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Docking studies of biologically active substances from plant extracts with anticonvulsant activity

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ABSTRACT

Virtual screening methods are promising and useful to design new herbal remedies to satisfy the numerous clinical needs. Major problem for extensive use of herbs is the lack of information concerning mechanisms of their action. In the previous pharmacological studies using different models of seizures, it was found that some members of the Solanaceae, Fumariaceae, Lamiaceae, Oleaceae, Viscaceae, and Betulaceae families show a high level of anticonvulsant properties but the role of individual components of these herbs in the realization of antiepileptic activity remains unknown. The aim of the present research was molecular docking of compounds identified in the studied herbs to find the binding mechanism with the suitable targets. The study resulted that the given herbal individual substances did not show any potential anticonvulsant properties. Anticonvulsant activity of the studied herbs is probably due to the fact that in contrast to individual substances they are characterized by a complex chemical composition and synergism of biologically active compounds.

INTRODUCTION

Nowadays, biologically active compounds are rather informative and important both for the search of new promising substances and for the further development of new drugs. Many scientists describe virtual screening methods as the most direct and rational approaches for the search of new promising substances, as well as for the discovery of drugs, whose advantages are low cost and high efficiency (Lavecchia and Di Giovanni, 2013; Tripathi and Misra, 2017). Therefore, a virtual screening method is often used by scientists and pharmaceutical companies engaged in development and implementation of new medicines (Cheng *et al*., 2012; Lionta *et al*., 2014; Pitt *et al*., 2013). Molecular docking method is considered to be the most prospective due to its possibility for the study of the affinity of a particular substance in relation to a certain biological target (Klebe, 2006; Ma *et al*., 2013; Schomburg *et al*., 2014; Scior *et al*., 2012; Shoichet, 2004; Vyas *et al*., 2008). Besides, it allows reducing time and money by its similarity to high-performance biological screening (Ferreira *et al*., 2015; Ruyck *et al*., 2016; Tripathi and Misra, 2017).

The mentioned aspect made virtual screening method promising and useful to design new herbal remedies to satisfy the numerous clinical needs. For example, traditional herbs are very useful and irreplaceable in epilepsy treatment effort. The literature analysis has shown that a lot of herbs members of the Solanaceae, Fumariaceae, Lamiaceae, Oleaceae, Viscaceae, and Betulaceae families are known for their anticonvulsant activity (Blyznyuk *et al*., 2016; Chauhan *et al*., 2011; Gupta and Reddy, 2013; Mittal *et al*., 2016; Okoli *et al*., 2010; Singh and Kumar, 2010; Wannang *et al*., 2008). Apparently, this activity is connected with the content of powerful pharmacologically active compounds. Different literature sources report about the anticonvulsant activity of herbs and some herbal compounds, e.g., tropane alkaloids, different groups of flavonoids, phenolic acids, etc. (Al-Ashaal *et al*., 2013; Diniz *et al*., 2015; Zhu *et al*., 2014). Nevertheless, anticonvulsant properties of any individual compounds identified from the mentioned herbs still have not been reported.

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In the previous pharmacological studies using different models of chemo-induced convulsions, including pentylenetetrazole as the main screening model (Tsyvunin *et al*., 2016), picrotoxin, thiosemicarbazide, strychnine, camphor, maximal electroshock seizures, as well as pentylenetetrazole-induced kindling, dry extracts of herbs members of the Fumariaceae, Lamiaceae, Solanaceae, Betulaceae, Viscaceae, and Oleaceae families have shown a pronounced anticonvulsant properties (Blyznyuk *et al*., 2016; Prokopenko *et al*., 2015; Tsyvunin *et al*., 2014; 2016).

As a result, anticonvulsant activity spectra for seizures with different pathogenesis was determined and summarized in Table 1. Results have shown that the mentioned extracts decreased the duration of seizures, lethality level, and severity of seizures compared with control (Table 2), but the role of individual components of the extracts in the realization of antiepileptic activity remains unknown. However, the data about the antiepileptic potential of individual substances of the mentioned herbs can be useful in the selection of markers for subsequent standardization of the finished drugs. It should be noted that the other problem for wider clinical application of herbs is the absence of information about mechanisms of their action.

Considering the accumulated experience about the components of the chemical composition of herbs, certain information concerning mechanisms of pharmacological activity of herbs can be obtained using molecular docking. Previous studies in the area of molecular docking for all chemical structures of *Bupleurum aureum* in relation to hepatoprotective biotargets have shown that the offered method can be promising and rather informative (Glushchenko *et al*., 2015).

