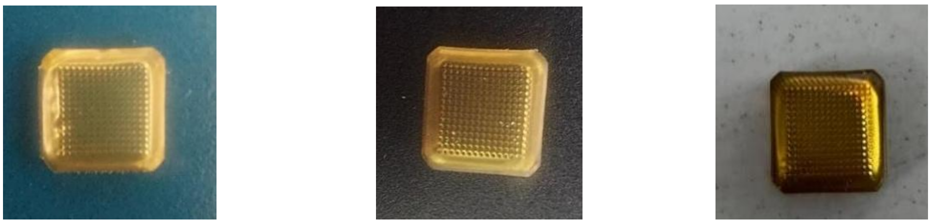
Folding Endurance Test

The test was conducted by repeatedly folding the same section of the MNs until the formulation was damaged. The acceptable standard for folding endurance was >200 folds [8].

3. Results and Discussion

The *Centella asiatica* herb extract produced a yield of 44.56%, which was higher than that reported in a previous study where the herb was extracted using the maceration method, resulting in a yield of 8.883% [9]. Compound identification using GC–MS revealed 18 active compounds, with three major compounds identified as 5-hydroxymethylfurfural, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, and n-hexadecanoic acid. The identified compounds were analyzed in silico to determine their potential to inhibit collagenase and elastase enzymes. Eighteen compounds were analyzed in silico using the molecular docking method to evaluate ligand–protein interactions, ADMET analysis (Absorption, Distribution, Metabolism, Excretion, and Toxicity), and evaluation of Lipinski's Rule of Five to determine the potential pharmacokinetic profile of the ligands. Validation (redocking) results showed an RMSD value of 0.99 Å for 966C and 1.92 Å for 1BRU.

9,12-Octadecanoic acid (Z,Z)-, 2-(acetyloxy)-1-(acetyloxy)methyl]ethyl ester and 2-Carbamyl-9-[β-D-ribofuranosyl]hypoxanthine did not cause skin sensitization based on ADMET analysis. 9,12-Octadecadienoic acid (Z,Z)-, 2-[(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester showed good binding activity toward the collagenase enzyme with a binding energy value of −8.46, fulfilled Lipinski's Rule of Five criteria, and did not cause skin sensitization. 2-Carbamyl-9-[β-D-ribofuranosyl]hypoxanthine showed good binding activity toward the elastase enzyme with a binding energy value of −4.26, fulfilled Lipinski's Rule of Five criteria, and did not cause skin sensitization. However, this compound exhibited limited skin permeability, as indicated by skin permeability values of −2.691 and −2.735, which are considered promising for the development of microneedle (MNs) formulations because MNs interact directly with the skin by penetrating the stratum corneum, which has high lipophilicity. Microneedles (MNs) are able to enhance drug penetration through transcellular and trans-appendageal pathways across the stratum corneum. The type of MNs used in this study was dissolving MNs, as they are capable of rapidly releasing macromolecules through a poke-and-release mechanism. MNs formulations were prepared with extract concentrations of 1%, 3%, and 5%, as shown in Figure 1.



(A) (B) (C)

Figure 1. Appearance of microneedle (a) F1 (b) F2 (c) F3.

The MN formulations were evaluated for their physical characteristics. The results of the physical characteristic tests of the MNs formulations are presented in Table 2.

Table 2. Characteristic of MNs Centella asiatica Herb Extract.

Evaluation		F1	F2	F3
Organoleptic	Color	Green	Dark green	Dark green
	Shape	Square patch	Square patch	Square patch
	Odor	Odorless	Odorless	Odorless
Homogeneity		Homogenous	Homogenous	Homogenous
Needle Morphology		Sharp	Sharp	Sharp
Average Weight (g)		0,1161±0,0111	0,1234±0,0081	0,1337±0,0070
Folding Endurance		>350	>350	>350
pH		5,81±0,06	5,61±0.01	5,54±0,06
MC		4,51±0,37	5,05±021	6,64±1,26

Information :

F1: MNs containing *Centella asiatica* herb extract at 1% concentration.

F2: MNs containing *Centella asiatica* herb extract at 3% concentration.

