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PHYTOCHEMICAL ANALYSIS AND MOLECULAR DOCKING OF BIOACTIVE CONSTITUENTS PRESENT IN *Sesbania grandiflora* LEAVES, TO CURE PEPTIC ULCERS

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ABSTRACT

Nonsteroidal anti-inflammatory drugs are major risk factor for peptic ulcers. Prostanoid E2 receptor 3 in parietal cell is a significant target for non-steroidal anti-inflammatory drugs. Misoprostol is a standard medication used for its treatment which has a lot of side effects. So people prefer herbs medicine to treat this disease. The phytochemicals present in the leaves of *Sesbania grandiflora* have the ability to heal peptic ulcers. In this study, the phytochemicals present in its leaves were identified through gas chromatography and mass spectroscopy. For this, the maceration method was used for phytochemical extraction with ethanol used as extraction solvent. The chromatogram revealed 37 bioactive compounds present in *S. grandiflora* extract. Molecular docking helps in drug discovery by providing clues about the behaviour of small molecule in binding sites of the target protein and fundamental biochemical process involved in the formation of protein-ligand complex. β-amyrin, linolenic acid and L-α-terpineol present in the leaves of *S. grandiflora* were considered for molecular docking studies. All three components showed high binding affinity to prostaglandin 3 receptor E2, with β-amyrin having the highest affinity (-57.10 kcal mol^{-1}). The study revealed the binding affinity of bioactive components of *S. grandiflora* with human prostaglandin E2 receptor 3, which may lead to the formulation of a novel drug for peptic ulcers.

Keywords: Gas chromatography-mass spectrometry, gastric acid, molecular docking, prostaglandin E receptor 3, *Sesbania grandiflora*

INTRODUCTION

Helicobacter pylori (communicable) and non-steroidal anti-inflammatory drugs (non-communicable) are the two major risk factors for peptic ulcer disease (Jose *et al.*, 2021). Annually about 4 million people are affected by peptic ulcer disease and the life time prevalence rate of this disease is estimated to be 5-10% (Abbasi-Kangevari *et al.*, 2022). Advances in the treatment and maintenance of personal hygiene have reduced the prevalence of communicable duodenal ulcers (Rowland, 2022). Although the prevalence of communicable peptic ulcers has decreased in last 15 years, the incidence of peptic ulcers has increased by 25.8%. The reason for this increase in incidence is the usage of nonsteroidal anti-inflammatory drugs (NSAIDs) (Xie *et al.*, 2022). NSAIDs are a class of medications used for the treatment of inflammations. The principal objective of NSAIDs is to inhibit the enzyme cyclo-oxygenase (COX-2) at the site of inflammation. But NSAIDs inhibit the enzyme cyclooxygenase 1 (COX 1), the main mediator for establishing mucosal integrity in addition to the inflammation inducer COX 2. Inhibition of COX1 blocks the production of prostaglandin PGE2 (McEvoy *et al.*, 2021) and increases the gastric acid secretion. PGE2 produces pharmacological action on the prostaglandin E2

receptor 3(EP3 receptor) (Engevik *et al.*, 2020). Therefore, the function of EP3 receptor is affected by NSAID. The EP3 is a G-protein coupled receptor (GPCR) located on the basal membrane of the parietal cell (Wallace and Sharkey, 2022).

The current therapeutic strategy for treating NSAID induced peptic ulcer is misoprostol. Misoprostol has cytoprotective and antisecretory properties (Krugh and Maani, 2022); in addition, misoprostol has many side effects (Drini, 2017). Considering the side effects of the drug, it is necessary to identify other molecules capable of solving NSAID-induced peptic ulcers. People depend on natural resources for managing the disease. Plant extracts are the sources of new drugs and offer promising results in treating a variety of diseases. The leaves of *Sesbania grandiflora* is one such resource that cures peptic ulcer (Vimala and Shoba, 2014). Southern Indian people traditionally eat the cooked leaves of *S. grandiflora* to overcome the peptic ulcers. Some studies have demonstrated the peptic ulcer curing property of leaves of *S. grandiflora* for NSAIDs induced peptic ulcers (Naik *et al.*, 2012; Bhoumik *et al.*, 2016). The objective of this study was to identify the bioactive phytochemical components present in the ethanolic leaf extract of *Sesbania grandiflora* using gas chromatography and mass spectroscopy. Using the *in silico* technique, some potential molecules of the extract were selected to target the EP3 receptor which is an important receptor for nonsteroidal anti-inflammatory drugs causing a peptic ulcer.

