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Original Article

IDENTIFICATION OF FLAVONOIDS FROM *ACALYPHA INDICA* L. (EUPHORBIACEAE) AS CASPASE-3 ACTIVATORS USING MOLECULAR DOCKING AND MOLECULAR DYNAMICS

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ABSTRACT

Objective: The purpose of this study was to determine the structural-based molecular interactions between flavonoids contained in *Acalypha indica* L. and caspase-3 by molecular docking and molecular dynamics (MD) simulations.

Methods: In a computer simulation, ten flavonoids contained in *A. indica* L. were evaluated for caspase-3 using the X-ray crystal structure of human caspase-3 (PDB ID 1NME). The AutoDock 4.2 software was used to study molecular docking, and MD simulations were done with GROMACS v2018.

Results: The results of molecular docking identified the top four compounds, namely nicotiflorin, naringenin, hesperetin, and kaempferol, with docking scores of-6.81,-6.45,-6.33, and-6.10 kcal/mol, respectively. According to the MD simulation results, nicotiflorin was most effective in stabilizing the complex with caspase-3, with a total energy (ΔG_{bind}, MM-PBSA) of-96.315 kcal/mol.

Conclusion: This study showed that nicotiflorin was the flavonoid in *A. indica* L. that activated caspase-3 the best.

Keywords: *Acalypha indica* L., Caspase-3, Molecular docking, Molecular dynamics

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INTRODUCTION

Caspase-3, a member of the cysteine protease group, plays an important role in apoptosis. There are many possible strategies for cancer treatment, and caspase-3-induced apoptosis activation and causing cytotoxicity is one of them [1]. Caspase-3 plays the most important role in cellular mechanisms and can be triggered by various initiators, so caspase-3 becomes the main target for anticancer therapy [2]. Caspase-3 is normally expressed in the secretory epithelial cells of the prostate. Caspase-3 expression was also found to be lower in moderately and poorly differentiated prostate tumors in comparison to adenocarcinoma of the well-differentiated prostate and the normal prostate [3]. Many compounds, including plant isolates, have been reported to cause caspase-3-mediated apoptosis-induced cytotoxicity [1, 2].

Acalypha indica L. is a member of the family Euphorbiaceae, which has been used in traditional medicine practices [4]. Numerous studies have shown that *A. indica* L. has a variety of beneficial properties, including anticancer, antibacterial, antifungal, antioxidant, and antidiabetic activities. Secondary metabolites such as flavonoids, alkaloids, steroids, saponins, tannins, and essential oils were found to be contained in the extract of *A. indica* L. [5]. Flavonoids are one of the most abundant components in *A. indica* L.; however, it has not been determined which flavonoids are responsible for the interaction with caspase-3.

Flavonoids can regulate cell proliferation, attract pollinators, and protect plants from biotic and abiotic stressors [6]. In humans, this chemical has anti-inflammatory, anticancer, antiaging, cardioprotective, neuroprotective, immunomodulatory, antidiabetic, antibacterial, antiparasitic, and antiviral properties [7]. Based on their chemical structure, flavonoids can be divided into the following six main classes: flavan-3-ols, flavones, flavanols, flavanones, and isoflavones [8]. More importantly, several *in vitro* and animal studies have shown that flavonoids have potential protective properties against the development and progression of various types of cancer [9, 10].

The involvement of computer-aided drug design (CADD) [11], which includes molecular docking and MD, plays an important role in producing compounds with a high probability of being the lead compound from the series of compounds being screened. The docking score is the result of assessing the interaction of compounds with macromolecules using molecular docking. This is followed by the evaluation of interactions using MD, focusing on the stability of interactions between complex macromolecules and ligands.

This study aims to identify the flavonoid compounds contained in *A. indica* L. that are the most responsible caspase-3 activators. In this study, we predicted how the flavonoids contained in *A. indica* L. would interact with the three-dimensional crystal structure of caspase-3 using molecular docking and MD simulations.

MATERIALS AND METHODS

Materials

The personal computer was equipped with an Intel® Core™ i7-7200U CPU @2.50 GHz (4 CPUs. 2.7 GHz), 20 GB of RAM, and two operating systems: Linux Ubuntu 18.04 64-bit and Windows 10 Pro-64-bit for molecular docking and MD simulation.

