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Exploring the anticancer activity and active mechanism of turkish propolis against hl-60 myeloid cancer cells: insights from molecular docking studies

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ABSTRACT: Turkish propolis has gained significant attention in the medical field due to its diverse biological activities, which are influenced by geographical origin and plant sources. This study aimed to explore the anticancer activity and active mechanism of Turkish propolis collected from the Trabzon region against HL-60 myeloid cancer cells. Computational calculations using the Schrödinger 2021-4 small molecule drug discovery suite were performed, and the three-dimensional structure of caspase-3 (PDB ID: 2C1E) and procaspase-3 (PDB ID: 4JQY) were obtained from the Protein Data Bank. Molecular docking simulations were conducted to investigate the binding pose of the propolis' active ingredients within the caspase-3 binding site and also allosteric site of procaspase-3. The identified binding sites were utilized, and the natural compounds present in the propolis extract, including quercetin, caffeic acid, campherol, galangin, naringenin, and chrysin, were prepared using the LigPrep module. The docking results revealed significant interactions of the active ingredients with amino acid residues in the caspase-3 binding site, highlighting the potential role of ARG207, GLN161, GLY122, and HIE122 in modulating caspase-3 activity. Additionally, in the site-3 binding region, quercetin, kaempferol, and galangin exhibited specific interactions with CYS170 and LYS260. Furtheremore all polyphenolic compouds make several interactions with crucial amino acid residues, especially ARG164 and TYR197 in allosteric site of procaspase-3. These findings provide valuable insights into the active mechanism of Turkish propolis against HL-60 myeloid cancer cells through its interactions with caspase-3 and procaspase-3. Further experimental validation is required to confirm the functional significance of these interactions and their potential as effective anticancer agents. This study contributes to the understanding of Turkish propolis' anticancer potential and supports its potential use as a therapeutic agent against HL-60 myeloid cancer cells.

KEYWORDS: Turkish propolis, myeloid cancer, caspase-3, molecular docking

1. INTRODUCTION

Acute myeloid leukemia (AML) is a highly lethal cancer characterized by the uncontrolled proliferation of malignant marrow stem cells, leading to infection, anemia, and bleeding. Advances in understanding its pathophysiology, diagnostic techniques, and therapeutic approaches have transformed the landscape of AML management. However, despite these advancements, the incidence of AML is on the rise. In the United States alone, it is estimated that there will be approximately 20,380 new cases of AML and 11,310 deaths from the disease in 2023. These alarming numbers highlight the urgent need for continued research and improved strategies to tackle this aggressive form of leukemia [1].

Propolis, commonly known as bee glue, is a natural mixture of products derived from honey bees. It is produced using a variety of plant species, resulting in variations in its composition and ingredient percentages based on the geographical origin, climate, and plant sources [2]. Due to its highly complex nature, propolis exhibits a wide range of biological activities, including antimicrobial, antibacterial, antiviral, antifungal [3], immune system modulation [4], and anticancer effects [5]. Propolis, with its rich content of polyphenolic compounds, exhibits notable anticarcinogenic properties. Among the prominent bioactive components of propolis, CAPE (caffeic acid phenethyl ester), artepillin C, and chrysin have been extensively studied for their ability to inhibit cancer cell growth across different types of malignancies. Moreover, Chrysin exhibits anticancer properties by triggering caspases and inducing both intrinsic and extrinsic apoptosis pathways. These compounds hold promise as potential therapeutic agents in the fight against cancer [6].

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This study aims to provide valuable insights for future research by elucidating the affinities and interactions of natural compounds, namely caffeic acid, campherol, chrysin, galangin, naringenin, and quercetin that were characterized previously using different spectroscopic methods [7] with the caspase-3 enzyme in Turkish propolis derived from specific plant sources (*Picea orientalis, Fagus orientalis, Castanea sativa, Rhododendron ponticum, Rhododendron luteum*, and *Rubus caucasicus*) in the Yomra region. In silico studies were conducted to investigate the binding characteristics and potential modulatory effects of these compounds on caspase-3 activity. The findings of this study contribute to a better understanding of the bioactive properties of Turkish propolis and provide a foundation for further investigations in the development of potential therapeutic strategies targeting caspase-3.