The aim was molecular docking of compounds identified in dry extracts of *Fumaria schleicheri* Soy-Willem. (Fumariaceae), *Ocimum basilicum* L. (Lamiaceae), *Capsicum annuum* L. (Solanaceae), *Hyoscyamus niger* L. (Solanaceae), *Corylus avellana* L. (Betulaceae), *Viscum album* L. (Viscaceae), and *Forsythia europaea* L. (Oleaceae) to determine antiepileptic potential of their individual components and thus to find the most appropriate standardization markers.

MATERIALS AND METHODS

Preparation of extracts

Bismaceration method according to the European Pharmacopoeia was used to obtain dry extracts of the mentioned herbal material. Water or water-ethanol mixture was used for extraction.

The duration of the extraction was 2 hours at a temperature of 80°C–90°C.

Then, the obtained herbal extracts were concentrated and evaporated to dryness.

European Pharmacopoeia methods were used for the determination of the content of the sum of biologically active compounds (alkaloids, flavonoids, phenolic acids, and polyphenols) in the extracts (Prokopenko *et al*., 2015; Tsyvunin *et al*., 2014; 2016).

Table 1. Spectrum of anticonvulsant activity of the dry herbal extracts.

Experimental model of seizures	*Fumaria schleicheri*	*Corylus avellana*	*Viscum album*	*Ocimum basilicum*	*Forsythia europaea*	*Hyoscyamus niger*	*Capsicum annuum*
Pentylenetetrazole	++	++	++	+	+	+	+
Picrotoxin	++	+	+	–	–	+	–
Thiosemicarbazide	+	–	+	+	+	+	+
Strychnine	+	–	–	+	–	+	+
Camphor	+	++	+	+	+	++	+
Strychnine	+	–	–	+	+	+	+
Maximal electroshock seizures	+	+	+	+	+	+	+
Pentylenetetrazole-induced kindling	++	+	–	–	+	–	+

Note: "++": a pronounced activity (a significant reduction in the lethality and/or other key parameters); "+": moderate activity (reduction in the severity of seizures without a significant reduction in lethality); "–": no significant activity.

Table 2. The efficacy criteria of the dry herbal extracts with significant anticonvulsant properties on the model of pentylenetetrazole-induced seizures in mice.

Animal group, drug or extract, plant, type of extract	Latency period, min	Number of clonic-tonic attacks for one mouse	Severity of seizures, points	Duration of convulsive period (minutes)
Control	4.4 ± 0.4	3.0 ± 0.2	5.4 ± 0.2	10.4 ± 1.5
Sodium valproate	32.7 ± 10.4	1.4 ± 0.6	3.0 ± 1.1	3.9 ± 1.9
Fumaria schleicheri (aqueous)	11.6 ± 1.5	1.7 ± 0.2	3.9 ± 0.6	4.2 ± 1.0
Corylus avellana (aqueous)	9.9 ± 0.4	2.2 ± 0.5	3.5 ± 0.2	7.0 ± 3.6
Viscum album (aqueous-ethanol)	7.1 ± 2.8	1.8 ± 0.5	4.0 ± 0.5	3.6 ± 2.2
Ocimum basilicum (aqueous)	5.7 ± 0.7	2.8 ± 0.3	4.7 ± 0.4	13.0 ± 2.0
Forsythia europaea (aqueous)	6.4 ± 3.1	2.1 ± 0.4	5.6 ± 0.4	4.5 ± 1.7
Hyoscyamus niger (aqueous)	5.0 ± 1.2	2.8 ± 0.5	5.5 ± 0.3	7.1 ± 2.1
Capsicum annuum (aqueous-ethanol)	6.1 ± 1.7	1.8 ± 0.3	6.0 ± 0.0	6.0 ± 1.9

Note: Results are significant at $p < 0.05$ compared with control group.

Molecular docking method

Scigress Explorer, 7.7 [Fujitsu, Fukuoka, Japan (License number 742F6852C191] software was used for the screening. The software was set on the computer with 3.4 Hz frequency, Intel Core 2 Duo 1 Gb RAM processor, and 160 Gb hard drive with Windows XP system.

