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Abstract

Introduction
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Material and methods
In the cellular and molecular part of the recent study, the treated cells with Hydroxysafflor yellow A were assessed by MTT assay for 48h about the cytotoxicity and anti-small cell lung cancer properties on SBC-3, DMS273, and DMS114 cell lines. The molecular docking study was carried out for the evaluation of the biological activity of Hydroxysafflor yellow A against the collagenase H from clostridium histolyticum.

Results
In our study, inhibition result of hydroxysafflor yellow A on collagenase showed lower value IC50 = 78.81 µg / mL. The viability of small cell lung cancer cell lines reduced dose-dependently in the presence of Hydroxysafflor yellow A. The IC50 of Hydroxysafflor yellow A were 539, 432, and 416 µg/mL against SBC-3, DMS273, and DMS114 cell lines, respectively. The results of the docking calculations revealed the considerable binding affinity of the inhibitor to the enzyme with a docking score of -9.238 (kcal/mol). This remarkable binding affinity could be attributed to the number of hydrogen bonds and hydrophobic contacts, which are 6 and 10, respectively.

Conclusions
Maybe the anti-human lung carcinoma properties of the recent molecule are related to their antioxidant effects.
Anti-small cell lung cancer and collagenase inhibition properties of Hydroxysafflor yellow A

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Abstract

Hydroxysafflor yellow A is the major active chemical ingredient of Carthamus tinctorius L. (safflower), which is widely used in patients with cardiovascular and cerebrovascular diseases in China. In our study, inhibition result of hydroxysafflor yellow A on collagenase showed lower value IC50 = 78.81 µg/mL. In the cellular and molecular part of the recent study, the treated cells with Hydroxysafflor yellow A were assessed by MTT assay for 48h about the cytotoxicity and anti-small cell lung cancer properties on SBC-3, DMS273, and DMS114 cell lines. The viability of small cell lung cancer cell lines reduced dose-dependently in the presence of Hydroxysafflor yellow A. The IC50 of Hydroxysafflor yellow A were 539, 432, and 416 µg/mL against SBC-3, DMS273, and DMS114 cell lines, respectively. The molecular docking study was carried out for the evaluation of the biological activity of Hydroxysafflor yellow A against the collagenase H from clostridium histolyticum. The results of the docking calculations revealed the considerable binding affinity of the inhibitor to the enzyme with a docking score of -9.238 (kcal/mol). This remarkable binding affinity could be attributed to the number of hydrogen bonds and hydrophobic contacts, which are 6 and 10, respectively.

KEYWORDS: hydroxysafflor yellow A; collagenase, molecular docking, Anti-small cell lung cancer
Introduction

The flower of the safflower plant, *Carthamus tinctorius L.*, has been used extensively in traditional Chinese medicine for treatment of cerebrovascular and cardiovascular diseases. The extracts from *C. tinctorius* contain yellow and red pigments including hydroxysafflor yellow A, safflomin A, safflomin C, safflor yellow B, as well as other chemicals. Hydroxysafflor yellow A, the main chemical component of the safflower yellow pigments, has been demonstrated to antagonize platelet activating factor receptor binding [1,2]. The hypotensive, antithrombosis and inhibition effects on platelet aggregation have been also reported. Many studies have demonstrated that safflower yellow possesses various physiological and pharmacological activities, including anti-thrombotic and anti-hypertensive activities [3]. Hydroxysafflor yellow A, which is the effective water-solubility monomer of safflower yellow, has been demonstrated to have anti-oxidative activities and myocardial and cerebral protective effects. More recent studies suggest that hydroxysafflor yellow A has therapeutic effects on Parkinson's disease [4,5]. Indeed, it reported that hydroxysafflor yellow A is able to inhibit endothelin release, increase myocardial flow, improve the metabolism of myocardial oxygen consumption, and inhibit myocardial ischemia. It recorded that hydroxysafflor yellow A could reduce myocardial mitochondrial swelling, decrease mitochondrial membrane fluidity, and inhibit mitochondrial over-oxidation [6].