2. RESULTS & DISCUSSION

In the realm of cancer treatment, the ability to induce apoptosis, which refers to programmed cell death, assumes a pivotal role. Apoptosis, as a highly regulated and controlled process, plays a vital role in maintaining cellular homeostasis by eliminating damaged, infected, or potentially harmful cells. Within the context of cancer, the dysregulation of apoptosis is a hallmark characteristic, often resulting in uncontrolled cell proliferation and tumor progression. Consequently, harnessing the mechanisms that govern apoptosis and leveraging them as therapeutic strategies has emerged as a promising avenue in combating cancer. Although apoptosis has relationship with multiple genes that the keys mediators of the process are the caspases [8].

Caspase-3, known as an effector caspase, is extensively researched and recognized for its critical involvement in two major apoptotic pathways: the death receptor pathway, which is initiated by caspase-8, and the mitochondrial pathway, where caspase-9 plays a key role. Understanding the multifaceted functions of caspase-3 in these pathways is crucial for unraveling the intricate mechanisms of apoptosis and exploring its potential as a therapeutic target in various diseases, particularly cancer.

Propolis, also known as bee glue, has been well known since ancient times moreover in recent years its' popularity getting sharply increased. Its' chemical composition is highly complex that's why propolis has various biological activities such as antimicrobial, antibacterial, antiviral, antifungal [3], immune system booster [4], anticancer [5], etc. However, propolis' biological activities are dependent on the geographical origin, climate, and plant sources [2]. Therefore, in our study in 2016, The Turkish propolis' anticancer activity was figured out by inducing the caspase-3 activity and now we are exhibiting its' chemical compositions affinities and interactions with caspase-3.

By consideration the literature, it is obvious that there is no much studies to show caspase-3 activation site. Almost all researchers has been interested to improve caspase-3 inhibitor for obtaning anti-inflammatory activity [9-10]. For this reason, the SiteMap method was used to identify the probable binding site of caspase-3. The method is used for the identification and characterization of the binding site of any protein not known as its' binding site. Additionally, In large-scale validation, SiteMap correctly identified the known binding site as the top-scaled site in 86% of the cases, with the best results coming for sites that bind ligands with subnanomolar affinity. Hence, This indicates the high correctness of the binding sites determined using this method [11].

As a results of the SiteMap method, 3 different regions were identified as probable binding site of caspase-3 (PDB ID: 2C1E). While Site-2 region (see Fig. 1.) known as allosteric inhibitor binging site. That's why, in this study, the active ingredients of Turkish propolis that are caffeic acid, kaempferol, chrysin, galangin, naringenin, and quercetin were docked into the identified site-1 and site-3 binding site.

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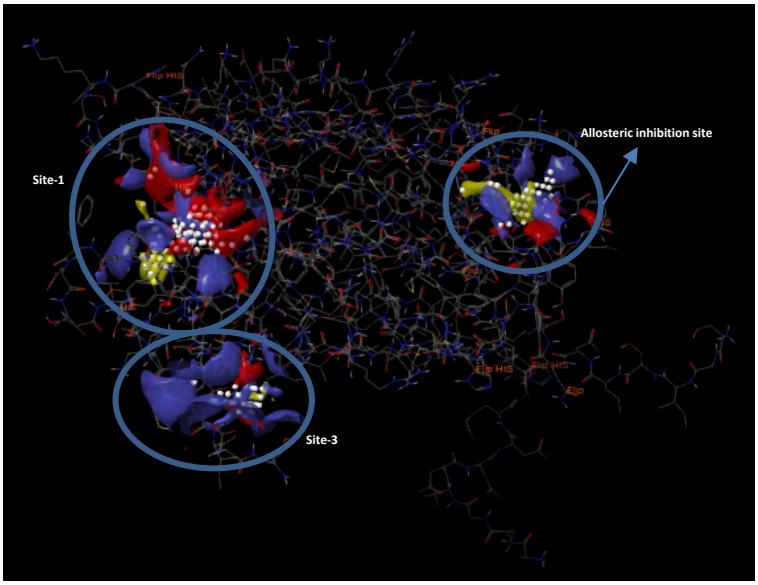

Figure 1. The probable binding sites and allosteric inhibition site of Caspase-3 (PDB ID: 2C1E)

In our previous study, we observed that the extract of Turkish propolis exhibits promising anti-cancer activity through the induction of apoptosis. Notably, we found that the activation of caspase-3 by the propolis extract resulted in a significant increase in caspase-3 activity, approximately 12-fold higher with 0.25 mg/mL against myeloid cancer [12]. These findings suggest a strong affinity between the active ingredients present in the propolis extract and caspase-3.

