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In silico investigation of wound healing potential of some compounds in tubers of *Asphodelus* species with GSK3-β protein

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ABSTRACT: Wound healing is a process that involves biochemical processes such as inflammation and cell proliferation and is controlled by many proteins. It is known that one of the most effective factors in this process is the inhibition of GSK3-β protein. In current study, in silico wound healing activity of the some compounds found in the tubers of *Asphodelus* species used as a wound healing in traditional medicine were investigated. For this purpose, the interactions between the compounds and GSK3-β protein were studied in silico. As a result of the study, it has been determined that and stigmasterol, β-sitosterol and emodin molecules are effective.

KEYWORDS: *Asphodelus* species; β-sitosterol; emodin; GSK3-β protein; stigmasterol; wound healing activity.

1. INTRODUCTION

Wound healing results in processes such as cellular infiltration, inflammation, and proliferation, in which new cell matrix is formed, tissue is remodeled, and mature scar tissue is eventually formed [1]. When the wound healing process is evaluated from this point of view, it is associated with many proteins. However, the most important of these is the GSK3-β protein, which is involved in the well-known Wnt pathway. Inhibition of GSK3-β leads to a change in the Wnt-beta catenin pathway. The Wnt-beta catenin pathway, which plays a role in processes such as cell division, is effective in accelerating wound healing [2]. Although the wound healing process is a complex process in which many proteins play a role, there are studies in the literature in recent years that the inhibition of the GSK3-β protein accelerates wound healing [3-5].

Evaluating the effects of many herbal products in wound healing studies is one of the most common studies [3]. In this study, we wanted to investigate the effect of *Asphodelus* plant on wound healing in terms of its potential to bind to the GSK3-β protein as in silico. Therefore, we selected the *Asphodelus* plant, which is known in the literature for its antimicrobial, antioxidant and anti-inflammatory effects and also has ethnobotanical use [6]. In order to do in silico study, the major compounds of this plant were examined. In phytochemical analysis studies on tubers of *Asphodelus* species, it was observed that they carried anthracene glycosides, flavonoids and triterpenes [7]. In our study, major compounds in this plant such as 1-beta-sitosterol, 2-stigmasterol, microcarpin, ramosin, chrysophanol, asphodelin, and emodin were first examined for toxicity and suitable ones were investigated by molecular docking method.

2. RESULTS

2.1. SwissADME results

When the β-sitosterol is evaluated with the Lipinski filter, it is concluded that it can be a drug candidate. However, it also violates one of Lipinski's rules. The MLOGP value was higher than this value, although it should be equal and lower than 4,15. Stigmasterol compound gave the same results with β-sitosterol compound. Chrysophanol compound, on the other hand, did not violate any of Lipinski's rules and draws a good profile as drug candidate compatibility. The asphodelin molecule violated one of the Lipinski rules. In terms of molecular weight, while it is preferred to be smaller than 500 g/mol, the molecular weight of this

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compound has been calculated as 506.5 g/mol. Similarly, the microcarpin molecule violates one of the Lipinski rules due to its high molecular weight. Emodin, on the other hand, has a very good result in terms of being a drug candidate without violating any rules. The Ramosin molecule does not comply with any of the Lipinski rules and has a poor profile as a candidate drug. As a result, the most suitable candidate molecules for drug likelihood were determined as emodin and chrysophanol.

2.2. Docking results

After the toxicity assessment, the docking results of the remaining molecules was investigated. Re-docking was performed for the validation of the method we used for docking. At the end of this process, the RMSD value was found to be 1.583 Angstrom (Figure 1).