Molecular docking of flavonoids with caspase-3

Flavonoid compounds were molecular docked to produce binding of their bioactive in the catalytic region of caspase-3. The docking method was carried out using AutoDock 4.2 software's genetic optimization, and hits were chosen based on clustering and docking scores. [12] The 3D crystallographic structure of caspase-3 with 2-Hydroxy-5-(2-mercapto-ethylsulfamoyl)-benzoic acid as the natural ligand (PDB ID: 1NME) was found on the RSCB website [13]. The protein and native ligand were prepared by removing water molecules and other ligands. The grid coordinates were 40.881 (X), 95.736 (Y), and 24.611 (Z), while the grid box sizes were 45 (X), 40 (Y), and 40 (Z). Refer to Febrina *et al.*, 2021 for further information on detailed techniques [14]. MD simulations were used to look at the potential flavonoids found through docking at the atomic level.

Molecular dynamics and energy components

The potential flavonoids found by the molecular docking procedure were put through MD simulation experiments in GROMACS v2018

with a timescale of 100 ns to test how stable they are in the water at the atomic level [15]. To begin the MD simulations, the best flavonoid-caspase-3 pose complexes were used to generate the protein and ligand topologies for use with the AMBER force field. After first using a cubic water box to solvate the system with the help of a TIP3P water model, the system was then neutralized by the addition of the Na^+counter ions. After the steepest descent energy minimization, a two-fold equilibration protocol was carried out, with NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) of 500 ps each. This was done so that the system could reach a state of equilibrium. For more information on the specific procedures, please refer to Febrina *et al.* 2021 [14]. It was determined whether or not the flavonoid molecules were stable by computing their root-mean-square deviation (RMSD) values over the course of the entire simulation production run. The orientation of their binding in the caspase-3 pocket was also investigated. In addition, the interaction of the best poses with the catalytic residues of caspase-3 was investigated and compared with the activity of the native ligand, 2-hydroxy-5-(2-mercapto-ethylsulfamoyl)-benzoic acid (ID: 159). Within the MD trajectories, 500 frames were chosen, and the free energy of binding, denoted by the symbol ΔG_{bind}, was calculated using the following equation from molecular mechanics, MM/GBSA:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

We employ a program called g_mmpbsa [16] to integrate high-throughput MD simulations with estimations of binding energy. This tool uses subroutines that were either built in-house or taken from the APBS and GROMACS packages to carry out the MM-PBSA method.

RESULTS

Docking analysis

Docking analysis of 10 test ligands (from various sources) at the 1NME receptor showed that 7 compounds had more negative energy than native ligand (table 1 and fig. 1).

Table 1: Docking score and estimated inhibition constant of ligands in the binding pocket of caspase-3 (1NME)

No.	Ligand	Code	Docking score (ΔG) (kcal/mol)	Estimated inhibition constant (Ki) (μM)	Amino acid involved in interaction with ligands
	2-Hydroxy-5-(2-mercapto-ethylsulfamoyl)-benzoic acid (ID: 159)	NL	-5.72	63.83	Tyr204, Arg207, Asn208, Ser249, Phe250
1	Naringin	Nar	-5.40	109.79	Arg207, Ser209, Glu248, Phe250, Asp253
2	Naringenin	Nari	-6.45	18.81	Trp214, Glu248, Phe250
3	Kaempferol	Kaem	-6.10	33.51	Arg207, Trp214, Glu248, Phe250, Ser251
4	Hesperetin	Hes	-6.33	22.83	Ser 205, Arg 207, Phe250
5	Chrysin	Chry	-6.03	37.91	Trp214, Glu248, Phe250
6	Galangin	Gala	-5.96	43.07	Arg 207, Trp214, Glu248, Phe250
7	Nicotiflorin	Nico	-6.81	10.15	Arg207, Ser209, Trp214, Phe250, Ser251, Phe252
8	Quercetin	Quer	-5.51	91.08	Asn208, Phe250, Phe252
9	Kaempferol Glucoside	KG	-5.56	83.80	Arg 207, Trp214, Glu248, Phe250, Ser251, Phe252
10	Rutin	Rut	-5.85	51.53	Arg207, Asn208, Ser209, Phe250