To further explore and enhance these affinities and interactions with caspase-3, we conducted *in silico* studies. As mentioned above, Docking simulations were performed using the identified site-1 and site-3 (refer to Fig. 1) as determined by SiteMap analysis.

Analysis of the docking results within the site-1 region of caspase-3 revealed multiple interactions of the six active ingredients with various amino acid residues, including hydrogen bonding and pi-pi interactions. Notably, several common interactions were observed with ARG207, GLN161, GLY122, and HIE122, suggesting the potential significance of these amino acid residues in modulating the activity of caspase-3.

Quercetin demonstrated excellent conformational compatibility within the hydrophobic pocket (refer to Fig. 2), along with multiple hydrogen bonds formed with GLY122, ARG207, and SER205. Similarly, caffeic acid exhibited a hydrogen bond interaction with ARG207. Another common interaction observed among caffeic acid, kaempferol, and Naringenin was a hydrogen bond with GLN161. Additionally, kaempferol and chrysin displayed pi-pi interactions with HIE122.

Overall, our findings suggest that interactions involving ARG207, GLN161, and HIE122 may play a significant role in enhancing the activity of caspase-3. Furthermore, quercetin and caffeic acid, with their respective compatibility to the hydrophobic pocket and solvent exposure, have the potential to exhibit higher activity and prolonged binding within the caspase-3 binding site.

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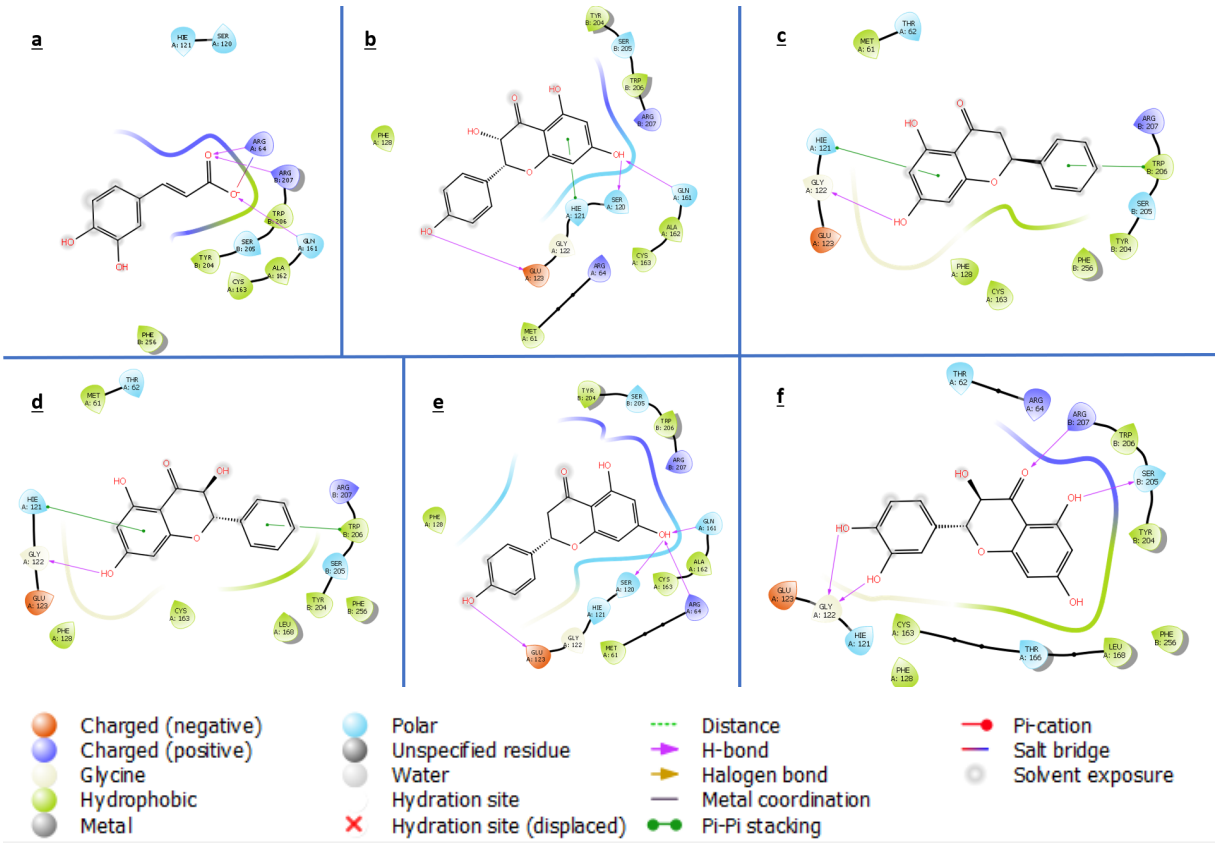