Collagenase (EC 3.4.24.3) enzyme is an enzyme belonging to the hydrolase class and breaks the 3-helix structure of collagen. Hydrolases provide hydrolysis reactions, that is, the destruction of molecules with the help of H\(^+\) and OH\(^-\)ions of water [7]. The enzyme named after its substrate is also known as matrix metallopeptidase-1 or matrix metalloprotease-1. Collagenases, depending on their type, have a weight ranging from 50-60 kDa, the cofactor is Zn metal. One group of proteases, which are extracellular proteolytic enzymes, are metalloproteases that require Ca\(^{2+}\) or Zn\(^{2+}\) ions in
bound state for their activation, while the other group is serine proteases containing reactive serine in their active site [8,9]. The breakdown of matrix proteins such as collagen, laninmin and fibronectin by metalloproteases and serine proteases facilitates cell migration. Collagenase is one of these enzymes. These enzymes play an important role in physiological conditions like tissue restructuring, normal structuring of tissues and systems, wound healing and normal developmental process, as well as in pathological processes such as the spread of tumor cells to surrounding tissues and disrupting their function [10,11].

The theoretical investigation of experimental results is an essential work that has to be done as a complementary study. One of the most significant things about theoretical studies is that they could provide sufficient insight into the experimental outcomes [12]. A popular method for such theoretical studies is molecular docking, which can contribute beneficial information about the interactions between various molecules and biological compounds. The prediction of mechanisms in which a molecule would inhibit the activity of an enzyme is predictable with molecular docking study [13]. The binding affinity of the ligands to the biological materials is an essential outcome of molecular docking.

We also investigated the Hydroxysafflor yellow A in the cytotoxicity studies against common small cell lung cancer cell lines i.e., SBC-3, DMS273, and DMS114, in vitro. Interestingly, we got significantly good results in the study. The best result was achieved in case of DMS114 cell line. Also, We investigated the enzyme inhibition and molecular docking studies in this study.

Matherials and methods

2.2. Determination of cell toxicity and anticancer effects of Hydroxysafflor yellow A
MTT is a colorimetric technique. Based on the fact that living cells can do oxidative metabolism, as a result, oxidation, breaks down the MTT dye and produces a dye ranging from yellow to blue. This test determines the number of living cells [14].

In this research, we used the following Cell lines to evaluating anti-human small cell lung cancer and cytotoxicity effects of Hydroxysafflor yellow A using an MTT method.

- Small cell lung cancer cell lines: SBC-3, DMS273, and DMS114.
- Normal cell line: HUVEC.

For this purpose, each cell line was placed separately in T25 flasks with a complete culture medium (including DMEM (Dulbecco's Modified Eagle Medium, 10% complementary bovine fetal serum, and 1% penicillin-streptomycin solution) and at 37°C in the incubator, cell culture was incubated with 5% CO₂. After obtaining 80% cell density, the sample was exposed to 1% trypsin-EDTA solution and after 3 minutes of incubation at 37°C in a cell culture incubator with 5% CO₂ and observation of cells removed from the bottom of the plate, the sample was centrifuged at 5000 rpm for 5 minutes and then the cell precipitate was decrypted by adding trypsin culture medium. Then, the cell suspensions after adding trypan blue dye were counted by neobar slide and cytotoxicity test was performed by MTT method [14].

Initially, 10,000 cells were implanted in cell culture plates and then the cells were treated at concentrations of 1-1000 μg/mL of Tiliroside. After 24 hours, 20 μL of MTT dye was added to the wells and incubated for 5 hours at 37 °C with 5% CO₂. DMSO was then added to the wells to dissolve the formazan crystals and the absorption rate of the wells at 570 nm was read by ELISA reader (ELISA Teknika Oraganon reader, Netherlands) and the cell viability rate was computed by the below formula [14]:
**Cell viability (%) = \frac{\text{Sample A.}}{\text{Control A.}} \times 100**