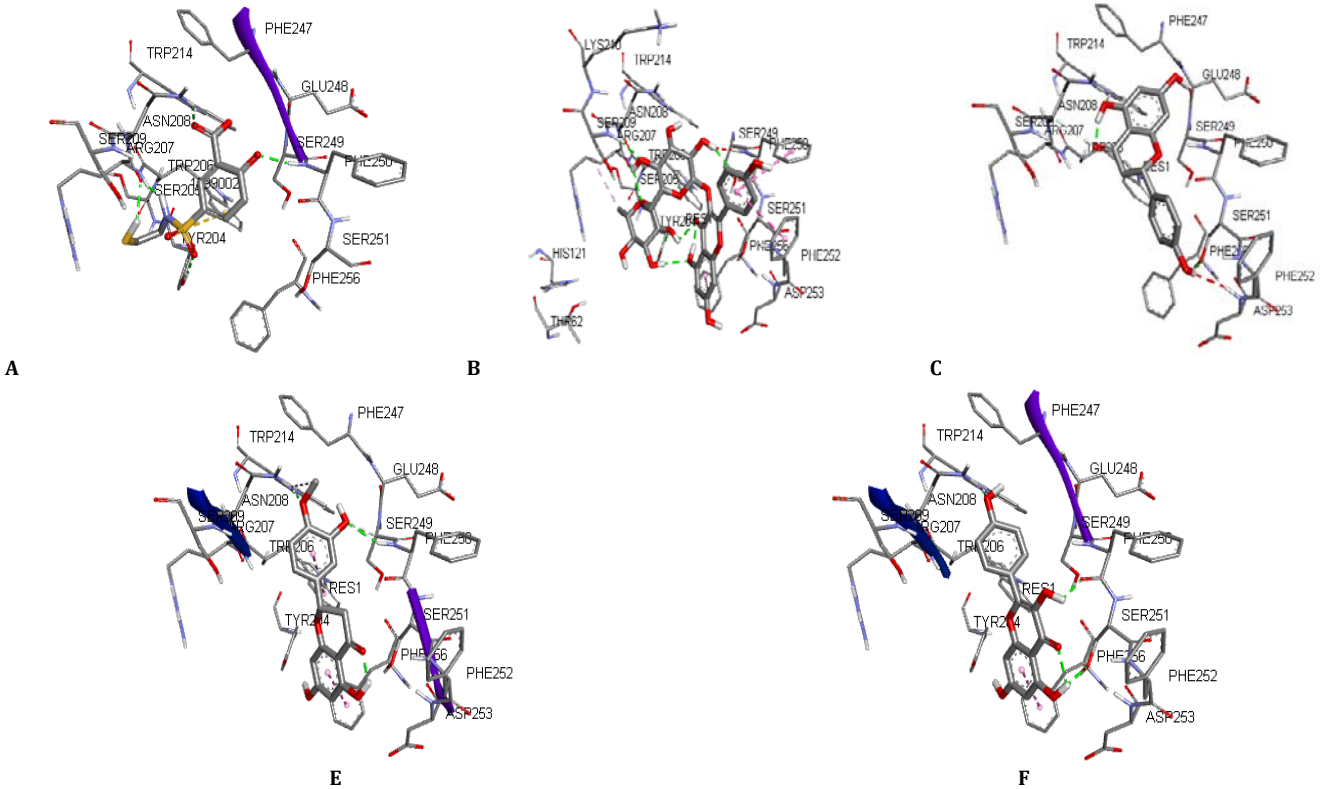

Fig. 1: 3D interactions of [A] native ligand (NL), [B] nicotiflorin, [C] naringenin, [D] hesperetin, and [E] kaempferol into the binding pocket of caspase-3 (1NME)

Molecular dynamics analysis

Root-mean-square deviation (RMSD)

The docking best poses were used to study how solvents and the flexibility of proteins affect how receptors and ligands interact. To assess the simulation's stability, the parameters of each complex, such as temperature, pressure, energy, and structure, were evaluated along the MD trajectory (fig. 2).

Root-mean-square fluctuation (RMSF)

The RMSF was employed in MD simulations to evaluate the stability of residues in the binding pocket. The RMSF of each residue near the ligand was determined using the data of the last one ns of the trajectory (fig. 3).

Energy component estimation

The MM-PBSA study determines the free energy required to bind macromolecules and ligands by combining molecular mechanics calculations and continuum solvation models (table 2).