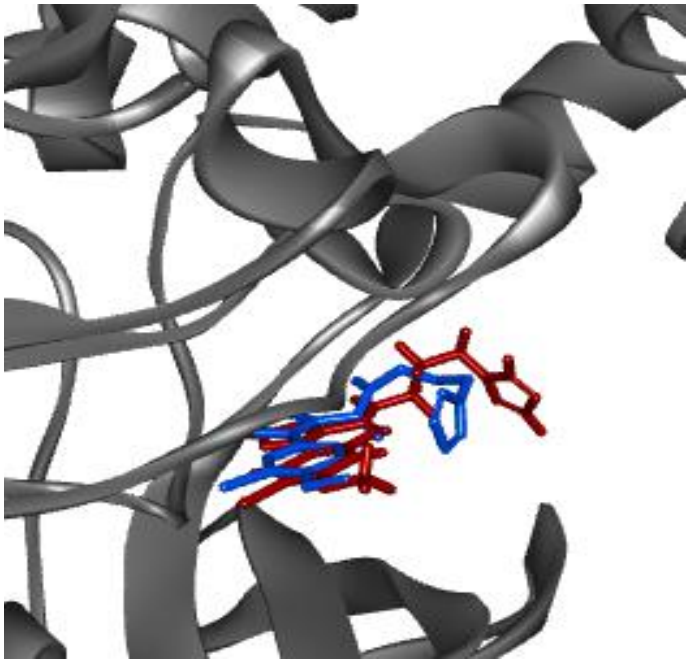

Figure 1. 3D representation of the re-docking process for validation. (Blue: Native ligand, Red: re-docked ligand).

When the binding energies obtained using Autodock Vina software are evaluated, it is seen that the major compounds of *Asphodelus* species give very good results compared to positive controls. When evaluated in terms of binding energy, it is seen that stigmasterol (-13.90 kcal/mol) and β-sitosterol (-12.62 kcal/mol) have the best results (Table 1). It has been determined that these two molecules give a better result than positive controls in terms of binding energy.

Table 1. Binding energies obtained as a result of in silico docking experiments.

Ligand Name	Estimated Free Energy of Binding (kcal/mol)	Estimated Inhibition Constant, Ki Values
Indirubin (positive Control)	-7.58	2.80 µM
PF-04802367 (positive Control)	-7.56	2.87 µM
β-Sitosterol	-12.62	559.72 pM
Stigmasterol	-13.90	65.12 pM
Chrysophanol	-7.11	6.10 µM
Emodin	-6.90	8.71 µM