**Enzymes methods**

PHL83510-Hydroxysafflor yellow A (CAS Number 78281-02-4) taken from sigma. The inhibitory effect on the collagenase enzyme has been modified by Thring et al. (2009) [15] was determined spectrophotometrically using the method. The solution of the compound and diluted solutions were prepared in our study. 50 µL of the solution containing 0.8 U / mL collagenase was taken, and 50 µL of the prepared chemical solution at different concentrations was added. Then 0.9 mL of Tricin buffer solution pH 7.5 was added [16]. The plant extract and chemical substance solution was not put into the control solution. For the blank, DMSO solution was used as much as the enzyme amount instead of enzyme. The blank, control and sample solutions were left to the first incubation at 25 °C for 30 minutes [17]. After this first incubation, 1 mM 0.05 mL of N-(3-[2-Furyl] acryloyl) -Leu-Gly-Pro-Ala (collagenase enzyme substrate) solution was added to all solutions and left for a second incubation for 15 minutes at 25 °C. Absorbance values against blank at 340 nm were read in the UV spectrophotometer of the sample solutions and the control solution. The experiments were repeated 2 times. The inhibitory effect of the sample solutions at different concentrations prepared in the study on collagenase enzyme was calculated according to the following equation [18].

\% inhibition = \frac{((\Delta A_{340} \text{ control} - \Delta A_{340} \text{ sample}) / \Delta A_{340} \text{ control}) \times 100}{\Delta A_{340} \text{ control}}

\Delta A_{340} \text{ control}: \text{Absorbance value of the control solution}

\Delta A_{340} \text{ sample}: \text{Absorbance value of the sample solution}
The IC$_{50}$ value, which is the amount of substance required for the collagenase enzyme to show 50% inhibition effect, was calculated by the regression equation obtained from the linear part of the curve drawn by applying enzyme inhibition data to the abscess concentration in the graph [19].

**Molecular docking study**

The biological activities of various compounds could be easily reached using theoretical approaches, and molecular docking study is among the most popular methods for this purpose. The enzymes used in this study were collagenase H from clostridium histolyticum (PDB ID: 4AR1) [20]. The biological activities of the hydroxysafflor yellow A were investigated against this enzyme. The structure of the enzyme was obtained from the protein data bank (http://www.rcsb.org/pdb) and prepared with the protein preparation module of the Schrödinger Suite [21]. The hydrogen bond addition and removal of water molecules were carried out using this module. An H-bond network was created using the optimization step. The structure was then minimized using the OPLS3e force field, and the prediction of active sites of the structure was performed utilizing SiteMap of Schrödinger [22]. The hydroxysafflor yellow A was obtained from the Pubchem database in SDF format and prepared with the LigPrep module of Schrödinger [23]. Finally, the calculations of molecular docking were accomplished utilizing the Glide of Schrödinger suites.
Results and discussion

Anticancer effect analysis

One of the cytotoxicity test methods to measure the rate of cell death is the MTT method, which is based on the formation of formazan dye by reducing the substance MTT (dimethyl thiazole 2 and 5 diphenyltetrazolium bromide) or other tetrazolium salts [24,25]. By breaking the MTT tetrazolium ring by mitochondrial enzymes in living cells, insoluble purple formazan crystals are formed. The formation of these crystals indicates the activity of respiratory chain enzymes and is a measure of cell viability. By measuring the amount of absorption by spectrophotometer at specific wavelengths, the number of living cells can be determined. This test is performed according to ISO 10993-5 and its purpose is in vitro evaluation of cytotoxicity. Cytotoxicity test is performed according to ISO10993-5 standard and in three ways: NRU test, CFU test, MTT test and XTT test [26,27]. The most common method for assessing cytotoxicity is to measure cell survival by MTT. The basis of this method is based on the intensity of dye produced by the mitochondrial activity of cells, that measured at a wavelength of 540 to 630 nm and directly proportional to the number of living cells, the increase or decrease in the number of living cells is linearly related to the activity of cell mitochondria. MTT tetrazolium dye is revived in active (metabolically) cells. Mitochondrial dehydrogenases in living cells produce NADH and NADPH, leading to an insoluble purple precipitate called formazan. This precipitate can be dissolved by isopropanol or dimethyl sulfoxide [28]. Dead cells, on the other hand, are unable to perform this conversion due to the inactivity of their mitochondria and therefore do not show a signal. In this method, dye formation is used as a marker for the presence of living cells [29]. In recent years, MTT testing has been the most important measurement method to evaluate the toxicity and anticancer effects of molecules [30].
In the recent study, the treated cells with different concentrations of the present Hydroxysafflor yellow A were assessed by MTT assay for 48h about the cytotoxicity properties on SBC-3, DMS273, and DMS114 cell lines. The absorbance rate was evaluated at 570 nm, which represented viability on normal cell line (HUVEC) even up to 1000μg/mL for Hydroxysafflor yellow A (Table 1; Figures 1,2).