DISCUSSION

Docking score and interaction

The structure of caspase-3 with tethered salicylate (PDB ID: 1NME) was used to generate a rigid structure for the purpose of molecular docking. This structure was created by using the crystal structure of the protein caspase-3 in a complex with its specific inhibitor. Before docking flavonoids, the AutoDock software was tested by re-docking the native ligand, 2-hydroxy-5-(2-mercapto-ethylsulfamoyl)-benzoic acid (ID: 159), in the caspase-3 binding pocket. The RMSD that was achieved as a result of applying this method was equal to 1.77 Å between the docked pose and the pose corresponding to the 3D crystal structure. This degree of variation was determined to be acceptable for docking procedure validation. This docking approach was then used to dock ten flavonoids. The findings indicate that seven flavonoids had a docking score that was more negative than NL (ΔG =-5.72 kcal/mol). The seven flavonoids were naringenin, kaempferol, hesperetin, chrysin, galangin, nicotiflorin, and rutin, with docking scores of-6.45,-6.10,-6.33,-6.03,-5.96,-6.81, and-5.85 kcal/mol, respectively.

Seven flavonoids formed an H-bond with the two key amino acid residues Arg207 and Phe250 in caspase-3's active pocket, whereas three flavonoids (naringenin, chrysin, and quercetin) exclusively interacted with Phe250 (fig. 1 and table 1). The NL was able to create six H-bonds to the side chains of the active pocket, which consisted of Tyr204, Arg207, Asn208, Ser249, and Phe250, respectively. The kaempferol was successful in forming five H-bonds with the side chains of the active pocket, which included Arg207, Trp214, Glu248, Phe250, and Ser251. The hesperetin was able to create four H-bonds to the side chains of the active pocket, which were located on Ser 205, Arg 207, and Phe250, respectively. The galangin was successful in forming four H-bonds to the side chains of the active pocket, which were located on Arg 207, Trp214, Glu248, and Phe250. The nicotiflorin was successful in forming eight H-bonds to the side chains of the active pocket, which consisted of Arg207, Ser209, Trp214, Phe250, Ser251, and Phe252. It was possible for the rutin to make three H-bonds to the side chains of the active pocket, which were located on Arg207, Asn208, Ser209, and Phe250. In contrast, the naringenin was able to create three H-bonds to the active pocket side chains of Trp214, Glu248, and Phe250. The chrysin was able to make three H-bonds to the side chains of Trp214, Glu248, and Phe250 in the active pocket. The number of H-bonds not listed in the table.

Following that, we carried out MD simulations in order to determine the extent to which flavonoids are able to maintain their complexes with caspase-3. For the purposes of MD simulations, we limit our attention to the top four flavonoids, which are nicotiflorin, naringenin, hesperetin, and kaempferol, in addition to NL.

Complex stability

Simulations of molecular dynamics (MD) have been used in terms of making estimates of thermodynamic properties, such as the binding affinity of protein-ligand complexes. The MD simulation used for the NL and top four flavonoids had a more significant amount of negative energy than the NL, which was evaluated for 100 ns. The RMSD, RMSF, and molecular mechanics/Poisson–Boltzmann surface area (MM-PBSA) of these flavonoids' backbones were examined in order to assess the binding stability of the flavonoids.

Root-mean-square deviation (RMSD)

The RMSD of backbone atoms is a measurement used to determine the average distance between the backbone atoms of superimposed proteins. We used the docking best poses to investigate how solvents and the flexibility of proteins affect the way receptors and ligands interact with one another. During the study of the MD simulation, the various characteristics of each complex were analyzed to determine how stable the simulation was. These parameters included temperature, pressure, energy, and structure.

Both the four flavonoids and the protein showed fluctuations that were consistent with the complex's stable range (fig. 2). During the range of 0 to 10 ns, the NL value for the complex 1NME-NL changed from 0.0 to 1.0 Å. In addition, the value varied from 0.6 to 1.0 Å between 10 and 30 ns. At the end of the simulation, which lasted for 100 ns, it oscillated around 1.0 Å. For the complex 1NME-Nico, the nicotiflorin fluctuated between 0.3 and 0.5 Å from 0 to 35 ns. Between 30 and 45 ns, the value ranged from 0.5 to 1.0 Å. Up until the end of the 100 ns, it stayed within a range of 0.65 to 1.0 Å. During the complex 1NME-Nari simulation, which lasted for over 100 ns, the value of naringenin fluctuated between 0.35 and 0.50 Å. For the complex 1NME-Hes, the hesperetin fluctuated progressively up to 0.0-1.50 Å from 0 to 50 ns. The value ranged from 1.3 to 1.5 Å between 50 and 70 ns. It stayed within a range of 1.25 Å all the way up until the end of the 100 ns simulation. During the period of 0-55 ns, the complex 1NME-Kaemp fluctuated between 0.5 and 1.0 Å. Up until the end of the simulation that lasted 100 ns, the kaempferol fluctuated somewhere about 0.5 Å.