Figure 2. Predicted 2D binding interactions of Caffeic acid (a), kaempferol(b), Chrysin (c), Galangin (d), Naringenin(e), and Quercetin (f) in identified site-3 region of Caspase-3 (PDB ID: 2C1E). H-bond and pi-cation interaction are displayed with purple one-headed arrow and red line, respectively.

Within the identified site-3 binding region of caspase-3, our analysis revealed that quercetin, campherol, and galangin exhibit specific interactions with certain amino acid residues. Notably, these three compounds form hydrogen bonds with CYS170 and LYS260, while also engaging in a pi-cation interaction with LYS260 (see Fig. 3). Conversely, caffeic acid and chrysin do not demonstrate substantial interactions with caspase-3 in the site-3 binding region.

The observed hydrogen bonding and pi-cation interactions between quercetin, campherol, and galangin with CYS170 and LYS260 highlight their potential significance in modulating the binding and activity of caspase-3 within the site-3 region (see Fig. 3). These specific interactions may contribute to the stabilization and favorable positioning of the compounds within the binding site, potentially influencing their efficacy as caspase-3 modulators.

Conversely, the limited interaction of caffeic acid and chrysin with caspase-3 in the site-3 region suggests that these compounds may have a different mode of action or exhibit activity through alternative mechanisms, independent of direct binding to this specific region.