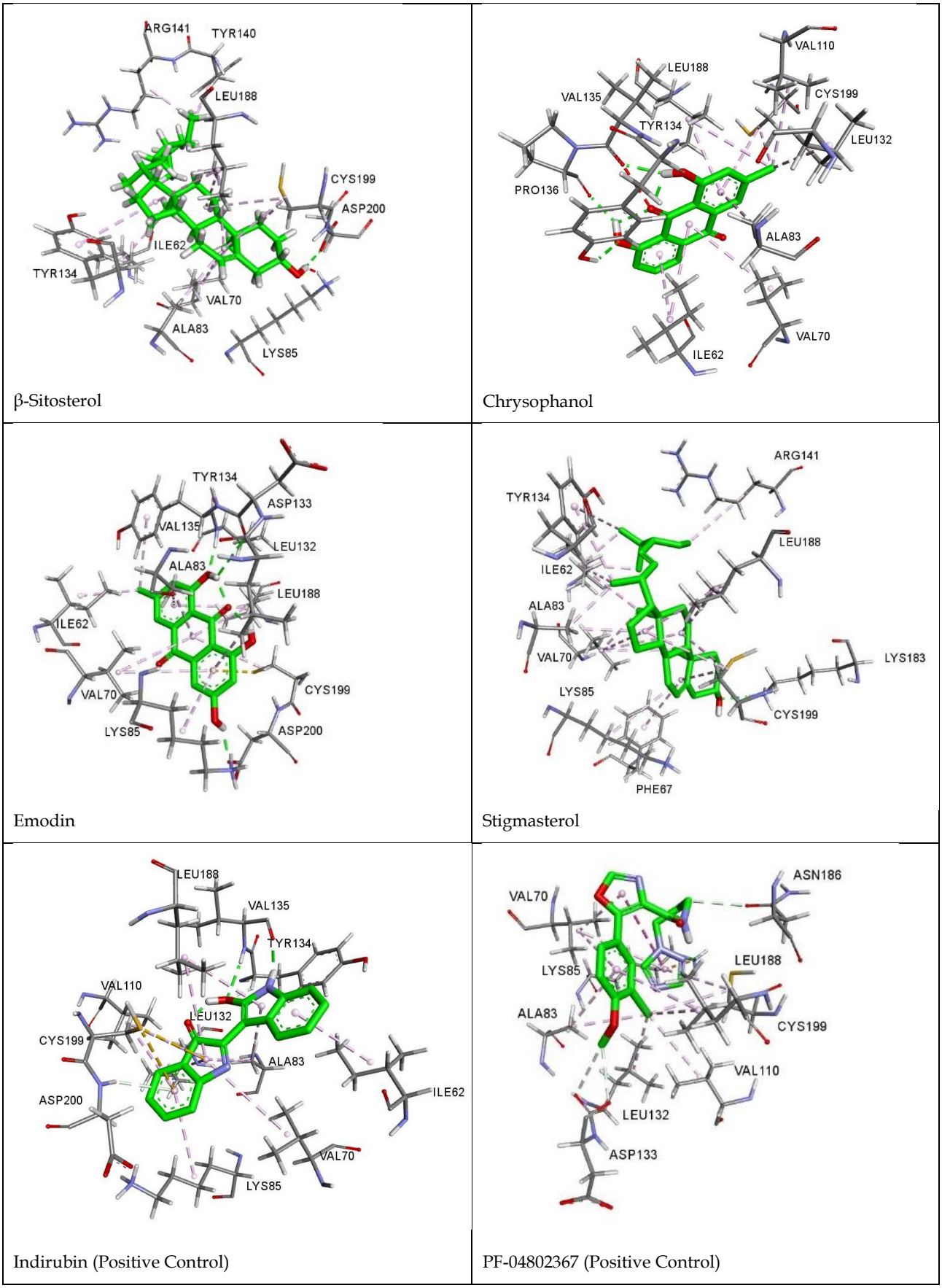

Figure 2. 3D representation of binding sites of ligands.