F3: MNs containing *Centella asiatica* herb extract at 5% concentration.

All results are presented as mean ± standard deviation (n = 3).

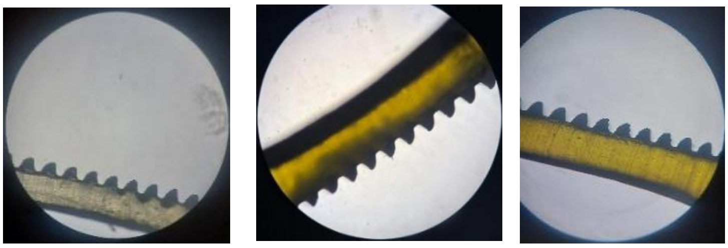
The organoleptic test showed that the formulations formed flexible patches, were homogeneous, and contained no visible air bubbles. Increasing the concentration of extract in the formulation resulted in a darker color intensity. PVP and PVA are polymeric materials that contribute to good physical appearance of the formulations.

The homogeneity test evaluated the uniform distribution of particles, both extract and excipients, within the MN formulations. The polymer matrix formed flexible and homogeneous patches without the presence of air bubbles [10]. As shown in Table 2, all MN formulations were declared homogeneous. These results are related to the properties of PVA and PVP, which are water-soluble polymers capable of forming stable formulations during the mixing and drying processes [11].

The pH test of the MN formulations showed values of 5.54 ± 0.06 for F1, 5.61 ± 0.01 for F2, and 5.54 ± 0.06 for F3. The pH values were within the acceptable range of 4–8 for topical skin preparations, and the base formulation exhibited a pH of 6.39. A previous study reported MN formulation pH values in the range of 5.61–7.15 [8]. Therefore, the MN formulations met the standard pH requirements for topical preparations and demonstrated good acceptability.

The moisture content results were 4.51 ± 0.37 for Formula 1, 5.05 ± 0.21 for Formula 2, and 6.64 ± 1.26 for Formula 3. The moisture content values met the standard requirements for MN formulation. A moisture content greater than 10% may increase elasticity and cause the formulation to tear easily, whereas very low moisture content may result in a rigid structure and lead to cracking.

The needle morphology test was conducted to assess the quality of needle formation and to determine the presence of any structural damage to the MNs [7]. The visual appearance of the MNs was observed using a light microscope at 4×10 magnification, as shown in Figure 4. F3 exhibited a sharper needle profile compared to F1 and F2.



(A) **(B)** **(C)**

Figure 2. Microscopic appearance of MN formulation Formula 1 (A), MN formulation, Formula 2 (B), and MN formulation Formula 3 (C).

The average weight results were 0.1161 ± 0.01 for F1, 0.1234 ± 0.01 for F2, and 0.1337 ± 0.01 for F3. The results of the three formulations were not uniform, as the standard deviation (SD) values were >0.005. The lack of uniformity was presumed to be influenced by the drying process of the MN formulations at uncontrolled room temperature [12].

The folding endurance test showed that all formulations exhibited good flexibility, as no damage was observed after 350 folds. These results met the standard requirement for MN formulations (>200 folds) [8]. Good folding endurance indicates that the formulations were flexible and not easily broken.

4. Conclusions

The *Centella asiatica* herb extract contained 18 compounds, with three major compounds identified as 5-hydroxymethylfurfural, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, and n-hexadecanoic acid. The best candidate compounds for anti-aging activity in inhibiting the MMP-1 enzyme (966C) were 9,12-octadecadienoic acid (Z,Z)-, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl, while 2-carbamyl-9-[β-D-ribofuranosyl]hypoxanthine showed the best activity against the elastase enzyme (1BRU). Both compounds passed the skin sensitization test. The results of the MN characterization tests showed significant differences ($P < 0.05$) in pH, moisture content (Formula 1 vs. Formula 3 and Formula 2 vs. Formula 3), and average weight (Formula 1 vs. Formula 3 and Formula 2 vs. Formula 3). The folding endurance test results for all three formulations were >350 folds. The needle morphology test showed sharp needle structures. Overall, the evaluation results met the quality standards for MN formulations.

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