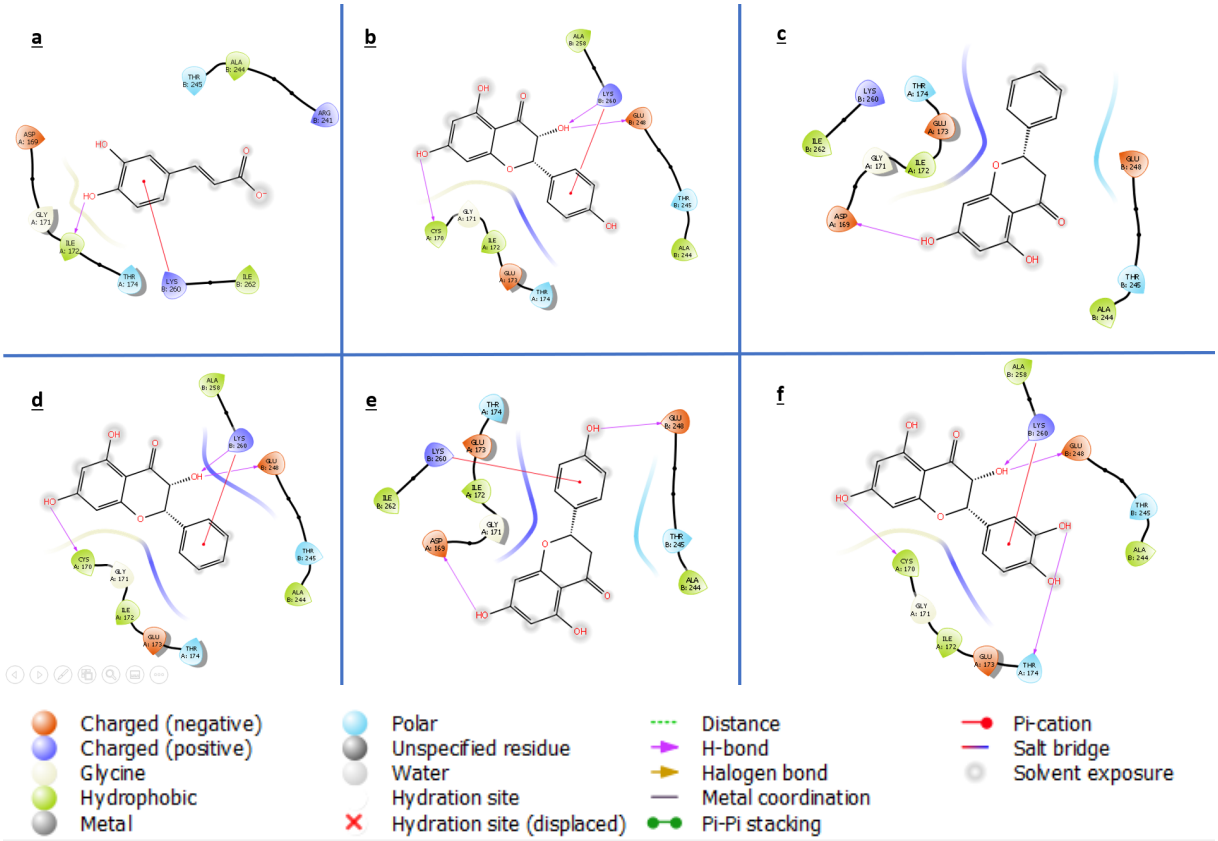

Figure 3. Predicted 2D binding interactions of Caffeic acid (a), kaempferol(b), Chrysin (c), Galangin (d), Naringenin(e), and Quercetin (f) in identified site-3 region of Caspase-3 (PDB ID: 2C1E). H-bond and pi-cation interaction are displayed with purple one-headed arrow and red line, respectively.

By considering the literature, embarked on a comprehensive exploration, focusing on also procaspase-3, as a key target for our molecular docking investigations. At the outset, our curiosity led us to delve into the intricate interactions between small molecules and caspase-3. This initial phase of our study sought to unravel the potential binding dynamics modulatory prospects inherent to caspase-3. Through computational simulations, we aimed to shed light on the structural nuances that govern these interactions, thereby discerning potential avenues for influencing caspase-3's catalytic behavior.

Drawing from the insights gained from the caspase-3 docking study, our investigation naturally evolved to encompass procaspase-3, a precursor imbued with its own unique significance within the apoptotic cascade. Procaspase-3's pivotal role as the dormant form, awaiting activation through processing by initiator caspases [13], was a driving force behind our decision to extend our study. By targeting procaspase-3 in our molecular docking analyses, we aimed to glean insights into its potential binding interactions and the plausibility of allosteric activation.

To address receptor flexibility, we adopted an advanced computational strategy known as induced-fit molecular docking. In contrast to conventional docking approaches assuming a static receptor structure, induced-fit docking accommodates receptor dynamics, permitting conformational adjustments during the docking procedure. This methodology accurately captures the intricate interplay between ligands and target proteins or enzymes, resulting in a more faithful representation of ligand-receptor interactions [14]. By integrating induced-fit molecular docking, we achieve a nuanced understanding of the binding site's conformational adaptability. This comprehensive approach enables the exploration of diverse ligand binding modes and the corresponding structural modifications within the target protein or enzyme. Our incorporation of the induced-fit mechanism furnishes valuable insights into the molecular underpinnings of high-affinity compound interactions, shedding illumination on their potential utility as therapeutic agents.

Analysis of the docking results within the allosteric site of procaspase-3 (PDB ID: 4JQY) revealed multiple interactions of the six active ingredients with various amino acid residues especially ARG164 and TYR197 that are significant for activation of procaspase-3 (See Fig. 4) [13]. Moreover, Phenyl and dihydroxyphenyl moieties in chrysin, galangin, and quercetin show great compatibility within the

hydrophobic pocket that is covered by crucial amino acid residues such as LEU136, ILE160, CYS264, and VAL266 (see Fig. 4) [13].