Root-mean-square fluctuation (RMSF)

The RMSF was utilized in MD simulations in order to determine the degree to which amino acid residues were stable within the binding pocket. The RMSF value that is larger suggests that there is more flexibility, whereas the RMSF values that are smaller suggest that there is a more stable or constrained region. This was done in five different locations (the 187–196, 197–213, 226–231, 248–262, and 265-274 regions) (fig. 3). The four flavonoids and NL exhibited fluctuations of up to 0.8 Å in the 187–196 region. In the region of 197–213, only NL showed fluctuations as high as 0.7 Å. This was true for all four flavonoids. Only NL showed a fluctuation of up to 0.4 Å in the 226–231 region out of the four flavonoids tested. Only NL showed a fluctuation of up to 0.5 Å in the 248–262 region out of the four flavonoids tested. Only NL showed a fluctuation of up to 0.4 Å in the 265–274 region out of the four flavonoids tested. For the most part, all of the complexes that were chosen displayed fluctuation patterns of amino acid residues that were, the most part, comparable to one another. As long as each of the five complexes changed by less than 0.8 Å in each of the most recent places, it is likely that the residue stayed in the stable category for each of these places.

Table 2: Energy components of the four flavonoids in complex with caspase-3 (1NME)

Energies types	Energy components of the four flavonoids in complex with caspase-3 (1NME) (kcal/mol)				
	NL	Nicotiflorin	Naringenin	Hesperetin	Kaempferol
Van der Waal energy	-113.839	-174.26	-77.15	-112.344	-55.669
Electrostatic energy	-226.37	-33.692	-89.358	-99.968	-115.032
Polar solvation energy	228.934	130.266	121.863	157.593	147.84
SASA energy	-12.467	-18.628	-11.228	-15.055	-10.179
Binding energy	-123.741	-96.315	-55.874	-69.773	-33.04