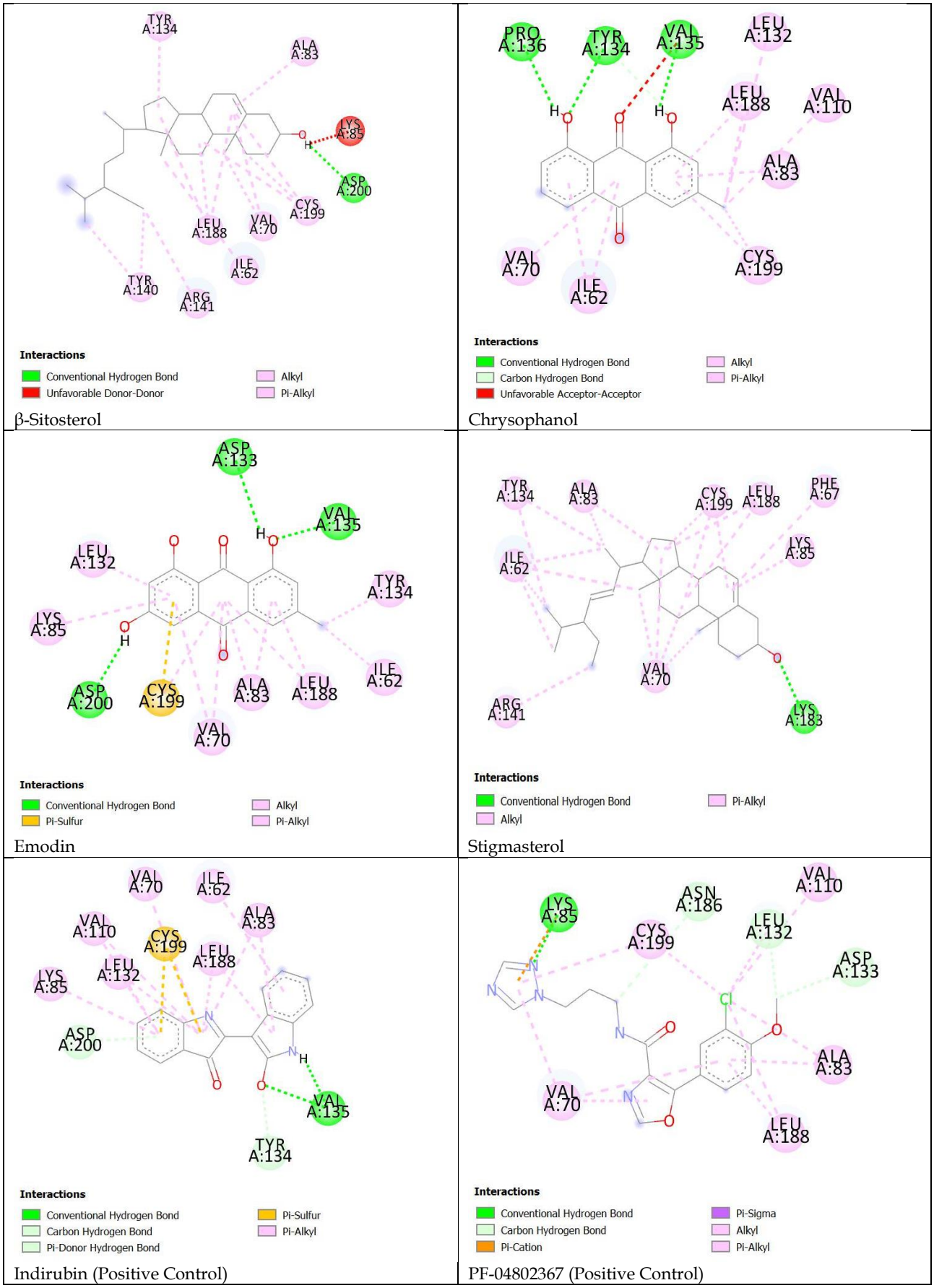

Figure 3. Types of interactions between ligands and protein.

Table 2. Hydrogen bonds formed between ligands and the receptor (GSK3-β).

Ligand Name	Protein residue that participates in hydrogen bonding	Length of each hydrogen bond (Angstrom)
Indirubin (positive control)	(VAL135)	2.23
PF-04802367 (positive control)	(VAL135), (ASP133), (LEU132)	2.49- 2.74 -3.52
β-Sitosterol	(ILE62, VAL70), (ASP70)	2.02 – 2.25
Stigmasterol	(LYS183), (ASP200)	2.22-3.02
Chrysophanol	(PRO136), (TYR134), (VAL135)	2.17 – 2.20 -2.48
Emodin	(ASP133), (VAL135)	3.03- 1.98

According to the results, it is seen that stigmasterol, the molecule with the best value in terms of binding energy, has the most effective value when evaluated in terms of the estimated inhibitor constant (65.12 pM). However, when looking at the interactions between the ligand and the protein, the conformation with the most bonds belongs to the emodin molecule (Table 2) (Figures 2 and 3).

3. DISCUSSION

Asphodelus species has local use against diseases such as abscesses, ear pain. There are also studies on these plant extracts regarding their antioxidant and anti-inflammatory properties [6]. Processes such as inflammation and cell proliferation in wound healing and the importance of antioxidant - oxidant balance that affect these processes are known [12]. Considering these properties, it was predicted that the *Asphodelus* plant may have wound healing properties. In our study, we aimed to investigate the effect of some major compounds on this plant species on GSK3-β protein as in silico. The relationship between the inhibition of the GSK3-β protein and the increase in wound healing has been reported in the literature. There are some studies in silico of some molecules that can affect this target protein [3, 13].