MATERIALS AND METHODS

Sample preparation

For experimental purposes, the bioactive constituents were separated from the fresh leaf tissues of *Sesbania grandiflora* by processes of pre-extraction and extraction. The pre-extraction process involved selecting, drying and grinding of the leaves. Fresh leaves, collected from a farm near Chennai (India), were thoroughly washed under tap water and shade-dried at room temperature with good ventilation for 10 days (Thamkaew *et al.*, 2021). The dried leaves were ground into fine powder using an electric blender. Maceration method was adopted for extraction process (Abubakar and Haque, 2020). Ethanol was used as extraction solvent. The powdered leaves (10 g) were soaked in 200 mL ethanol in a beaker and its mouth tightly closed by using aluminium foil for 3 days. The mixture was stirred well with a magnetic stirrer for 20 min at 500 rpm each day. On 4th day, the blend was removed by Whatman No. 1 filter paper. The filtrate was collected in crucibles and their mouths covered by an aluminium sheet with holes. Crucibles were held at ambient temperature to evaporate ethanol. The bioactive mixture was collected for gas chromatographic and mass spectroscopic studies.

Gas chromatography and mass spectrometric studies

In order to identify the bioactive constituents in ethanolic extract, GC-MS analysis was conducted using gas chromatograph (Perkin Elmer GC Clarus 580) from the Department of Chemistry, SRM University, Kattankulathur, Chennai (India). Inert gas was used as carrier gas and the sample injected into the injection port in split-less mode. The injector temperature was initially set to 55°C. The injector was kept at this temperature for 2 min after GC started its analysis. It was then raised to 245°C at 5°C min^{-1} and stays in place for 10 min. It was equipped with a flame ionization detector and a thermal conductivity detector.

Molecular docking

Molecular docking allows elucidation of binding affinity between protein and ligands by computational methods. For molecular docking studies of bioactive components, identified in the leaves of *S. grandiflora,* the 3-dimensional structural data of target protein, prostaglandin E2 receptor 3 bound with a ligand available in the Protein Data Bank (PDB ID: 6M9T) with a resolution of 2.50 A° were utilized. A ligand within its own crystalline structure and the ligands identified from the

plants were docked to the binding site of EP3 receptor (Audet *et al*., 2019; Morimoto *et al.*, 2019). Using docking program, Glide (from Schrodinger with Maestro methodology) available in the Centre for Advanced Studies in Crystallography & Biophysics, University of Madras, Chennai, the molecular docking was conducted.

Ligand preparation

The 3D structure of identified bioactive components and self-docking ligand were extracted from PubChem, an open chemistry database. The ligand structures, available in PDB file format, were converted to SDF (structure data files). Ligands were prepared using the LigPrep module and the ligand energy was minimized using OPLS 2005 force field. The 3D molecular structures were generated with reduced energy (Schrodinger LLC, 2019).

Protein preparation

The 3D crystal structure of EP3 receptor (Audet *et al.*, 2019) was obtained from the protein databank (PDB ID: 6M9T). Using a menu, Prep Wizard the protein was prepared. The protein was pre-processed by adding missing hydrogen atoms and removing co-crystallised water molecules. Within the Schrodinger software suite, the energy of pre-processed protein was minimized and optimised using the OPLS-2005 force field.