Molecular docking stages using Scigress software were as follows:

Biological targets selection and determination of their active sites

Protein Data Bank (PDB) code proteins of GABAA receptor (PDB code 1GNU) and GABA-AT receptor (GABA aminotransferase) (PDB code 1OHW) were selected according to Usman Abdulfatai and Yvonne Thielmann publications (Abdulfatai *et al*., 2017; Thielmann *et al*., 2008). Docking into the membrane protein crystal 1BYY (sodium channel activator) has not been performed since the protein is a single spiral and has two unstructured areas, so it does not have more or less a definite site for ligand binding. Consequently, the obtained results of the docking into the 1BYY protein crystal will be unreliable (Rohl *et al*., 1999) (Fig. 1).

PDB (www.rcsb.org) was used for the search of crystallographic models of the 3D structure of proteins of $GABA_A$ receptor (PDB code 1GNU), glutamate receptor Glu-1 (PDB code 1EWK), and GABA-AT receptor (GABA aminotransferase) (PDB code 1OHW) (Fig. 2).

3D optimization of chemical structures from the herbs members of Fumariaceae, Lamiaceae, Solanaceae, Oleaceae, Viscaceae, and Betulaceae families (molecular mechanics MM3 method)

Ligands against the mentioned proteins were selected from *Fumaria schleicheri* Soy-Willem. (Fumariaceae), *Capsicum annuum* L. (Solanaceae), *Hyoscyamus niger* L. (Solanaceae), *Ocimum basilicum* L. (Lamiaceae), *Corylus avellana* L. (Betulaceae), *Viscum album* L. (Viscaceae), and *Forsythia europaea* L. (Oleaceae) dry water extracts. Selection of the ligands was carried out according to the information data concerning the most specific substances for the mentioned herbs, which are used as chemical markers for both qualitative and quantitative assessments (Table 1) (Prokopenko *et al*., 2015; Tsyvunin *et al*., 2016). It is known that the specific components must be unique ingredients of herbs, as well as they may contribute to the therapeutic effects.

Considering the obtained results, the most specific components of the above-mentioned extracts were selected for the further molecular docking study and a deeper understanding of the mechanisms of their anti-seizure activity (Table 3).

ISIS Draw 4.0 (http://accelrys.com) software tool was used to draw 2D structures of the mentioned compounds; the obtained chemical structures were saved as .mol files. Then, the structures were imported to SCIGRESS program and converted to 3D structures. After this procedure, the obtained 3D structures were optimized and saved as .csf format (Fig. 3A–D). Geometry optimization was carried out by the MM3 method with the preliminary addition of hydrogen atoms.

In Figure 3, oxygen atoms are red-colored and nitrogen atoms are blue-colored. Carbon chain is shown in ash-grey color.

The next stage was the optimization of the geometry of the mentioned structures using molecular mechanics MM3 method. Optimization of the molecules was performed by Myelin oligodendrocyte glycoprotein (MOG) function using Scigress Explorer, 7.7 [Fujitsu, Fukuoka, Japan (License number 742F6852C191)] software tool, which calculates and minimizes the energy associated with formation heat (Fig. 4). To optimize molecules to the smallest steady energy stage, MM3 molecular mechanics method was used. Conformers of individual 3D optimized ligands have been generated using Monte Carlo conformational simulation, which was chosen randomly.

3D molecular docking

All chemical components of the herbs members of Fumariaceae, Lamiaceae, Solanaceae, Oleaceae, Viscaceae, and Betulaceae families were put into the active regions of the crystallized body using the Fast Dock and Dock into active site functions.

Genetic algorithm (GA) was used within automatic docking. The GA is an evolutionary search algorithm used to solve problems of optimization and modeling by successive selection, combination, and variation of the studied parameters (Li *et al*., 2005). The use of the GA allows to study all areas available for the ligand by parameters (Population size: 500; Crossover: 0.2; Elitism: 50; Max generations: 50.000; Mutation rate: 0.0; Convergence 0.1; Max iterations: 1,000; and Rate: 0.01).

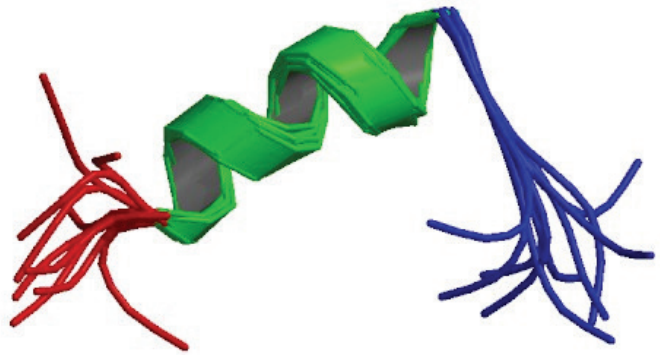


Figure 1. Crystallographic model of 1BYY protein 3D structure.