In our study, some major compounds in *Asphodelus* species were selected based on the literature. Subsequently, the properties of these compounds in terms of drug likelihood, ADME tests were performed in silico, and toxic molecules were eliminated. As a result of the ADME test, the two best molecules that did not violate any rules of Lipinski were chrysophanol and emodin.

Docking operations were performed using Autodock Vina software. It has been shown that molecules belonging to the indirubin family inhibit the GSK3-β enzyme [14]. Re-docking was performed for the validation of our method. Native ligand was docked back into the respective crystal structure(5N5K) and the RMSD value with respect to reference ligand was found to be< 2A^o (RMSD value:1,583 A^o). In the light of this information, indirubin was preferred as the positive control. The molecule named PF-04802367 was chosen as our second positive control based on the work on the complex of GSK3 B protein, which we use as target protein (PDB ID: 5K5N), with the inhibitor named PF-04802367 [15]. As a result of the docking study, it was observed that all ligands were bound to the selected active site in the protein. While the dock scores of the indirubin and PF-04802367 molecules that we chose as positive control were -7.58 and -7.56, respectively (kcal/mol), the stigmasterol and β-sitosterol molecules showed a much better score. (respectively -13.90, -12.62 kcal/mol). In another docking study with inhibition of glycogen synthase kinase-3β, in parallel with our findings, the minimum binding energy of the molecule named taraxerol, which is one of the molecules with a sterol ring obtained from the extract of *Naravelia zeylanica* (L.), is -12.59 and that of β-sitosterol is - It was found to be 11.25 [16]. In another study, the binding score of the molecule Tideglusib, which had significant results in clinical trials, was determined as -9.4 in docking studies [17]. According to a study conducted with the inhibitor coded PF-367, hydrogen bond formation occurred between the amino acids Val 135 and Asp 133 and the ligand, and it was found that there was a π-bond interaction with Arg 141 [10]. According to our findings, emodin molecule similarly formed hydrogen formation with Val133 and Asp 133 amino acids(Figure 3). The inhibitor coded PF-04802367, which we used positively in our study, was found to form hydrophobic interaction with the protein A chain through the amino acids Val70, Leu132, Thr 138, and hydrogen bond formation through Tyr134 and Val135 amino acids [15]. According to our study, it is seen that the chrysophanol molecule forms hydrogen bonds with Tyr 134, Val 135 and Pro136 (Figure 3). Amino acids with which ligands interact with GSK3-β (Val135, Asp200, Val70, Gln185, Arg141, Pro136, Tyr134, Leu188, Asn186 and Cys199) are similar to each other in accordance with the literatüre [18]. If a general evaluation is made, it can be said that the most interaction with the protein is seen in the emodin molecule (in terms of hydrogen bond, pi-sulfide, pi-alkyl and alkyl bonds).

4. CONCLUSION

As a result, it was observed that the ligands used in our study had high binding potentials with GSK3-β. Among these molecules, emodin, stigmasterol and β-sitosterol stand out as the best. Although it is predicted that these molecules may have potential inhibitory effects, in vitro and in vivo studies are needed. In the light of the literature, although it is seen that there is a relationship between GSK3-β and wound healing, considering that the formation of the wound is a complex phenomenon, there is a need for such studies with other proteins that play a role in wound formation.

5. MATERIALS AND METHODS

5.1. Data, database and tools

Ligands have been selected from some of the major compounds in the *Asphodelus* species. Drug candidate likelihood values for these compounds in terms of toxicity and bioavailability criteria were calculated using the Swiss ADME web server [8]. AutoDock Tools software was used for molecular docking studies [9].

5.2. Work flow

The study was carried out according to the work flow chart shown in the Figure 4 in order to have an idea about which molecule the wound healing effect of *Asphodelus* species originates from.

