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Original Article

HR-LCMS-BASED METABOLITE PROFILING, AND ANTI-COLAGENASE PROPERTIES OF ETHANOLIC EXTRACT OF PIDADA MERAH: COMPUTATIONAL AND *IN VITRO* STUDY

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ABSTRACT

Objective: Extract of pidada merah (*Sonneratia caseolaris*) leaves has very strong antioxidant activity and has potential as anti-aging. This study aimed to determine the anti-collagenase activity *in silico* and *in vitro*. Molecular docking includes exploring proteins or nucleotides, modeling 3D structures, and calculating bond energies. Collagenases are enzymes that can hydrolyze native collagen into fragment collagen peptides.

Methods: Investigation of *in silico* docking activity for collagenase receptors (966C). We performed metabolomics analysis through HR-LCMS on the extract pidada merah. To explore the use value of anti-collagenase, we analyzed the molecular docking of metabolites profiling pidada merah. *In vitro* study used a collagenase assay kit.

Results: Metabolite profiling on the HR-LCMS from Pidada Merah extract are A (AL_8810), B (NP_001596), C (NP_018716) and D (NP_021797). The anti-collagenase test showed the IC50 value = 26.74±0.40 ppm, which is the very strong category. NP_018716 has the lowest binding energy value with the target protein, which is -6.0, and binds to THR241 (2.24Å) and SER239 (3.35Å) and is the best compound according to calculations.

Conclusion: The results of this study indicate that the Extract Pidada merah has the Potential to be developed as a new drug for antiaging.

Keywords: Anti-collagenase, *In silico*, *In vitro*, docking molecular, Pidada merah

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INTRODUCTION

The use of Indonesian medicinal plants for the treatment of a disease is only based on empirical experience passed on from generation to generation without supporting data that meets the requirements. To be accepted in modern medicine, several conditions must be met, especially the active substance, efficacy, and safety level [1]. Pidada Merah (*Sonneratia caseolaris*) is a woody mangrove species that is widely distributed in tropical coastal areas [1]. Extracts from various parts of the mangrove trees such as *Sonneratia caseolaris*, *Sonneratia avatar, Sonneratia apetala, Sonneratia alba* Compounds with antioxidant, antiobesity, antibacterial, and antidiabetic [2-4]. This plant is very easy to find in coastal and estuarine areas where other plants are difficult to grow [2]. Several studies reported that almost all parts of the plant have pharmacological properties, including antidiabetic [3], antioxidant [4], antiseptic, analgesic [5], anti-inflammatory [6], and antimicrobial [7, 8].

The biochemical processes catalyzed by enzymes, providing useful molecular insights for the biochemistry of organisms at any given time, are known as metabolites. Metabolism produces primary metabolites that affect plant growth and development, and secondary metabolites can help plants withstand environmental stresses [8]. In Addition, metabolomics technology is widely used in the evaluation of plant quality [9-11]. Pidada merah contains secondary metabolites such as alkaloids, terpenoids, flavonoid phenols, and steroids [12-15]. Traditional use for use of traditional medicine has been carried out. Antioxidant activity Pidada merah which grows in East Kalimantan is one of the sources of anti-aging compounds that have not been widely disclosed [16]. *Sonneratia caseolaris* is used as a traditional cosmetic product by the Dayak and Banjar tribes (Indigenous People on the island of Borneo, Indonesia) which is called "Pupur Dingin". It contains some herbal medicines for skin care, but unfortunately, scientific evidence about it has not been known yet. Based on that circumstance, the study aims to investigate the potential antiaging medicine from *Sonneratia caseolaris* leaves.

MATERIALS AND METHODS

Plant materials and sample preparation

Pidada merah (*Sonneratia caseolaris*) leaves were collected from Sanga-sanga, Kutai Kartanegara, East Borneo, Indonesia induring Januari 2022. It is identified in Departement Biology, Faculty of Math and Natural Science, Universitas Mulawarman, Indonesia. The leaves were cleaned, separated, and then dried. After that, it is ground into a fine powder. The sample was sieved to obtain a uniform particle size, then stored in an air-tight container until further analysis.

Extraction

As many as 100 grams of powdered *Sonneratia caseolaris* leaves were put in a maceration container and added the ethanol 95% until the simplicia was submerged. Put aside it for 24 h and stir occasionally. The simplicia filtered and separated from the dregs. Furthermore, the dregs were macerated again using a new ethanol filter. It was conducted for three consecutive days. The ethanol 95% extract of *Sonneratia caseolaris* leaves was concentrated by a rotary evaporator.