The viability of small cell lung cancer cell lines reduced dose-dependently in the presence of Hydroxysafflor yellow A. The IC50 of Hydroxysafflor yellow A were 539, 432, and 416 µg/mL against SBC-3, DMS273, and DMS114 cell lines, respectively (Table 1; Figures 1,2). The best result was achieved in case of DMS114 cell line. It looks that the anti-human small cell lung cancer effect of recent molecule is due to their antioxidant effects. Because tumor progression is so closely linked to inflammation and oxidative stress, a compound with anti-inflammatory or antioxidant properties can be an anticarcinogenic agent [31].

Many molecules have pharmacological and biochemical properties, including antioxidant and anti-inflammatory properties, which appear to be involved in anticarcinogenic and antimitogenic activities. Today, molecules synthesized by biological methods play a vital role in treating many diseases, including cancer [32]. Molecules synthesized by biological methods are no longer the only ones in traditional medicine, in addition, they have been able to adopt an industrial line of natural products for treating various cancers. Various cell lines from cancers of the prostate, ovary, lung, liver, and pancreas have been treated with herbal molecules synthesized [31,32].
Fig. 1. The anti-small cell lung cancer properties of Hydroxysafflor yellow A against SBC-3, DMS273, and DMS114 cell lines.
**Fig. 2.** The cytotoxicity effects of Hydroxysafflor yellow A against normal (HUVEC) cell line.

**Table 1.** The IC50 of Hydroxysafflor yellow A in the anti-small cell lung cancer test.

| IC50 against HUVEC | Hydroxysafflor yellow A (µg/mL) |
|--------------------|---------------------------------|
| IC50 against SBC-3 | 539±0<sup>b</sup>              |
| IC50 against DMS273 | 432±0<sup>a</sup>              |
| IC50 against DMS114 | 416±0<sup>a</sup>              |

**Enzymes results**

During the experiments, N- (3- [2-furyl] acryloyl) -Leu-Gly-Pro-Ala (FALGPA) (Sigma F5135) as a collagenase substrate and collagenase (Sigma) C0130 Collagenase *Clostridium histolyticum*
2U / mL) was used. It was observed that the chemical substance we used in our study inhibits the collagenase enzyme. It can be suggested that the chemical substance, which has a high level of collagenase inhibitory effect, may be suitable for use as a collagenase inhibitor in addition to drug therapy in skin and dental diseases [33]. However, further studies are needed to prove the collagenase enzyme inhibition of this chemical substance through in vivo experiments. In our study, hydroxysafflor yellow A was found to inhibit the collagen enzyme at a high rate. This compound is an antioxidant substance and contains a hydroxy group in its structure [34]. It can be suggested that the hydroxy group in the catechin may have an effect on the collagenase enzyme, as is the case with other hydroxy group containing compounds [35]. In the hydroxysafflor yellow A we used in our study, the inhibitory effects on the collagenase enzyme have an IC50 value at micromolar level. hydroxysafflor yellow A is a flavonoid substance, it is reported that this substance prevents many damages in the body. In the trials of collagenase enzyme inhibition by Galardy and Grobelny (1983) [36] with phosphoric and phosphonic amides containing glycine, proline, leucine, phenyl and alanine, it was found that phosphoric amides containing glycine, proline and alanine inhibited this enzyme with an inhibition value of IC50 = 14 ± 6 µM. In our study, inhibition result of hydroxysafflor yellow A on collagenase showed lower value IC50 = 78.81 µg / mL. Based on all this information, the inhibitory effects of various plant extracts, various chemicals, acids, vitamins, amino acids and peptides on this enzyme were investigated, considering the role of collagenase enzyme in skin aging and wound healing [37,38].
Molecular modeling results