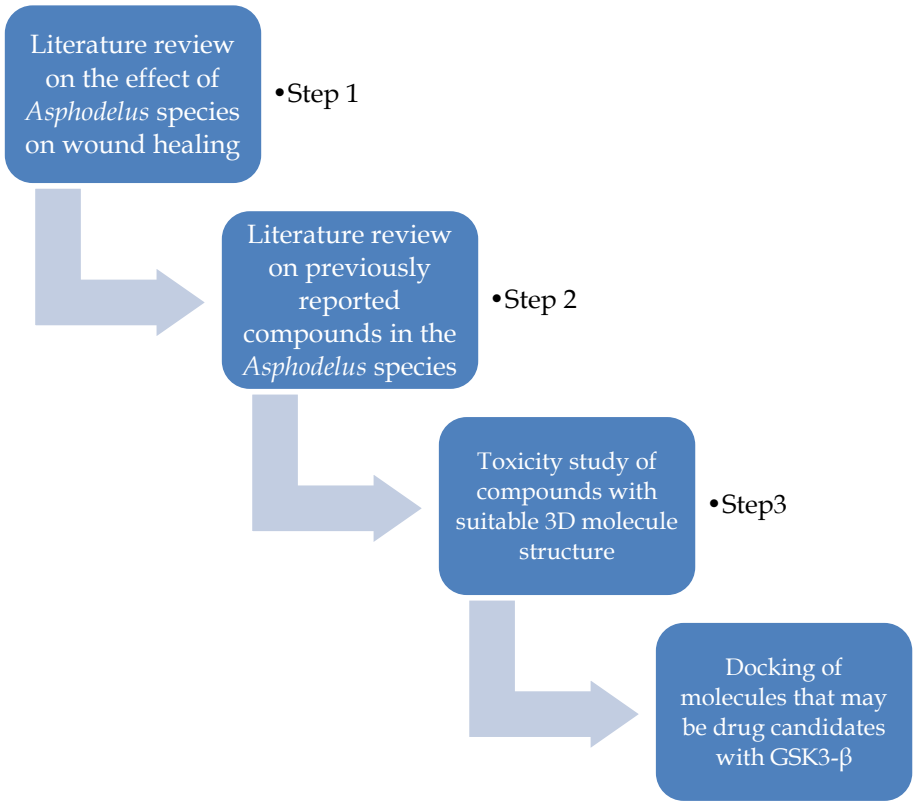

Figure 4. Work flow diagram of our study.

5.3. Docking

Crystal structures used as target proteins in wound healing studies were selected by conducting a literature search. It was observed that GSK3- β protein was frequently used in silico methods in wound healing studies. The crystal structure suitable for GSK3-β was downloaded from the Protein Data Bank with reference to a study by Adewale J. Ogunleye and his colleagues. (PDB Code: 5K5N) [10].

The X-Ray Crystallographic 3D structures of the protein 5N5K were downloaded from Protein DataBank website. Native ligands, water molecules and salt ions were removed from the protein by using

BIOVIA DS 4.5 , and also hydrogens atoms or needed loops were added by the "Prepare Protein" protocol. In addition, protein is minimized and clean geometry is applied. For each ligand which are namely 1-beta-sitosterol, 2-stigmasterol, microcarpin, ramosin, chrysophanol, asphodelin and emodin were prepared using the "Prepare Ligands" protocol by BIOVIA DS 4.5. They are all properly protonated, optimized and saved as SDF file format for further docking calculations. Docking procedure is done by AutoDock 4.2. [9]. XYZ coordinates of the proteins' catalytic binding site was set to X=0.753, Y=7.449 , Z=27.544 by checking the native ligand. Grid box dimensions were set to 60x60x60 Angstrom, Lamarkian Genetic Algorithm was used with 2.500.000 energy evaluations for each conformation. All ligands were saved as PDBQT using AutoDock Tools and results files were saved in DLG format for the DeltaG and inhibition constant (Ki) analysis [11].

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