Induced fit docking

To know the binding characteristic of ligands with EP3 receptor, docking was carried out through induced fit docking. Schrodinger glide (grid-based ligand docking with energetics) with Maestro methodology was used for docking. The optimized energy protein was uploaded into Maestro environment. Using Glide´s receptor generation wizard, a 3D grid of 20 x 20 x 20A$^{\circ}$ was generated to cover the binding sites. Glide docks ligands up to 300 atoms and 50 rotatable bonds at a time. The self-docking ligand and cross-docking ligands were loaded onto the binding site. Docking was carried out in extra precision (XP) mode. Twenty binding poses were proposed for each ligand. The docked complexes were analysed using 2D maestro view for ligand interactions and the docked complexes are stored as PDB files.

Docking validation

The protein structure already possesses a ligand in its binding site. In present study, both self-docking or re-docking process and cross docking process were performed. A ligand was self-docked within its own binding site. Bioactive components identified from plants that were different from ligand complexes with protein structure were cross-docked in binding site. After docking, based on docking score and glide energy, RMSD value was calculated for each ligand using PyMol (TM) 2.3.3 molecular graphics system. This was achieved by retrieving the protein structure of EP3 receptor (PDB ID 6M9T) and the protein structure of each docked complex stored as PDB files were fetched. The protein structure of protein data bank and docked complex were opened in PyMol and using the command "Align" both protein structures were aligned and RMSD values noted. RMSD values provide information about the deviation between re-docked ligand and cross-docked from the co-crystal of known conformation. DruLiTo, a screening tool was used to analyse the drug-likeness properties of selected molecules.

RESULTS AND DISCUSSION

Ligand selection

Sesbania grandiflora leaf extract contained 37 bio-active components as revealed by GC-MS studies. The retention time, probability percentage and area percentage of these 37 components are given in Table 1, and the corresponding chromatogram in Fig. 1. Based on biological activity to achieve the

beneficial effect of curing peptic ulcers from the components present in extract, the saturated fatty acids, n-hexadecanoic acid (RT-49.126), octadecanoic acid (RT-55.062), steric acid (RT-55.478), unsaturated fatty acid linolenic acid (RT-54.638), L-α-terpinol (RT-18.371), β-amyrin (RT-85.192), phytol (RT-53.073), and vitamin E (RT-78.789) are of great importance. However, from health perspective, saturated fatty acids do not have anti-ulcerogenesis properties but increased LDL cholesterol levels. Consequently, they were not considered for molecular docking. Antioxidants are capable of curing and preventing NSAIDs-induced peptic ulcers (Arulselvan *et al.*, 2016). L-α-terpineol, linolenic acid, β-amyrin, vitamin E and phytol are important due to their anti-ulcerogenic antioxidant nature. But poor oral bioavailability nature of both vitamin E and phytol (synthetic vitamin E) (Zaffarin *et al.*, 2020) has rendered them of lesser significance. Hence, L- α-terpineol (Khaleel *et al.*, 2018), linolenic acid (Anderson *et al.*, 2014; Djuricic and Calder, 2021), and β-amyrin (Asnaasharia *et al.*, 2018; Cardoso *et al*., 2020) were considered for molecular docking studies. The drug-likeness properties of L- α-terpinol, linolenic acid and β-amyrin are verified using Lipinski's rule of five.