The biological and chemical activities of the hydroxysafflor yellow A were assessed utilizing the molecular docking study. Fig. 3 shows hydroxysafflor yellow A in the docking pose, and Fig. 4 indicates the interactions of the hydroxysafflor yellow A with collagenase. As could be seen, the molecule has created some hydrogen bonds with Lys353, Asp398, Arg464, Tyr465, and Lys641. As it is apparently obvious, the residue Lys353 has created two hydrogen bonds with the ligand. This residue and Asp398, Arg464, and Tyr465 are the residues of the catalytic subdomain, and Lys641 comes from the helper subdomain. 6 of 10 residues with hydrophobic contacts are also from the catalytic subdomain. Table 2 presents the parameters determined through the calculations. The docking score, as the most important calculated parameter [39], indicates the binding affinity between the ligands and enzyme. Another essential parameter is the Glide Ligand Efficiency, which shows the efficiency of the molecules numerically. Some other parameters like Glide Evdw and Glide Ecoul are the parameters that are related to the interactions. The Glide energy is the energy of interaction that is numerically calculated, and the value of interaction pose is calculated and presented with Glide Emodel [40]. These results indicated that hydroxysafflor yellow A could be considered as a potential inhibitor for collagenase. Generally, this inhibitor creates various interactions like hydrogen bonds and hydrophobic contacts with the catalytic subdomain of the enzyme.
Fig 3. The docking pose of Hydroxysafflor yellow A among the residues of collagenase
Fig. 4. The interactions of Hydroxysafflor yellow A and collagenase. Green dashed lines indicate the hydrogen bonds, and semicircles show the hydrophobic contacts.
Table 2. The parameters obtained from the molecular docking calculations.

|                          | Hydroxysafflor yellow A |
|--------------------------|-------------------------|
| **IC₅₀ (mM)**            | 78.81                   |
| **Docking score (kcal/mol)** | -9.238                 |
| **Glide ligand efficiency (kcal/mol)** | -0.215                 |
| **Glide E Coul (kcal/mol)** | -18.820                |
| **Glide E Vdw (kcal/mol)** | -30.047                |
| **Glide E Model (kcal/mol)**  | -70.443                |
| **Glide energy (kcal/mol)**  | -48.867                |

Conclusions

Based on these results, we suggest Hydroxysafflor yellow A as a promising source for new anti-collagenase agents. In view of the importance that the maintenance of collagen structure has in preventing the skin ageing and photoageing processes, this molecule can enter as active ingredients in wrinkle-care cosmetics. The viability of small cell lung cancer cell lines reduced dose-dependently in the presence of Hydroxysafflor yellow A. The IC₅₀ of Hydroxysafflor yellow A were 539, 432, and 416 µg/mL against SBC-3, DMS273, and DMS114 cell lines, respectively. The results of experimental studies could be further assessed by the theoretical approaches to provide adequate insight into the mechanisms. Molecular docking study is one of these approaches that can reveal the biological activities of compounds in the presence of enzymes. In this study,
the activities of Hydroxysafflor yellow A against collagenase were evaluated using docking calculations. It was concluded that this compound has a considerable binding affinity to the enzyme by creating suitable bonds and interactions.

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References

[1] Y. Zhang, J. Guo, H. Dong, X. Zhao, L. Zhou, X. Li, J. Liu, Y. Niu
Hydroxysafflor yellow A protects against chronic carbon tetrachloride-induced liver fibrosis
European Journal of Pharmacology, 660 (2011), pp. 438-444.

[2] L. Zhang, P.H. Nie, G.H. Zhang, W.F. Rong, J.M. Zhi
Endothelium-independent vasodilation effect of hydroxysafflor yellow A in thoracic aorta of Wistar rats. Journal of Medicinal Plants Research, 5 (2011), pp. 2187-2191

[3] W. Wu, J.R. Li, Y.Z. Piao, N.N. Dong, M. Jin. Inhibitory effect of hydroxysafflor yellow A against rat myocardial mitochondrial injury Chinese Pharmaceutical Journal, 41 (2006), pp. 1225-1227.