Physicochemical examination

Physicochemical examination of the extract was identified using the High-Resolution Liquid Chromatograph Mass Spectrophotometer (HR-LCMS).

Collagenase test

Add 2 µl (25, 50, and 100 ppm) of sample extract into desired well(s) in 96 well plates and adjust the volume to 100 µl with collagenase Assay Buffer. For control, add 10 µl of provided collagenase (0.35U/ml). For inhibitor Control, add 10 µl of provided collagenase (0.35U/ml) and 2 µl of inhibitor (1.10 Phenanthroline) into desired well (s). Adjust the volume of ncontrol and inhibitor control wells to 100 µl with Collagenase Assay Buffer. For Reagent background control, add 100 µl of collagenase Assay Buffer.

Dissolve the test inhibitor to 100 x Final test concentration in an appropriate solvent to test collagenase inhibitors. Add 2 µl of test inhibitor and 10 µl of collagenase (0.35U/ml) into the test inhibitor well(s). Prepare a parallel well as Enzim Control (EZ) by adding 10 µl provided collagenase. Adjust the volume of the test inhibitor and Enzyme control well to 100 µl with collagenase Assay Buffer. Incubate at room temperature for 10 min. For each reaction, prepare 100 µl of Reaction Mix, containing 40 µl of collagenase substrate (FALGPA), ad 60 µl of collagenase assay buffer. Immediately measure in kinetic mode the absorbance (A) at 345 nm in a microplate reader at 37 °C for 5-15 min. Low-activity samples can be measured for 1-3 h.

$$\%\text{anticollagenase} = \frac{(\text{Abs sample} - \text{Abs control})}{(\text{Abs sample})} \text{x} 100\%$$

***In silico* validation**

Collagenase (966C) is associated with antiaging received from the protein database, used to create the 3-D crystal structure of the protein. Protein databases are three-dimensional structural data repositories for biological substances. The active site or receptor binding is determined using an online server. They are visual datasets that show the different molecules that make up their structure and a diagram of how those molecules are connected. The primary ID of the protein database from the protein structure was input into the home search box. The protein preparation wizard is used to create complex protein structures. Hydrogen ions are added automatically while the design is minimized and refined. All ligands were prepared for docking studies using Autodock Vina. Tautomer development and optimization for each ligand [17]. The force field calculates the partial atomic charge.

Molecular docking

The extra precision option (XP) links inflexible protein structures with elastic ligands. Created 100 poses for each docking calculation. To determine the potential non-polarity of the protein and ligand elements, the Van Der Waals (VDW) bonds were adjusted to 1.0 with a net atomic charge cut-off of 0.25 sub-units. In contrast, other elements, such as Van der Waals (VDW) bonds, are not adjusted. Glide docking with a cluster-based conventional method to find the best ligand binding region in a particular receptor lattice plane. The ligand with the lowest score had the strongest binding affinity for the enzyme [18, 19]. Molecular docking studies were performed for the collagenase target protein (966C) using the Autodock Vina software. The best-docked conformation determined by vina scoring was employed for the visual analysis [20]. Pose View, a program available through Protein PDB, was used to infer the intermolecular interactions of the protein-ligand combination.

RESULTS

Results show the new structure content, namely A (Formula: $C_{24}H_{31}FO_4$, Molecular Weight: 424.21, RT [min]: 20.046, Area (Max.): 17589246.47, mz Cloud Best Match: 89.6 is AL 8810); B (Formula: $C_{16}H_{30}O_4$, Molecular Weight: 308.19, RT [min]: 19.85, Area (Max.): 16939416.67, mz Cloud Best Match: 93.7 is NP_001596); C (Formula: $C_{11}H_{20}O_4$, Molecular Weight: 216.13, RT [min]: 12.18, Area (Max.): 14706854.10, mz Cloud Best Match: 84.9 is NP_018716); D (Formula: $C_{12}H_{22}O_3$, Molecular Weight: 214.16, RT [min]: 13.32, Area (Max.): 20043113.85, mz Cloud Best Match: 71.1 is NP_021797).