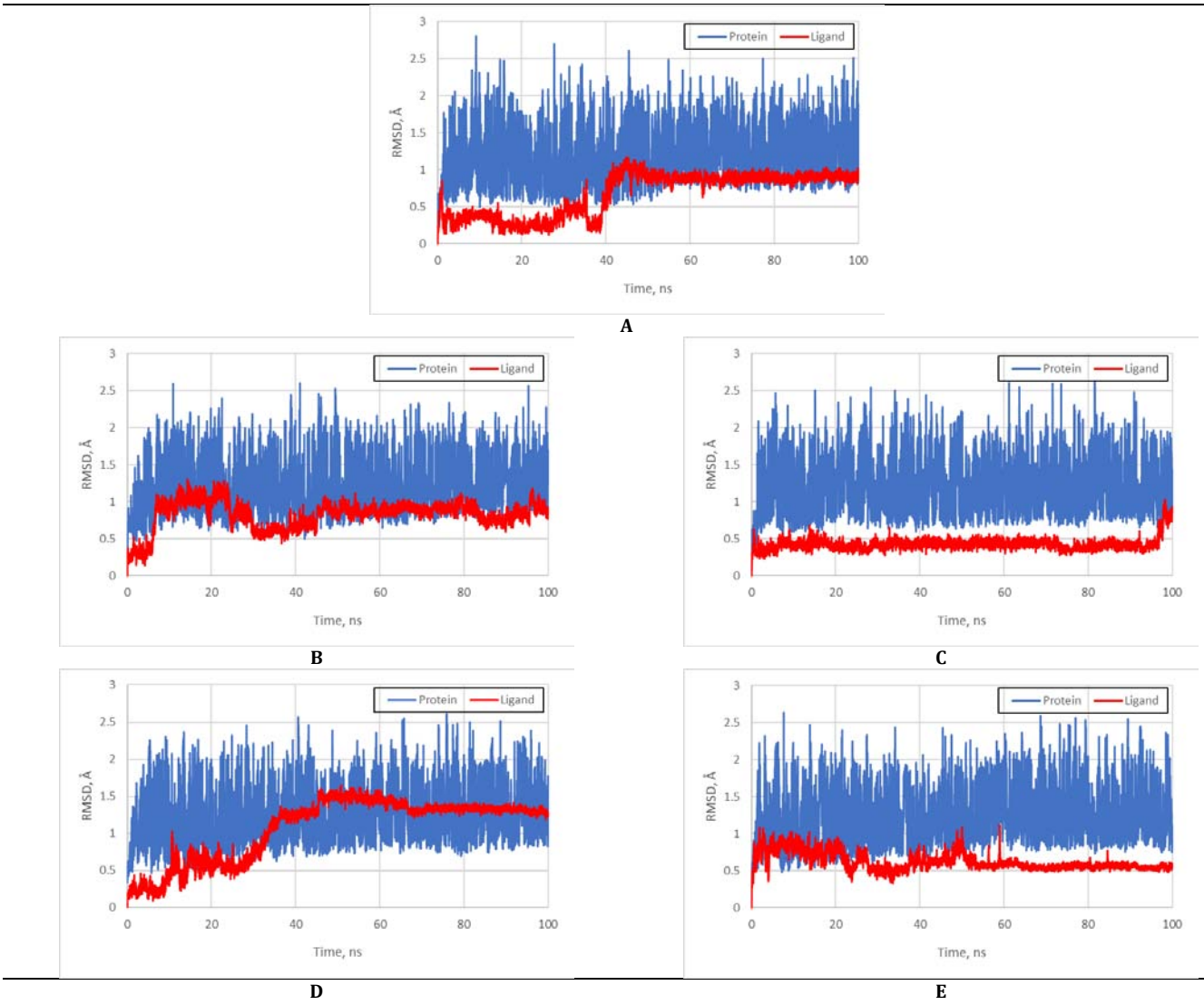

Fig. 2: The root mean squared deviation (RMSD) of the flavonoids (red) [A] native ligand (NL), [B] nicotiflorin, [C] naringenin, [D] hesperetin, and [E] kaempferol into the binding pocket of caspase-3 (1NME) backbone (blue)

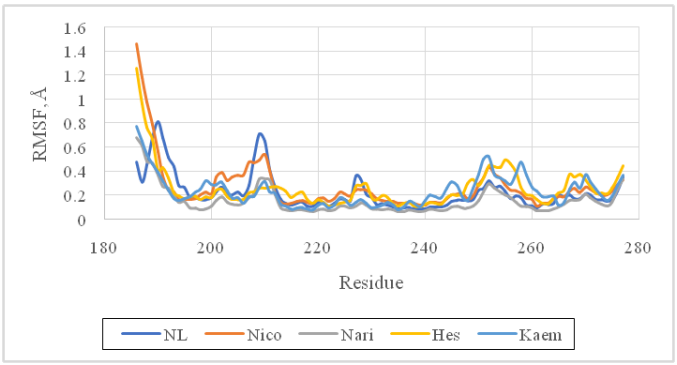

Fig. 3: The root mean squared fluctuation (RMSF) of residues at caspase-3 (1NME) backbone

Molecular mechanics/Poisson–Boltzmann surface area (MM-PBSA)

The MM-PBSA study uses both molecular mechanics simulations and continuum solvation models to fig. out how much free energy is needed for macromolecules and ligands to bond together [17]. The MM-PBSA method is implemented by the tool *g_mmpbsa* [16], which uses subroutines either written in-house or sourced from the APBS and GROMACS packages, respectively. The description of this tool follows. The goal of the *g_mmpbsa* project is to combine high-throughput molecular dynamics (MD) simulations with binding energy calculations. The application provides options to select alternative atomic radii and various nonpolar solvation models, such as models based on the solvent-accessible surface area (SASA),

solvent-accessible volume (SAV), and a model that contains both repulsive (SASA-SAV) and attractive components (described using a Weeks–Chandler–Andersen like integral method). The binding energies of the complexes of NL, nicotiflorin, naringenin, hesperetin, and kaempferol were, in order,-123.741,-96.315,-55.874,-69.773, and-33.04 kcal/mol, respectively. Electrostatic energy and polar solvation energy are the types of energy that have a substantial impact on the 1NME-NL complex. Van der Waal energy and SASA energy are the energy components that have been shown to have an effect on binding energy, in particular for the flavonoids that were tested (table 2). In general, the results of RMSD calculations showed that NL and the four flavonoids were able to stabilize the complex.

CONCLUSION

The docking scores of seven out of ten flavonoids were lower than those of the native ligand. MD simulations revealed that nicotiflorin was the most effective flavonoid from *A. indica* L. in stabilizing the complex with caspase-3.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have equally contributed to the current study.

CONFLICT OF INTERESTS

All the authors declare no conflicts of interests.

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