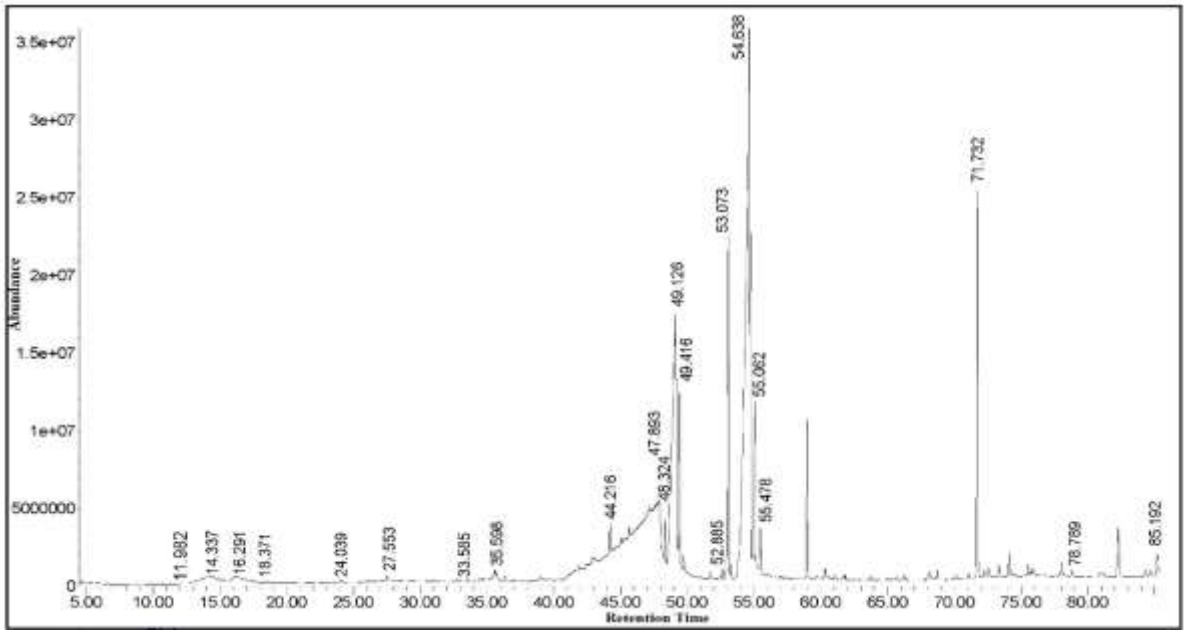

Fig. 1: Chromatogram of the ethanolic leaf extract of *Sesbania grandiflora*

Drug-likeness properties

The drug-likeness properties are tested based upon Lipinski's rule of five. The bioactive components from the ethanolic leaf extract of *S. grandiflora* was tested for drug-likeness properties along with misoprostol. According to the Lipinski's rule of five, the molecular weight of a molecule should be ≤ 500, Log P ≤ 5, HBD ≤ 5, HBA ≤ 10. The results are given in Table 2. The Lipinski rule suggests that bioavailability problem occurs when the molecule contravenes more than one rule (Benet *et al.*, 2016). Implementation of Lipinski's rules on the three bioactive components namely, L-α-terpineol, Linolenic acid and β-amyrin indicates that all the three components are effective via oral availability and satisfy drug-like properties. Therefore, they can be developed as drugs.

Molecular docking studies

The crystalline structure of the EP3 receptor bound to the misoprostol ligand already deposited into the protein databank with PDB ID 6M9T is selected as the target protein. Glide, the ligand docking program can accommodate up to 300 atoms and 50 rotating bonds at a time. The self- docking of misoprostol within its own binding site was done. Meanwhile, the ligands L-α-terpineol, linolenic acid and β-amyrin are cross-docked in the binding site of EP3 receptor. The glide energies of l-α-terpineol, linolenic acid and β-amyrin were -33.68, -48.29 and -57.10 kcal mol^{-1}, respectively. For the associated