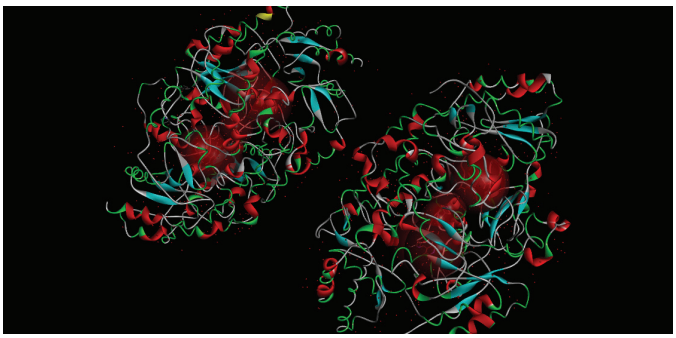
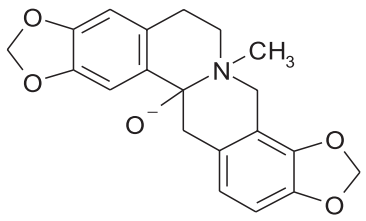
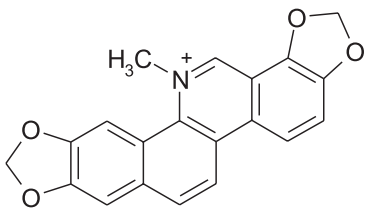


Figure 2. Protein GABA aminotransferase (code 1OHW) with active site in form of spheres.

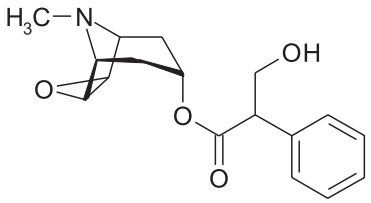
Table 3. Chemical structures of the herbs members of Fumariaceae, Lamiaceae, Solanaceae, Oleaceae, Viscaceae, and Betulaceae families.



