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Research Article

In Silico Study of Pomegranate (*Punica granatum* L.) Peel Extract Anti-Aging Activity through MMP-1 and Elastase Inhibition and Microneedle Formulation

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Abstract: Premature skin aging can be induced by ultraviolet radiation, which increases the activity of matrix metalloproteinases (MMPs) and elastase, resulting in accelerated degradation of collagen and elastin in the extracellular matrix (ECM). Pomegranate peel exhibits anti-aging potential; however topical delivery is limited by low penetration through the stratum corneum. Therefore, microneedles were developed as an alternative delivery system. This study aimed to evaluate the anti-aging activity of pomegranate peel extract compounds using in silico approach and to assess the physical characteristics of a microneedle formulation containing the extract. Ultrasound-assisted extraction (UAE) was employed to obtain the extract and the compounds were identified by GC–MS. Molecular docking was performed using AutoDock Tools against MMP-1 (PDB ID: 966C) and elastase (PDB ID: 1BRU). ADMET profiling and Lipinski's rule of five were analyzed using the pkCSM platform. Microneedles were formulated and evaluated for organoleptic properties, homogeneity, needle morphology, folding endurance, pH, average weight, and moisture content. The results showed that three major compounds exhibited strong binding affinity toward MMP-1 and elastase based on binding energy, and inhibition constant. All microneedle formulations (0.5%, 1%, and 2% extract) met quality requirements, with no significant differences in pH, average weight, and moisture content.

Keywords: Elastase; In Silico; Microneedle; MMP-1; Pomegranate.

1. Introduction

Premature skin aging, characterized by features such as loose skin, uneven tone, and textural changes, is a common and growing concern among adults. In a recent cross-sectional survey of over 1400 participants, nearly one-third reported dissatisfaction with their skin, and 44% stated that they felt less happy about their skin now compared with five years earlier, with aging-related features among the top concerns assessed. These findings suggest that concerns about aging skin affect adults broadly and are shaped by both intrinsic factors and changing social perceptions [1]. The main component of dermis is extracellular matrix (ECM), with its main component is collagen fiber that give dermis its strength and elasticity. Type-1 collagen is regulated by MMP-1, an endopeptidase enzyme that degrade and destroy ECM protein mainly type 1 and type 3 collagen [2]. Elastic fibers are the other components that makes ECM, these fibers recover the skin to its original form after stretched and deformed. These fibers will be degraded by elastase enzyme if the skin is exposed to UV radiation [3]. One of the plants that have a popular reputation on its antiaging activity is pomegranate (*Punica granatum* L) [4]. The fruit contains ellagic acid and gallic acid that have a great antiaging ability through the inhibition of MMP-1 and elastase enzyme [5]. Recent research on pomegranate peel extract showed that it could inhibit enzymes such as collagenase and elastase, and docking analysis supported that several pomegranate peel compounds were able to bind to the active sites of these enzymes.

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This suggests that pomegranate peel may contribute to anti-aging effects by helping reduce the breakdown of collagen and elastic fibers in the skin [6]. Bioactive compounds found in pomegranate (gallic acid, ellagic acid, punicalin, punicalagin) has high molecular weight and polar to semipolar properties, which makes their penetration through stratum corneum really low. Most polyphenol compounds will be accumulated in stratum corneum if not formulated in particular delivery system, or without any specific carrier such as in conventional cream or emulsion [7].

Microneedles usage results in higher compounds absorption than topical application [8]. Small micro-sized needles formed microcanals through stratum corneum, resulting in effective compound delivery inside the skin with concentration to cause effect.[9]. Vora et al (2018) test the permeation of calciferol which has a high molecular weight with nonpolar property the calciferol nanosuspension formulated in microneedles resulting in higher penetration with 489,19 μg than the one formulated in film patch with the penetration of only 73,2 μg [10].

This study aim to investigate compounds contained in pomegranate peel extract and its antiaging activity through MMP-1 and elastase inhibition using in silico method, also formulating a microneedles system containing the extract.