Table 1: Chromatogram for leaves of *Sesbania grandiflora*

S. No.	Retention time (min)	Compound name	Molecular weight	Formula	Prob%	Area%
1.	11.982	2,4-Heptadienal,2,4-dimethyl-	138	$C_9H_{14}O$	8.8	0.04
2.	14.337	Glycerine	92	$C_3H_8O_3$	90.9	1.53
3.	16.291	1-Butanol,2-methyl-, acetate	130	$C_7H_{14}O_2$	24.9	0.88
4.	18.371	L-α-Terpinol	154	$C_{10}H_{18}O$	22.2	0.03
5.	18.705	Octadecane,6-methyl-	268	$C_{19}H_{40}$	5.29	0.40
6.	24.039	p-Vinyl guaiacol	150	$C_9H_{10}O_2$	51.9	0.08
7.	27.553	Tetradecane	198	$C_{14}H_{30}$	16.7	0.11
8.	33.585	Phosphoric acid, diethyl octyl ester	266	$C_{12}H_{27}O_4P$	12.8	0.04
9.	35.598	Pentadecane,7-methyl-	226	$C_{16}H_{34}$	5.1	0.07
10.	36.304	3-Hydroxy-β-damascone	208	$C_{13}H_{20}O_2$	84.9	0.12
11.	44.216	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	32.0	0.36
12.	47.893	3-O-Methyl-d-glucose	194	$C_7H_{14}O_6$	34.5	18.51
13.	48.324	Dibutyl phthalate	278	$C_{16}H_{22}O_4$	21.6	0.65
14.	49.126	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	69.6	14.90
15.	49.416	Hexadecanoic acid, ethyl ester	284	$C_{16}H_{36}O_2$	78.7	2.68
16.	49.713	Butyric acid,2-(2,4-di-tert-pentylphenoxy)-	320	$C_{20}H_{32}O_3$	25.6	0.35
17.	51.719	Heptadecanoic acid	270	$C_{17}H_{34}O_2$	51.1	0.12
18.	52.619	Linolenic acid, methyl ester	292	$C_{19}H_{32}O_2$	36.9	0.15
19.	52.885	Phytol	296	$C_{20}H_{40}O$	67.5	0.12
20.	53.073	Phytol	296	$C_{20}H_{40}O$	76.5	5.98
21.	53.241	Butyric acid,4-amino-3-(4-methoxyphenyl)-	209	$C_{11}H_{15}NO_3$	16.3	0.23
22.	54.638	Linolenic acid	278	$C_{18}H_{30}O_2$	50.0	35.89
23.	55.062	Octadecanoic acid	284	$C_{18}H_{36}O_2$	86.0	3.85
24.	55.478	Stearic acid, ethyl ester	312	$C_{20}H_{40}O_2$	20.9	0.74
25.	58.984	Octinoxate	290	$C_{18}H_{26}O_3$	81.2	2.36
26.	60.321	Arachic acid	312	$C_{20}H_{40}O_2$	58.4	0.19
27.	61.799	Isopropyl 9,12,15-octadecatrienoate	320	$C_{21}H_{36}O_2$	22.6	0.08
28.	63.731	Eicosane	284	$C_{20}H_{42}$	10.4	0.06
29.	71.732	Squalene	410	$C_{30}H_{50}$	82.7	6.66
30.	73.321	Eicosane	282	$C_{20}H_{42}$	7.0	0.23
31.	78.016	Octacosyl trifluoroacetate	506	$C_{30}H_{57}F_3O_2$	4.3	0.32
32.	78.789	Vitamin E	430	$C_{29}H_{50}O_2$	32.9	0.14
33.	80.988	Phytol, acetate	338	$C_{22}H_{42}O_2$	42.6	0.29
34.	82.266	Stigmasterol	412	$C_{28}H_{48}O$	81.5	1.31
35.	84.301	γ-Sitosterol	414	$C_{29}H_{50}O$	79.3	0.21
36.	84.687	Cyclohexane,1,1'-(2-tridecyl-1,3-propanediyl) bis-	390	$C_{28}H_{54}$	18.6	0.19
37.	85.192	β-amyrin	426	$C_{30}H_{50}O$	76.5	0.49

ligand misoprostol, the glide energy was -55.84 kcal mol^{-1}. The negative value indicated that all the ligands can easily interact with residues of EP3 receptor. Based on the crystal structure of EP3 receptor, Arg333, Tyr114, Val110, Ser336, Thr206, Trp207, Gln339 and Gly141 were the binding site residues interacting in the EP3 receptor region (Audet *et al.*, 2019) . In addition, in terms of their bond with the residues in the active site, the bioactive components of leaf extract of *S. grandiflora* showed that L-α-terpineol, β-amyrin have hydrogen bond in the form of Arg333 and Tyr114 and hydrophobically interact with Thr206. Linolenic acid had a hydrogen bond with the guanidine group of Arg333 and a hydroxyl group of Tyr 114 and Thr206 like misoprostol. Since the residues Arg333, Thr206 and Tyr114 are the conserved residues of EP3 receptor (Morimoto *et al.*, 2019; Krishna Deepak *et al.*, 2021). These ligand- residue interactions indicated the presence of ligands within the binding site and an increase in cellular signalling responses that may result in conformational change.