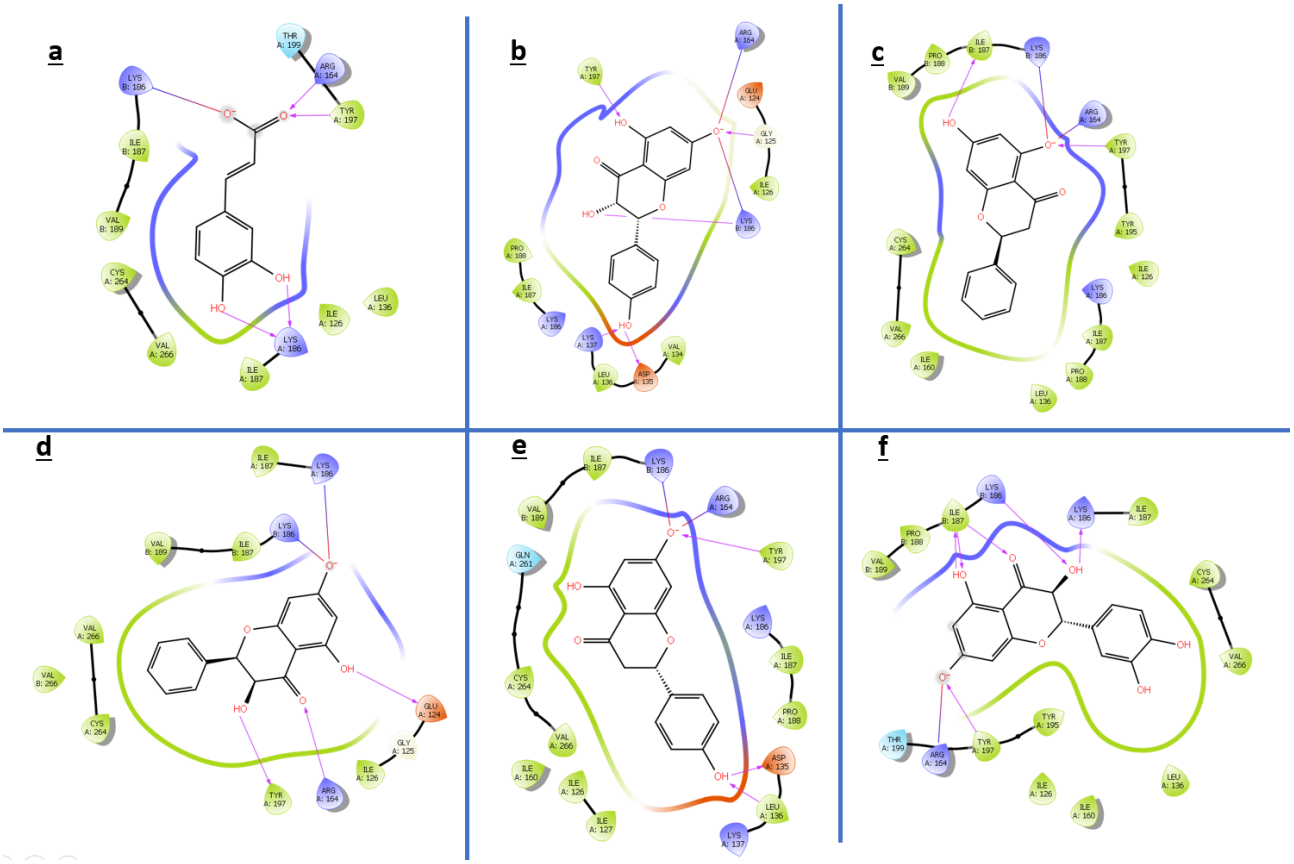

Figure 4. Predicted 2D binding interactions of Caffeic acid (a), kaempferol(b), Chrysin (c), Galangin (d), Naringenin(e), and Quercetin (f) in allosteric site of Pro-caspase-3 (PDB ID: 4JQY). H-bond and pi-cation interaction are displayed with purple one-headed arrow and red line, respectively.

Afterward, chemo-informatic properties were calculated using QikProp module in Maestro Schrödinger. It is crucial in especially the novel drug discovery which will be administrated as oral that the permeability and solubility of the compounds are estimated by making use of these evaluations. Lipinski's rule of 5 foretells whether compounds have good absorption and permeation. with respect to the rule of 5, it is inevitable that the compound's absorption and permeation are most probably good if its HBD is less than 5, HBA is less than 10, MW is less than 500 mg/mol and LogPo/w value is less than 5 [15] . All natural compounds from Turkish propolis showed acceptable values and obeyed the rule of 5 (see Table 1). However, LogPo/w values of caffeic acid, quercetin, and kaempferol extremely low. It means that they are highly lipophilic. Therefore, they can be faced a challenge regarding absorption from the membrane. In 2022, as a result of a study it was reported that quercetin usually absorbed via different mechanism instead of passive diffusion [16]. The findings of physicochemical properties from our *in silico* studies, provide additional support for the aforementioned result (see Table 1).