Fig. 1: Structure A (AL_8810), B (NP_001596), C (NP_018716) and D (NP_021797)

Table 1: The docking score, number of H-bonds, interacting residues, and bond length of the selected compounds to collagenase (966C)

Collagenase (966C)				
Ligan	**Docking score**	**Number of H-bonds**	**Interacting residues**	**Bond Length (Å)**
Native	-9.6	6	VAL215	5.33
			ASN180	3.05
			LEU181	1.96
			ALA182	5.24
			GLU219	2.74
			HIS228	1.88
				2.42
				2.37
				2.07
AL_8810	-5.8	5	HIS228	5.08
			ALA182	3.61
			GLU219	2.84
			LEU181	3.21
			HIS218	5.12
				5.29
				3.59
NP_001596	-5.7	2	THR241	2.25
			SER239	3.33
NP_018716	-6.0	2	THR241	2.24
			SER239	3.35
NP_021797	-5.8	2	TYR240	2.84
			HIS228	2.60

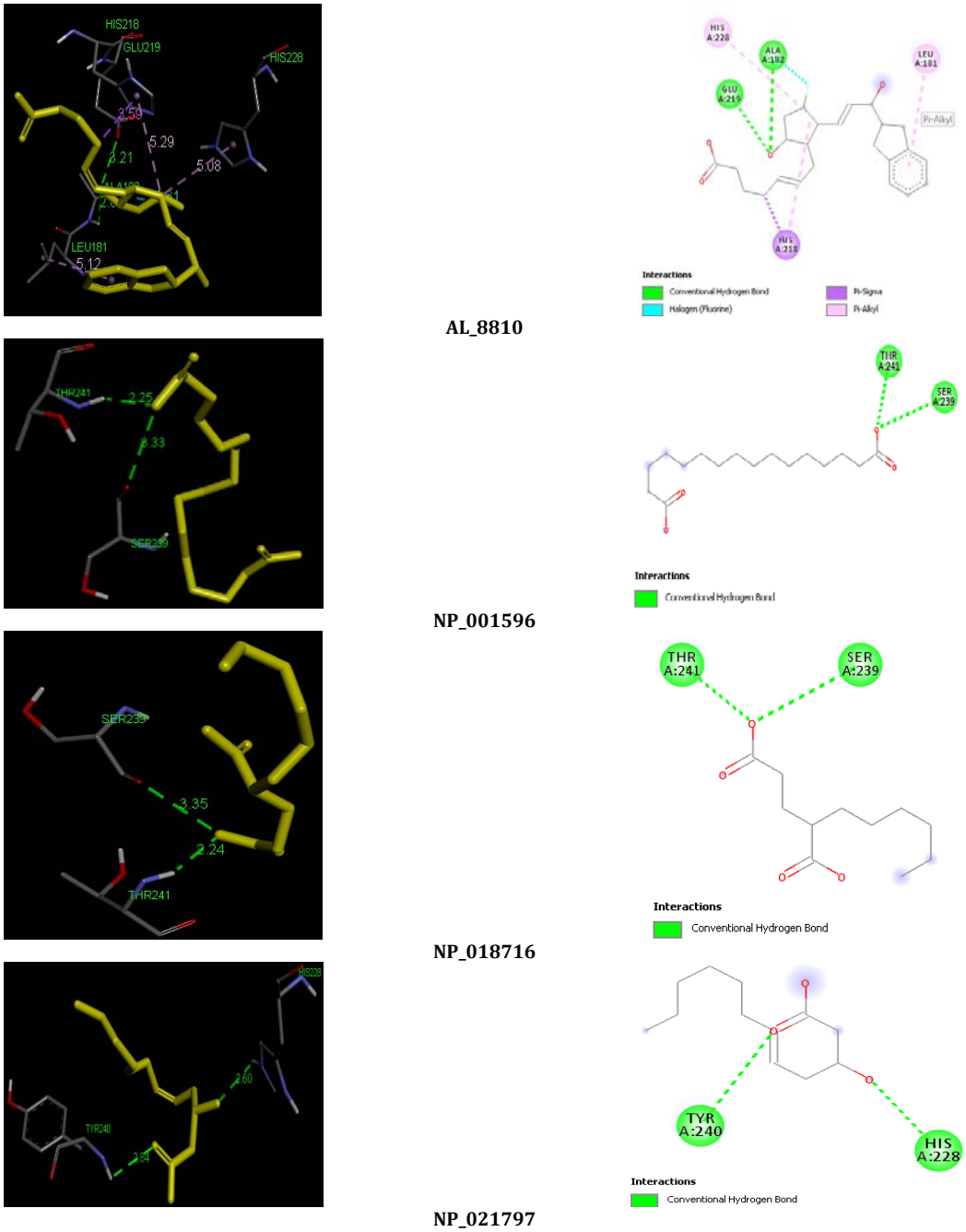

Fig. 2: Molecular docking of collagenase (966C) with AL_8810, NP_001596), NP_018716 and NP_021797