Table 2: Drug-likeness properties of L-α-terpineol, linolenic acid and β-amyrin and misoprostol

Molecules	Lipinski's rule of five			
	MW	Log P	HBD	HBA
L-α-terpineol	154.25	1.8	1	1
Linolenic acid	278.4	5.9	1	2
β-amyrin	426.7	9.2	1	1
Misoprostol (co-crystal)	382.5	3.7	2	5

MW: Molecular weight; Log P: Log of octanol-water partition coefficient; HBD: Hydrogen bond donor; HBA: Hydrogen bond acceptor

The glide energy and a similar interaction between the ligands and the residues of the target receptor molecular docking results were assessed on the basis of docking score. The ability of a ligand to interact with the receptor was determined from the glide energy values. Glide energy with a more negative value indicated that the reaction is spontaneous. Spontaneous reactions make it easier for the ligand to interact with target. Docking score and glide energy of the compounds are compared in Table 3. The interaction images of α-terpineol, linolenic acid, β-amyrin, and the co-crystal misoprostol are presented in Fig 2.

Table 3: Docking score and Glide energies of ligands with EP3 receptor

Compounds	Docking score (kcal mol^{-1})	Glide energy (kcal mol^{-1})	Hydrogen Bonding
L- α-terpineol	-6.10	-33.68	Tyr114 (O-H… O)
			Arg333 (N-H… O)
Linolenic acid	-8.73	-48.29	Tyr114 (O-H… O)
			Arg333 (N-H… O)
			Thr206 (O-H… O)
β-amyrin	-11.79	-57.10	Tyr114 (O-H… O)
			Arg333 (N-H… O)
Misoprostol (co-crystal)	-11.41	-55.84	Arg333 (N-H… O)
			Thr206 (O-H… O)
			Tyr114 (O… O-H)
			Thr107 (O… O-H)

Validation of docking was done by determining the root mean square deviation between the co-crystallized misoprostol docked to EP3 receptor binding pocket and the original molecule. The RMSD value of co-crystalline with molecule was 0.150A°. Similarly, RMSD value for α-terpineol, linolenic acid and β-amyrin with co-crystalline were obtained as 0.146, 0.152 and 0.150A°. Docking validations are acceptable only if the RMSD value is < 2A° (Ramírez and Caballero, 2018). The RMSD values showed that the position of atoms in the docked ligand were not too different from the position of atoms in the co-crystallised pose of ligand and were within the acceptable range. This indicated the validity of the molecular docking process.

The results of molecular docking studies suggest that L-α-terpineol, linolenic acid and β-amyrin are capable of targeting the EP3 receptor which is an important pathway of peptic ulcer induced by NSAIDs. Therefore, these leaf molecules need to be studied in detail so that their application in pharmacology is explored for the development of drugs from traditional herbs.

Conclusion: *Sesbania grandiflora* leaves have medicinal value and are traditionally used for treating peptic ulcers. The GCMS study of its ethanolic extract showed a total of 37 constituents present in the mixture. Based on anti-ulcerogenic properties, the drug-likeness properties and bioavailability of components, L-α-terpineol, linolenic acid and β-amyrin, were considered for molecular docking. These three components had high binding affinity with β-amyrin depicting the highest binding affinity. This study suggests that L-α-terpineol, linolenic acid and β-amyrin have the ability to target