2. Method

This study aim to analyze the antiaging activity of compounds identified with gas chromatography-mass spectrometry (GC-MS) from pomegranate peel extract (PPE) using in silico method, also the physical characteristics of microneedles containing PPE. Tools used in this study are ultrasonic homogenizer, rotary vacuum evaporator, waterbath, evaporating dish, GC-MS, laptop, software Autodock Tools, Biovia Discovery Studio, microneedles formulated using 3d printer, centrifuge, glassware, desiccator, light microscope, moisture analyzer.

Materials used in this study include pomegranate peel powder, technical grade materials include ethanol 96%, PVP, PVA, and distilled water. Pomegranate peel powder mixed with 96% ethanol with 1:5 ratio. ultrasound-assisted extraction (UAE) is performed using 60% of power ratio, horn 6 for 60 minutes. Filtrate then put on the evaporator to reduce the solvent. PPE bioactive compounds identified using GC-MS with HP-5MS column (30 m × 250 μm × 0,25 μm), oven temperature of 60 °C-325 °C, 8,2317 psi pressure, flow rate of 1 mL/min, helium (He) as the gas carrier, and MSD detector.

The 3D structures of MMP-1 (PDB ID: 966C) and elastase (PDB ID: 1BRU), which were bound to their natural inhibitors, were obtained from the RCSB Protein Data Bank. The 3D structures of compounds identified from GC-MS were obtained from PubChem. The enzyme structures of MMP-1 and elastase were prepared using BIOVIA Discovery Studio, including structure cleaning and residue arrangement. In addition, the structures were confirmed to contain no residues with non-integral charges, in accordance with the receptor file requirements in AutoDock Tools. The ligand structures were prepared using ChemDraw 3D for energy minimization, followed by the addition of hydrogen atoms and torsion settings using AutoDock Tools. Validation was performed using the re-docking method, by docking the natural inhibitor of each enzyme back into its enzyme structure. This validation aimed to ensure that the docking method was able to accurately place the natural inhibitor into the active site, similar to the reference complex downloaded from the Protein Data Bank. The grid box parameters used for MMP-1 docking were X = 9.166; Y = −10.353; Z = 38.398 with a dimension of 40 Å, while for elastase the grid box was X = 23.204; Y = 44.660; Z = 17.090 with a dimension of 25 Å and a spacing of 0.375 Å. The resulting complex from re-docking was compared with the reference complex, and the root mean square deviation (RMSD) value was calculated.

Table 1. Microneedles formula.

Materials	Concentration (w/w)		
	F1	F2	F3
PPE	0,5%	1%	2%
Base added until	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 formulation was started by preparing the mold. The mold was produced using a 3D printing method with water washable resin material, with a hole depth of 1500 μm, diameter of 350 μm, and a distance between holes of 500 μm. The microneedle were prepared by first dissolving and swelling PVA and PVP at 90°C for 5 minutes. The polymer base and extract were then mixed according to the concentration ratio shown in Table 1, weighed, and stirred until homogeneous. The mixture was poured into the microneedle mold and centrifuged at 4000 rpm for 10 minutes. The mold containing the mixture was then dried in a desiccator for 6 days. After drying, the microneedle were evaluated for physical characteristics including organoleptic properties, homogeneity, pH, needle morphology, average weight, folding endurance, and moisture content.

3. Results and Discussion

UAE extraction produced a pomegranate peel extract yield of 21.19%. Compound identification using GC-MS showed 22 compounds, with the three compounds with the largest peak areas being Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl; 5-Hydroxymethylfurfural; and Isopropyl palmitate.

The 22 identified compounds were then tested for their anti-aging activity through inhibition of MMP-1 and elastase enzymes. Validation of the grid box parameters resulted in an RMSD value of 1.24 Å for MMP-1 re-docking with its natural ligand N-hydroxy-2-[4-(4-phenoxy-benzenesulfonyl)-tetrahydro-pyran-4-yl]-acetamide, and 1.92 Å for elastase re-docking with 2-(2-hydroxy-cyclopentyl)-pent-4-enal. These results indicate that the docking parameters were valid and could be used for in silico testing. The three compounds with the strongest binding are shown in Table 2.