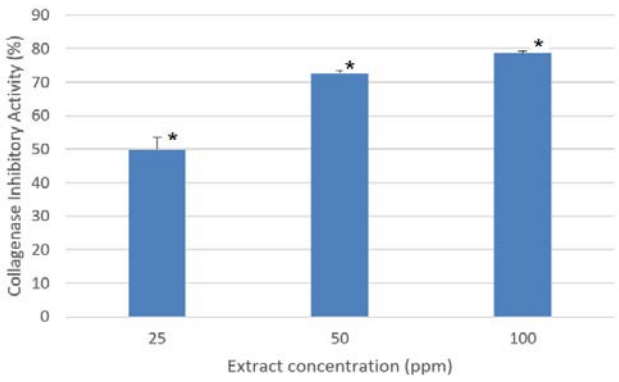

Fig. 3: Collagenase inhibitory activity of Pidada merah extract, fluoresence was measure in kinetic mode, the absorbance (A) at 345 nm in a microplate reader. The data are representative of three concentration, *P<0,05 when compare with control

DISCUSSION

Fig. 1 showed that metabolite profiling on the HR-LCMS from Pidada merah extract are A (AL_8810), B (NP_001596), C (NP_018716) and D (NP_021797) and table 1. Molecular docking was used to simulate the potential binding mechanism of phytochemicals from *Sonneratia caseolaris* to a protein associated with aging. Collagenase (966C) have their three-dimensional structures taken from the RCSB protein data bank. The data was then cleaned up by removing any co-crystallized ligand and crystallographic water.

To propose a molecular-level explanation of Collagenase inhibition enzymes by the best inhibitors (docking scores is-6.0). NP_018716 principally attributed to the hydrogen bond interactions related to the residues THR241 and SER239. A molecular docking study was conducted to assume the model where the protein and ligand were considered rigid and flexible during the docking procedure [21]. Unfortunately, the sameness in the estimate shown in table 1. The reason there why there is a limitation of the docking model for how the compounds could arrive at the active site.

The molecular docking showed in fig. 2. Collagenase (966C) with AL_8810, NP_001596), NP_018716, and NP_021797. The protein showed as surface representation. The ligand showed as a stick representation. Aging is a natural process affecting various body organs. The binding validation of the native ligands is needed to find the 3D conformation of the natural ligand to the receptor protein by taking into account the coordinates of the center of mass of the structure and the grid box arrangement of the binding site pocket in units of (angstroms) or a number of points.

It is often shown by Reactive Oxygen Species (ROS) build-up in cells [22]. In a normal situation, ROS holds a vital role in various biological processes, such as immune response, but radical homeostasis is impaired through various stimulus. Increased cytoplasmic ROS can induce the synthesis related to the degradation of the extracellular matrix, causing tissue structural diminishment that manifests as the formation of wrinkles and sagging elasticity [22]. The antioxidant phytochemical compound helps decrease ROS-induced skin damage. Not only relieves oxidative stress, but it also decreased collagen-degrading enzyme activity [23].

The collagenase inhibitory effect was carried out using the assay, and the results are shown in fig. 3. The test extract inhibited collagenase in a dose-dependent manner. Regarding anti-collagenase activity, extract concentation 25 ppm (collagenase inhibitory activity 49.91±3.51%), 50 ppm (72.67±0.67%), 100 ppm (78.61±0.69%), IC_{50} 26.74±0.40 ppm. The data are representative of three concentration, *P<0,05; when compare with control, it has higher anti-collagenase activity. This study indicates that the extract of pidada merah leaves the potential for anti-collagenase activity. Free radicals can also speed up the aging process. Physical and physiological changes are a feature of the aging process. Wrinkles can characterize physical changes, reduced skin elasticity, uneven pigmentation, brown spots, sagging, and a rough appearance [24]. In the *in vitro* study, the combination of fruits extract showed a higher antioxidant activity which was comparable with the positive standard (ascorbic acid, butylated hydroxyanisole, and Trolox). The data also showed a dose-dependent inhibition of collagenase. In the *in vivo* study, treatment with 2% formulated cream for 56 d significantly reduced the percentage of wrinkle depth, length, and area with 11.5, 10.07, and 29.55, respectively [25].