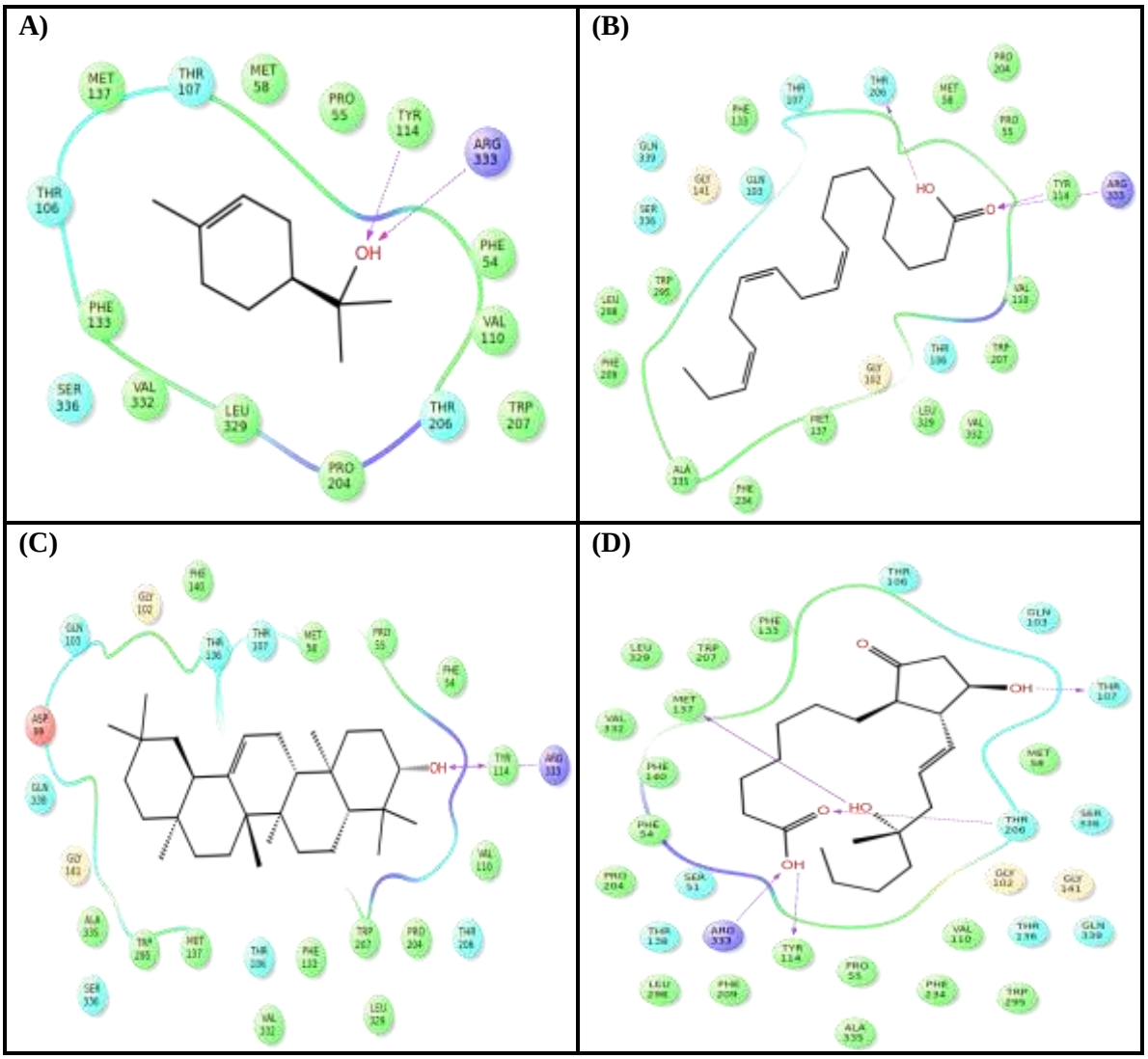

Fig. 2: Interaction image of bioactive constituents: A) L-α-terpineol, B) Linolenic acid, and C) β-amyrin and (D) Misoprostol (co-crystal)

the EP3 receptor which is an important target for NSAIDs- induced peptic ulcers. The glide energy gives idea about the strength of protein-ligand interaction which is an important hint in the field of pharmacology for the development of drugs from traditional herbs for NSAID inductive peptic ulcers.

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