Table 2. Molecular docking results.

	Compound name	Molecular docking results		
		Run	Binding energy	Inhibition Constant
	MMP-1			
1	*2,6,10,14-Hexadecatetraene, 1-benzyloxy-9-(phenylthio)-3,7,11,15-tetramethyl-*	30	-9,4	129.63 nM[1]
2	*2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane*	97	-9,12	208.09 nM
3	*2,7,11-Trimethyl-4-phenylthiododeca-2,6,10-triene*	32	-8,92	287.86 nM
	Elastase			
1	*2,7,11-Trimethyl-4-phenylthiododeca-2,6,10-triene*	66	-5,88	49.36 μM[2]
2	*(5R,10R)-6,10-Dimethyl-2-(propan-2-ylidene)spiro[4.5]dec-6-en-8-one*	62	-5,14	170.68 μM
3	*trans-Z-.alpha.-Bisabolene epoxide*	44	-5,13	173.61 μM

[1] nanomolar. [2] micromolar

Lipinski's rule of five test showed that the six compounds had relatively large molecular weights in the range of 218.34–488.781 Da and high Log P values (>3). A suitable Log P value for skin penetration is 1–3; if Log P < 1, the compound will not be able to penetrate the stratum corneum, and if Log P > 3, the compound may penetrate but will tend to accumulate in the stratum corneum (Schwinn & Jackson, 2020). Therefore, microneedle are considered an appropriate delivery system to deliver these compounds into the dermis, where MMP-1 and elastase enzymes activity happened.

ADMET parameter prediction showed that 2,6,10,14-Hexadecatetraene,1-benzyloxy-9-(phenylthio)-3,7,11,15-tetramethyl-; 2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane; and tridec-2-yn-1-yl trans-4-methylcyclohexyl ester did not show potential to cause skin sensitization.

Initial ADMET prediction screening also indicated potential skin sensitization for the major compound Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl. This finding is an important consideration from a safety aspect, however ADMET prediction cannot be directly equated with actual toxic effects, because toxicity is influenced by the compound concentration in the formulation, exposure dose, and the delivery system used.

Microneedle were formulated using PVA and PVP polymers because they have good water solubility, good biocompatibility, and have been widely used in microneedle formulations. PVA functions to provide mechanical strength to the needles to ensure skin penetration, while PVP functions to increase water solubility, which helps the needles dissolve faster after insertion. Glycerin was used as a plasticizer to increase flexibility and prevent needle tip damage, and it also acts as a humectant to hydrate the skin. The physical characteristics results are shown in Table 3.

Table 3. Physical characteristics of microneedle.

Characteristics		F1	F2	F3
Organoleptic	Form	Square patch	Square patch	Square patch
	Color	Light brown	Brown	Dark brown
	Odor	No smell	No smell	No smell
	Taste	Sweet	Sweet	Sweet
Homogenity		Homogenous	Homogenous	Homogenous
Needle morphology		Sharp	Sharp	Sharp
Average weight (g)		0,11303 ± 0,009	0,10463 ±0,011	0,13116 ±0,003
Folding endurance		>350	>350	>350
pH		5,65 ± 0,24	5,43 ± 0,21	5,21 ± 0,29
MC (%)		5,65 ± 0,29	7,47 ± 1,19	7,85 ± 0,41

Organoleptic evaluation is needed as an initial physical test. Changes in color or odor may indicate degradation of active compounds or polymer base during formulation or storage. The organoleptic evaluation showed that all microneedle formulas (F1, F2, and F3) had consistent characteristics, with the same shape, odor, and taste, but different color intensity. The microneedle color became darker as the extract concentration increased. All formulas also produced homogeneous preparations. The appearance of the microneedle can be seen in Figure. 1.