[4] J. Tian, G. Li, Z. Liu, F. Fu
Hydroxysafflor yellow A inhibits rat brain mitochondrial permeability transition pores by a free radical scavenging action
Pharmacology, 82 (2008), pp. 121-126.

[5] D.B. Ji, L.Y. Zhang, C.L. Li, J. Ye, H.B. Zhu. Effect of Hydroxysafflor yellow A on human umbilical vein endothelial cells under hypoxia. Vascular Pharmacology, 50 (2009), pp. 137-145.

[6] B. Han, H. Zhao. Effects of hydroxysafflor yellow A in the attenuation of MPTP neurotoxicity in mice. Neurochemical Research, 35 (2010), pp. 107-113.

[7] M.R. Villegas, A. Baeza, A. Usategui, P.L. Ortiz-Romero, J.L. Pablos, M. Vallet-Regí
Collagenase nanocapsules: an approach to fibrosis treatment. Acta Biomater, 74 (2018), pp. 430-438
M.R. Villegas, A. Baeza, M. Vallet-Regí

Hybrid collagenase nanocapsules for enhanced nanocarrier penetration in tumoral tissues. ACS Appl. Mater. Interfaces., 7 (2015), pp. 24075-24081.

S. Vaccaro, M. Caputo, C. Cuppari and G. Gennari, Fidia Farmaceuticals SpA, New process for the production and purification of the collagenase enzyme from vibrio alginolyticus, US Pat., 9,738,883, 2017.

K. Tanaka, N. Teramura, O. Hayashida, T. Okitsu and S. Hattori, The C-terminal segment of collagenase in Grimontia hollisae binds collagen to enhance collagenolysis, FEBS Open Bio, 2018, 8(10), 1691–1702.

H. Alipour, A. Raz, S. Zakeri and N. D. Djadid, Therapeutic applications of collagenase (metalloproteases): a review, Asian Pac. J. Trop. Biomed., 2016, 6(11), 975–981.

S. Mittal, A. Malde, C. Selvam, K.H.S. Arun, P.S. Johar, S.M. Jachak, P. Ramarao, P.V. Bharatam, H.P.S. Chawla Bioorg. Med. Chem. Lett., 14 (2004), pp. 979-982

C. H. Jhong, J. Riyaphan, S. H. Lin, Y. C. Chia, and C. F. Weng, “Screening alpha-glucosidase and alpha-amylase inhibitors from natural compounds by molecular docking in silico,” BioFactors, vol. 41, no. 4, pp. 242–251, Jul. 2015.

Arunachalam KD et al. One-step green synthesis and characterization of leaf extract-mediated biocompatible silver and gold nanoparticles from Memecylon umbellatum. Int J Nanomedicine. 2003; 8: 1307-1315.

Thring, T.S., Hili, P. & Naughton, D.P. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Complement Altern Med 9, 27 (2009).

Barrantes E, Guinea M: Inhibition of collagenase and metalloproteinases by aloins and aloe gel. Life Sci. 2003, 72: 843-850.

Van Wart HE, Steinbrink DR: A continuous spectrophotometric assay for Clostridium histolyticum collagenase. Anal Biochem. 1981, 113: 356-365.

Wittenauer J, Mäckle S, Sußmann D, Schweiggert-Weisz U, Carle R. Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity. Fitoterapia. 2015;101:179–87.

Chatatikun M, Chiabchalard A. Thai plants with high antioxidant levels, free radical scavenging activity, anti-tyrosinase and anti-collagenase activity. BMC Complement Altern Med 2017;17:487.

U. Eckhard, E. Schönauer, and H. Brandstetter, “Structural basis for activity regulation and substrate preference of clostridial collagenases G, H, and T,” J. Biol. Chem., vol. 288, no. 28, pp. 20184–20194, Jul. 2013.

“Schrödinger Release 2020-4: Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY 2016; Impact, Schrödinger, LLC, New York, NY 2016; Prime, Schrödinger, LLC, New York, NY 2020.”

A.K. Chakraborti, R. Thilagavathi Bioorg. Med. Chem., 11 (2003), pp. 3989-3996.