Table 1. Docking scores and ADME properties of all natural compounds in Turkish Propolis

Compounds	DS[a]/Site-1	DS/Site-3	DS/procaspase-3	MWb	HBDc	HBAd	LogPo/we	HOA%f
Caffeic acid	-6.081	-4.304	-6.435	180.16	3	3.50	0.519	54
Quecetin	-6.089	-5.824	-6.041	304.26	4	6.45	0.078	52
Kaempferol	-5.937	-5.052	-6.409	288.26	3	5.70	0.724	64
Chrysin	-5.478	-4.461	-6.668	256.26	1	3.25	2.339	87
Naringenin	-5.658	-5.003	-7.071	272.26	2	4.00	1.603	74
Galangin	-5.658	-5.041	-5.044	272.26	2	4.95	1.429	77

[a]Docking score (kcal/mol).
[b]Molecular weight (g/mol) (recommended value ≤ 500)
[c]Hydrogen bond donar (recommended value ≤ 5)

[d]Hydrogen bond acceptor (recommended value ≤ 10)
[e]Logarithm of the octanol/water ratio coefficient of compound (recommended value < 5).
[f]Percentage oral absorption (< 25% weak and > 85% strong).

4. CONCLUSION

This study explored the anticancer activity and active mechanism of Turkish propolis collected from the Trabzon region against HL-60 myeloid cancer cells. In summation, our research journey transitioned seamlessly from caspase-3 to procaspase-3, offering a more comprehensive perspective on the molecular interactions underlying apoptosis regulation. These insights into the specific interactions and molecular features influencing the activity of caspase-3 provide valuable information for the design and development of potential therapeutic agents targeting this key apoptotic protein. Further experimental validation is warranted to confirm the functional significance of these interactions and their potential as effective anti-cancer agents. This dual-focus investigation not only enriches our understanding of apoptotic pathways but also paves the way for the translation of these findings into meaningful clinical applications.

5. MATERIALS AND METHODS

5.1. Molecular Docking

The computational calculations were conducted using the Schrödinger 2021-4 small molecule drug discovery suite. The three-dimensional structure of caspase-3 (PDB ID: 2C1E) (Shen et al., 2009) at a resolution of 1.77 Å and Human procaspase-3 (PDB ID: 4JQY) [15] at a resolution 2.50 Å were obtained from the Protein Data Bank. Molecular docking simulations were performed to investigate the binding pose of the active ingredients from Turkish propolis which was obtained from *Picea orientalis, Fagus orientalis, Castanea sativa, Rhodendron ponticum, Rhododendron luteum,* and *Rubus caucasicus* found in the region of Yomra, Trabzon within the caspase-3 binding site and procaspase-3 allosteric site.

Bond orders were determined using the protein preparation wizard, and any absent hydrogen atoms were included. Water molecules and heteroatoms, including native ligands, were eliminated from the binding site. Subsequently, optimization and restraint minimization were carried out utilizing the OPSL4 force field.

The SiteMap module was used in order to identify the probable binding sites of caspase-3. Afterward, using the identified binding sites, GrideBoxes were created and carried out molecular docking simulations [11]. However, ARG164 and TYR197 were retrieved as the center for creating the Girde Boxes of procaspase-3. Additionally, the natural products (cafeic acid, campherol, chrysin, galangin, naringenin, and quercertin) found in propolis extract were prepared using the LigPrep module of Schrödinger (Schrödinger Release 2023-1: LigPrep, Schrödinger, LLC, New York, NY, 2021). This process generates all possible conformations, ionization states, and tautomers at pH 7.0 ± 2.0 by converting each structure from 2D to 3D.

Furthermore, Molecular modeling calculations for Procaspase-3 were calculated using the induce fit module.

Additionally, The Absorption-Distrubition-Metabolism-Elimination (ADME) properties of the chose compounds such as molecular weight, Hydrogen Bond acceptor (HBA), Hydrogen Bond Donor (HBD), logPo/w were determined by QikProp module [17] of Schrodinger suit.

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