UV radiation increases MMP levels in the skin, accelerating the photoaging process [26]. Collagenase is the enzyme responsible for the degradation of Matrix collagen. There are three types of collagenases in humans, MMP (Matrixmetallo protease)1, MMP-8, and MMP-13 [27, 28]. This study proves that pidada merah leaf extract has the potential as an anti-aging treatment through its anti-collagenase activity.

CONCLUSION

In this study, the extract of pidada merah leaves showed inhibitory activity for antiaging. In terms of collagenase inhibitory activity assay, extract of pidada merah leaves had a lower value of IC50 (26.74±0.40 ppm). It has higher anti-collagenase activity. NP_018716 has the lowest binding energy value with the target protein, which is-6.0 and binds to THR241(2.24Å) and SER239 (3.35Å). This study indicates that the extract of pidada merah leaves the potential for an antiaging treatment through anti-collagenase activity.

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AUTHORS CONTRIBUTIONS

All authors contributed to the manuscript, arranged, read, revised, and approved the submitted version.

CONFLICT OF INTERESTS

The authors declare no conflct of interest.

REFERENCES

1. Kuspradini H, Kusuma IW, Bang TH, Yamashita S, Katakura Y, Shimizu K Arung. Effects of isolated compound from *Sonneratia caseolaris* leaf: a validation of traditional utilization by melanin biosynthesis and antioxidant assays. J Appl Pharm Sci. 2015;5(10):39-43.
2. Rani PU, Sandhyarani K, Vadlapudi V, Sreedhar B. Bioefficacy of a mangrove plant, Sonneratia caseolaris and a mangrove associate plant, Hibiscus tiliaceus against certain agricultural and stored product pests. J Biopest. 2015;8(2):98-106.
3. Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses. Second edi. Dhaka: Asiatic Society of Bangladesh; 1998.
4. Das SK, Samantaray D, Patra JK, Samanta L, Thatoi H. Antidiabetic potential of mangrove plants: a review. Front Life Sci. 2016;9(1):75-88. doi: 10.1080/21553769.2015.1091386.
5. Varghese J, Belzik N, Nisha A, Remi S, Silvipriya K. Pharmacognostical and phytochemical studies of a mangrove (Sonneratia caseolaris L.) from Kochi of Kerala State in India. J Pharm Res. 2010;3(11):2625-7.
6. Bandaranayake WM. Bioactive compounds and chemical constituents of Mangrove plants. Wetlands Ecology and Management. 2002;10(6):421-52. doi: 10.1023/A:1021397624349.
7. Buatong J, Phongpaichit S, Rukachaisirikul V, Sakayaroj J. Antimicrobial activity of crude extracts from mangrove fungal endophytes. World J Microbiol Biotechnol. 2011;27(12):3005-8. doi: 10.1007/s11274-011-0765-8.
8. Simlai A, Rai A, Mishra S, Mukherjee K, Roy A. Antimicrobial and antioxidative activities in the bark extracts of Sonneratia caseolaris, a mangrove plant. Excli J. 2014;13:997-1010. PMID 26417316.
9. Sadhu SK, Ahmed F, Ohtsuki T, Ishibashi M. Flavonoids from sonneratia caseolaris. J Nat Med. 2006;60(3):264-5. doi: 10.1007/s11418-006-0029-3, PMID 29435876.
10. Gemperline E, Keller C, Li L. Mass spectrometry in plant-omics. Anal Chem. 2016;88(7):3422-34. doi: 10.1021/acs.analchem.5b02938, PMID 26889688.
11. Worley B, Powers R. Multivariate analysis in metabolomics. Curr Metabolomics. 2013;1(1):92-107. doi: 10.2174/2213235X11301010092, PMID 26078916.
12. Syamsul ES, Umar S, Wahyuni FS, Martien R, Hamidi D. Anti-aging activity, *in silico* modeling and molecular docking from sonneratia caseolaris. Open Access Maced J Med Sci. 2022;10(A):1471-7. doi: 10.3889/oamjms.2022.10558.
13. Jariyah J, Widjanarko SB, Yunianta Y, Estiasih T. Phytochemical and acute toxicity studies of ethanol extract from Pedada (Sonneratia caseolaris) fruit flour (PFF). International Journal

on Advanced Science, Engineering and Information Technology 2015;5(2):95. doi: 10.18517/ijaseit.5.2.485.