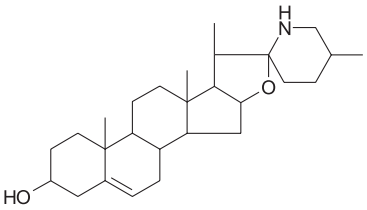
Protopine



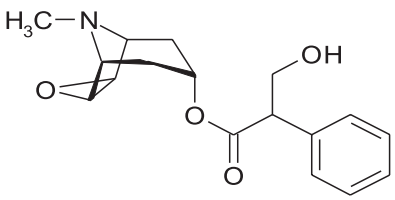
Sanguinarine



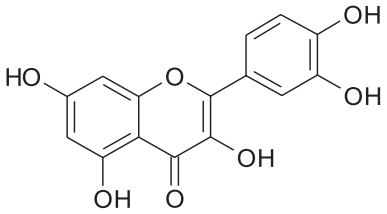
Scopolamine



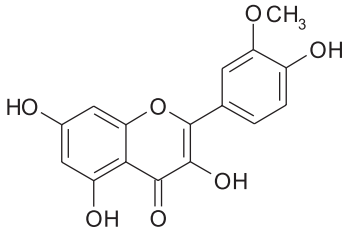
Solasodine



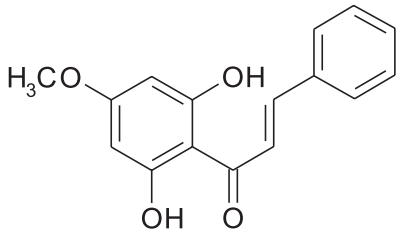
Hyoscyamine



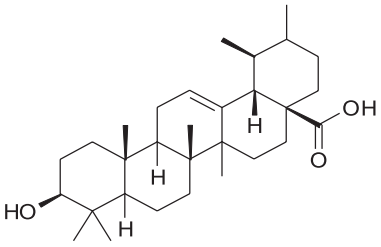
Quercetin



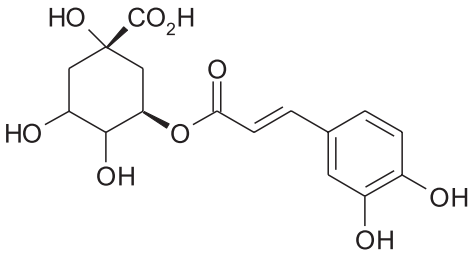
Isorhamnetin



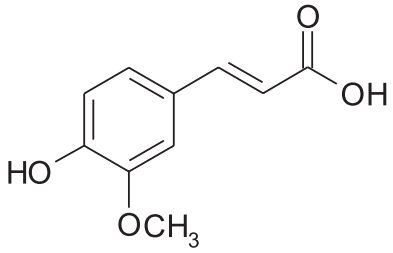
2’6’-Dihydroxy-4’-Methoxychhalcone



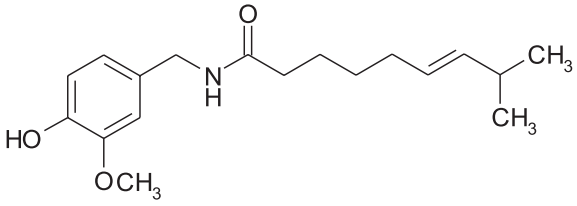
Ursolic acid



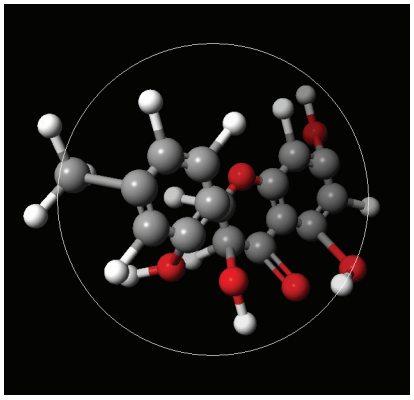
Chlorogenic acid



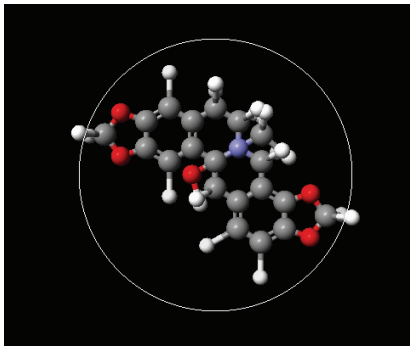
Ferulic acid



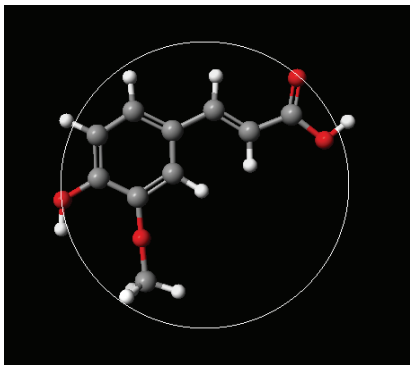
Capsaicin



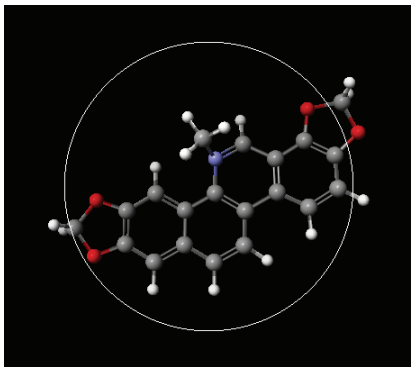
A



B



C



D

Figure 3. Quercetin (A), protopin (B), ferulic acid (C), and sanguinarine (D) chemical structure in 3D csf format.

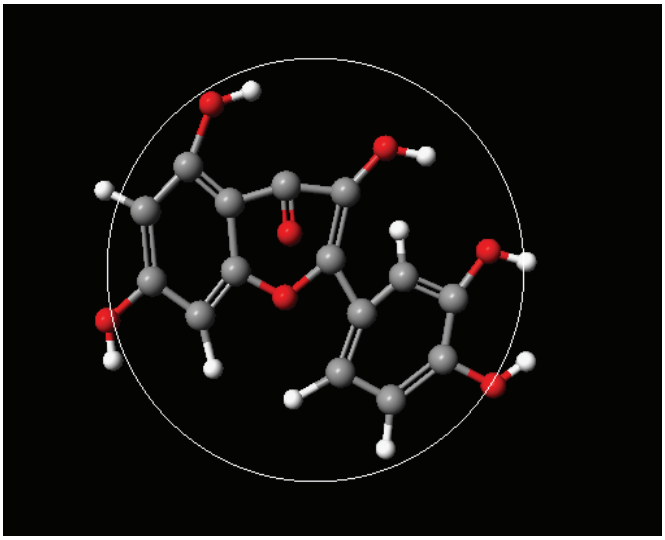


Figure 4. Quercetin structure after MM3 optimization.