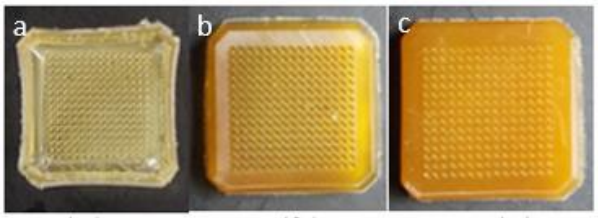

Figure 1. Appearance of microneedle (a) F1, (b) F2, (c) F3.

The pH values of microneedle in this study ranged from 5.21 to 5.65. Compared to a previous study reporting pH values of 5,6 to 6,4 [11], these results indicate that pomegranate peel extract microneedle are safe for topical use and are not likely to cause skin irritation. Statistical analysis showed that extract concentration did not have a significant effect on pH, suggesting that the polymer base has a stable buffering system, and the extract did not cause significant pH changes despite concentration variation.

Needle morphology testing was performed to evaluate the needle shape and to observe any damage in the molded needles. In this study, morphology evaluation showed that all formulas had needle tips that were sharp but slightly leaning sideways, as shown in Fig. 2. This sideways needle tip appearance may be influenced by the mold fabrication process using a different material compared to other studies.

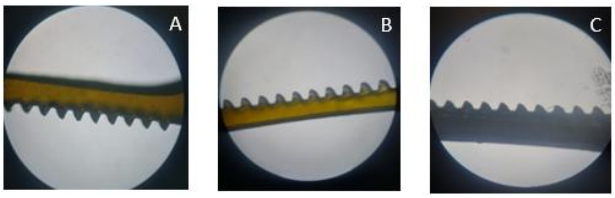

Figure 2. Needle morphology (a) F1, (b) F2, (c) F3.

The average weight analysis showed that only F3 met the requirement for average weight uniformity with SD = 0.003, while F1 and F2 did not produce uniform weight. This may be caused by a printing or casting process that was not yet optimal. This result indicates the need for further optimization in the formulation stage, particularly related to the casting volume. Statistical analysis did not show a significant effect of extract concentration variation on the average weight.

Folding endurance evaluation was performed to assess the microneedle's flexibility and durability. Good folding endurance is required to ensure the patch remains stable during application and storage. The folding endurance test showed that all formulas had folding endurance >350 folds, which meets the requirement of >200 folds [11]. This value indicates that the microneedle have good flexibility and are not easily broken.

Moisture content testing was performed because water content in microneedle can affect flexibility, brittleness, mechanical strength, and dissolution behavior in the skin. In this parameter, the moisture content is expected to be not more than 11% [12]. The moisture content results showed an increasing trend across formulas with a range of 5.65–7.85%, and no significant effect of extract variation on moisture content was found. The increase on each formula is likely due to the higher extract concentration; since the extract is in a viscous form, it increases the viscosity of the base mixture that is molded, thereby making it more difficult for water to evaporate during the drying process.

Based on the physical characteristic evaluation, pomegranate peel extract microneedle with extract concentration variations of 0.5%, 1%, and 2% did not show significant differences across all tested physical parameters. The initial hypothesis stated that increasing extract concentration would affect the physical characteristics of the preparation. However, these results indicate that within the concentration range of 0.5%, 1%, and 2%, the extract amount was not sufficient to cause significant changes in the evaluated physical parameters.

4. Conclusions

Pomegranate peel extract (*Punica granatum* L) contains 22 compounds, with three major compounds being phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl; 5-Hydroxymethylfurfural; and Isopropyl palmitate. The three compounds that showed the strongest anti-aging activity in silico through MMP-1 and elastase inhibition, and did not show skin sensitization potential, were 2,6,10,14-Hexadecatetraene, 1-benzyloxy-9-(phenylthio)-3,7,11,15-tetramethyl-; 2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane; and tridec-2-yn-1-yl trans-4-methylcyclohexyl ester. Different extract concentrations did not have a significant effect on physical characteristics, including organoleptic properties, homogeneity, pH, needle morphology, average weight, folding endurance, and moisture content.

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