14. Jariyah AL, Widjanarko S, Estiasih T, Yuwono S, Yunianta. Hypocholesterolemic effect of Pedoda (Sonneratia caseolaris) fruit flour in wistar rats. Int J Pharm Tech Res. 2013;5(4):1619-27.
15. Wu SB, Wen Y, Li X W, Zhao Y, Zhao Z, Hu JF. Chemical constituents from the fruits of *Sonneratia caseolaris* and *onneratia ovata* (Sonneratiaceae). Biochemical Systematics and Ecology. 2009;37:1-5.
16. Syamsul ES, Supomo, Jubaidah S, Wijaya H, Lestari D, Poddar S. Antioxidant activity test of pidada merah leaves (Sonneratia caseolaris L.) using ABTS method (2,2-azinobis-(3-ethylbenzothiazolin)-6-sulfonicacid). Research Journal of Pharmacy and Technology. 2022;15(9):3957-1. doi: 10.52711/0974-360X.2022.00663.
17. Cavasotto CN. *In silico* drug discovery and design: theory, methods, challenges, and applications. CRC Press; 2015.
18. Genheden S, Ryde U. The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. Expert Opin Drug Discov. 2015;10(5):449-61. doi: 10.1517/17460441.2015.1032936, PMID 25835573.
19. Magalhaes LM, Segundo MA, Reis S, Lima JLFC. Automatic method for determination of total antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl assay. Anal Chim Acta. 2006;558(1-2):310-8. doi: 10.1016/j.aca.2005.11.013.
20. Sliwoski GR, Meiler J, Lowe EW. Computational methods in drug discovery prediction of protein structure and ensembles from limited experimental data View project antibody modeling, antibody design and antigen-antibody interactions view project. Comp Methods Drug Discov. 2014;66(1):334-95.
21. Davalli P, Mitic T, Caporali A, Lauriola A, D'Arca D. ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. Oxid Med Cell Longev. 2016;2016:3565127. doi: 10.1155/2016/3565127, PMID 27247702.
22. Farage MA, Miller KW, Elsner P, Maibach HI. Intrinsic and extrinsic factors in skin ageing: a review. Int J Cosmet Sci. 2008;30(2):87-95. doi: 10.1111/j.1468-2494.2007.00415.x, PMID 18377617.
23. Tanigawa T, Kanazawa S, Ichibori R, Fujiwara T, Magome T, Shingaki K. (+)-Catechin protects dermal fibroblasts against oxidative stress-induced apoptosis. BMC Complement Altern Med. 2014;14(1):133. doi: 10.1186/1472-6882-14-133, PMID 24712558.
24. Tsai ML, Huang HP, Hsu JD, Lai YR, Hsiao YP, Lu FJ. Topical N-acetylcysteine accelerates wound healing *in vitro* and *in vivo* via the PKC/Stat3 pathway. Int J Mol Sci. 2014;15(5):7563-78. doi: 10.3390/ijms15057563, PMID 24798751.
25. Cui N, Hu M, Khalil RA. Biochemical and biological attributes of matrix metalloproteinases. Prog Mol Biol Transl Sci. 2017;147:1-73. doi: 10.1016/bs.pmbts.2017.02.005. PMID 28413025.
26. Ghimeray AK, Jung US, Lee HY, Kim YH, Ryu EK, Chang MS. *In vitro* antioxidant, collagenase inhibition, and *in vivo* anti-wrinkle effects of combined formulation containing punica granatum, ginkgo biloba, ficus carica, and morus alba fruits extract. Clin Cosmet Investig Dermatol. 2015;8:389-96. doi: 10.2147/CCID.S80906. PMID 26203268.
27. Fisher GJ, Kang S, Varani J, Bata Csorgo Z, Wan Y, Datta S. Mechanisms of photoaging and chronological skin aging. Arch Dermatol. 2002;138(11):1462-70. doi: 10.1001/archderm.138.11.1462, PMID 12437452.
28. Ricciarelli R, Maroni P, Ozer N, Zingg JM, Azzi A. Age-dependent increase of collagenase expression can be reduced by alpha-tocopherol via protein kinase C inhibition. Free Radic Biol Med. 1999;27(7-8):729-37. doi: 10.1016/s0891-5849(99)00007-6, PMID 10515576.