RESULTS AND DISCUSSION

In result of the molecular docking, a number of the consensus scoring function values, which estimate quality and binding energy of the studies substances with the molecules of two biological targets, were obtained using the Fast Dock function (Table 4).

It should be noted that affinity of the given chemical structures to proteins was not detected.

As to the low values of the scoring function in the case of docking into the 1GNU crystal using the Dock into active site functions, it should be mentioned that the binding site is an area where the hydrophobic fragment of the ligand is immersed (Fig. 5). In the middle of this pocket, hydrophobic interactions (usually with aromatic fragments of the ligands) are formed and the hydrogen bonds with the hydrophilic environment are formed outside (Klebe, 2006). Docking studies to determine interaction possibility of the chemical components of herbs members of the Fumariaceae, Lamiaceae, Solanaceae, Oleaceae, Viscaceae, and Betulaceae families from the active site of the crystalline structures of the GABAA receptor and the GABA-aminotransferase enzyme were carried out. The obtained scoring functions and the visualization of docking into the proteins active site indicate that it is impossible to form stable complexes.

The analysis of results has shown that the given individual substances of herbal origin did not show any anticonvulsant properties. Anticonvulsant activity of the studied herbs is probably due to the fact that in contrast to individual substances they are characterized by complex chemical composition and synergism of biologically active compounds (Tsyvunin *et al*., 2016). Interactions between these compounds can lead to synergistic effects and increase pharmacological activity. However, synergistic compounds can both reinforce the bioactivities of other components and modulate the therapeutic effects of the herbs (Prokopenko *et al*., 2015; Zhou *et al*., 2016). The present study has demonstrated that the study of individual substances in some cases is not applicable to herbs, especially when their active components responsible for the certain pharmacological effect remain unknown and molecular targets are unclear.

Table 4. Scoring function values for the studied substances.

Substance	Scoring function values	
	$GABA_A$ protein (PDB code 1GNU)	GABA aminotransferase protein (PDB code 1OHW)
Protopine	0.23	0.00
Sanguinarine	0.51	24,712.19
Scopolamine	0.75	13,927.31
Hyoscyamine	0.90	2,880.99
Solasodine	0.74	50,277.67
Quercetin	0.71	220,518.47
Isorhamnetin	0.70	3,561.22
Ferulic acid	0.80	52.95
Ursolic acid	1.6	159,350.02
Chlorogenic acid	2.34	597.06
2'6'-Dihydroxy-4'-Methoxychalcone	2.99	0.00

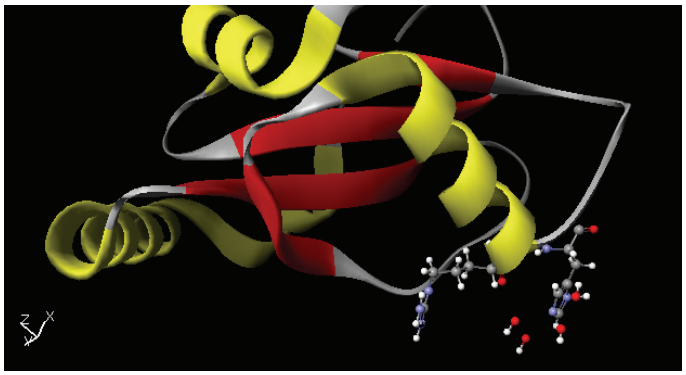


Figure 5. Protein-ligand interaction (1GNU and protopine).

CONCLUSIONS

Molecular docking studies are a promising tool for the creation of more effective and potential drugs through ligand-based drug designing approaches. Based on the obtained docking results in the present study, individual compounds from herbs members of Fumariaceae, Lamiaceae, Solanaceae, Oleaceae, Viscaceae, and Betulaceae families have not shown any potential anticonvulsant activity. However, there is a need to carry further studies to analyze whether the pharmacological activity of the mentioned herbs is due to the synergistic effect of their compounds.

CONFLICT OF INTEREST

There are no conflicts of interest.

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