“Schrödinger Release 2020-4: LigPrep, Schrödinger, LLC, New York, NY 2020.”
[24] Arunachalam KD et al. One-step green synthesis and characterization of leaf extract-mediated biocompatible silver and gold nanoparticles from Memecylon umbellatum. Int J Nanomedicine. 2003; 8: 1307-1315.

[25] (a) Veisi, H.; Tamoradi, T.; Karmakar, B. Mohammadi, P.; Hemmati, S.; Mat. Sci. Eng. C. 104 (2019) 109919; (b) M. Hamelian, K. Varmira, H. Veisi, Journal of Photochemistry and Photobiology B: Biology 184 (2018) 71-79.

[26] You C, Han C, Wang X, et al. The progress of silver nanoparticles in the antibacterial mechanism, clinical application and cytotoxicity, Mol. Biol. Rep. 2012; 39: 9193-9201. https://doi.org/10.1007/s11033-012-1792-8.

[27] Mao BH et al. Mechanisms of silver nanoparticle-induced toxicity and important role of autophagy. Nanotoxicol. 2016; 10: 1021–1040.

[28] Namvar F, Rahman HS, Mohamad R, et al. Cytotoxic effect of magnetic iron oxide nanoparticles synthesized via seaweed aqueous extract. Int J Nanomedicine 2014; 19: 2479-88.

[29] Sankar R, Maheswari R, Karthik S, et al. Anticancer activity of Ficus religiosa engineered copper oxide nanoparticles. Mat Sci Eng C 2014; 44: 234-239.

[30] Katata-Seru L, Moremedi T, Aremu OS, et al. Green synthesis of iron nanoparticles using Moringa oleifera extracts and their applications: Removal of nitrate from water and antibacterial activity against Escherichia coli. J Mol Liq 2018; 256: 296-304.

[31] Beheshtkhoo N, Kouhbanani MAJ, Savardashtaki A, et al. Green synthesis of iron oxide nanoparticles by aqueous leaf extract of Daphne mezereum as a novel dye removing material. Appl Phys A 2018; 124: 363-369.

[32] Sangami S, Manu M. Synthesis of Green Iron Nanoparticles using Laterite and their application as a Fenton-like catalyst for the degradation of herbicide Ametryn in water, Environ. Technol. Innov. 2017; 8: 150–163.

[33] Barrantes E, Guinea M: Inhibition of collagenase and metalloproteinases by aloins and aloe gel. Life Sci 2003, 72:843-850.

[34] Van Wart HE, Steinbrink DR: A continuous spectrophotometric assay for Clostridium histolyticum collagenase. Anal Biochem 1981, 113:356-365.

[35] Demina NS, Lysenko SV. [Collagenolytic enzymes synthesized by microorganisms]. Mikrobiologiya. 1996;65(3):293-04.

[36] Obayashi K, Akamatsu H, Okano Y, Matsunaga K, Masaki H. Exogenous nitric oxide enhances the synthesis of type I collagen and heat shock protein 47 by normal human dermal fibroblasts. J Dermatol Sci. 2006;41(2):121-26.

[37] Abdul Wahab N, Abdul Rahman R, Ismail A, Mustafa S, Hashim P, Assessment of antioxidant capacity, anti-collagenase and antielastase assays of Malaysian unfermented cocoa bean for cosmetic application, Nat Prod Chem Res 2, 2014.

[38] Thring TS, Hili P, Naughton DP, Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants, BMC Complement Altern Med 9, 27, 2009.

[39] S. Subhani, A. Jayaraman, and K. Jamil, “Homology modelling and molecular docking of
MDR1 with chemotherapeutic agents in non-small cell lung cancer,” *Biomed. Pharmacother.*, vol. 71, pp. 37–45, 2015.

[40] A. Türe, D. C. Kahraman, R. Cetin-Atalay, S. Helvacıoğlu, M. Charehsaz, and İ. Küçükgüzel, “Synthesis, anticancer activity, toxicity evaluation and molecular docking studies of novel phenylaminopyrimidine—(thio)urea hybrids as potential kinase inhibitors,” *Comput. Biol. Chem.*, vol. 78, pp. 227–241, 2019.