



Research Article

In-silico Evaluation of Wound Healing Potential of *Euphorbia tithymaloides*

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Abstract

Background and Objective: The wound healing after the injury is a complex process by which the skin and the tissue under the skin fix themselves. *Euphorbia Tithymaloides* has various therapeutic uses, such as antiprotozoa, anti-inflammatory, antiplasmodial, antimicrobial and so on. The goal of this study was to predict wound healing medicinal products with a focus on the *in silico* effect of chemical constituents on wound healing process. **Materials and Methods:** *In silico* molecular docking of reducing sugar present in *Euphorbia tithymaloides* was performed using two receptors of leukotriene such as structural basis of the proinflammatory signaling complex mediated by TSLP PDB ID: 4NN5, Crystal structure of the type-I interleukin-1 receptor complexes with interleukin-1 beta PDB ID: 1ITB and second two receptor of NF- κ B which are Cryptic glucocorticoid receptor-binding sites pervade genomic NF- κ B response elements PDB ID: 5E69 and Structure–function analyses of the bacterial zinc metalloprotease effectors protein GtgA+ uncover key residues required for deactivating NF-B PDB ID: 6GGR. **Results:** As a result, D-Ribose has a dock score of -51.51 and -75.56 Kcal mol⁻¹ against TSLP and interleukin-1 receptor type-I in the first test. And second result D-Ribose dock score of -76.42 and -64.69 Kcal mol⁻¹ against two NF- γ B receptors that predicted interaction with selected protein structure via strong hydrogen bonding, with good docking score ensuring significant binding affinity with selected protein structure. **Conclusion:** The current research offers a significant approach to the reducing sugar derivative's structural requirements that would make it possible for the wound healing mechanism to interact with the receptors concerned.

Key words: *In-silico* molecular docking, inflammatory diseases, phytochemical, inflammatory mediators, signaling complex, pharmacological activities, vasoconstriction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chronic inflammatory disease is a medical situation characterized through chronic inflammation which defines a delayed and determined pro-inflammatory condition is noticeable effectively via new connective tissue formation. Inflammation is explained as the local reaction of living mammalian tissues to injury appropriate mediator. It is body defense reactions categorized into eliminate or limit the spread of injurious agent, followed by elimination of the necrosis cells and tissues². Traditional nonsteroidal anti-inflammatory drugs (NSAIDs) represent the most widely prescribed, efficacious and cost-effective pharmacological treatment of rheumatologic and inflammatory disorders. In addition, this class of drugs is widely used to treat mild to moderate pain. Most currently used nonsteroidal anti-inflammatory drugs (NSAIDs) have limitations for therapeutic use since they cause gastrointestinal and renal side effects that are inseparable from their pharmacological activities. Wound healing is simply repairing process of injured cells. The dermis and epidermis form a protective barrier against the external environment during undamaged skin. Once the mediator busted the barrier there is development of damages and regulate the sequence of biochemical procedures be situated to cure / repair the damage. External wound healing is a complex procedure with four important stages that occur constantly. The first stage is Hemostasis is occurs right away after the injury to stop the bleeding by platelet aggregation and platelet-mediated vasoconstriction⁵.

In the second stage (Inflammation), the injured tissue cells and capillaries activate the release and function of several cytokines which induce phagocytosis to remove debris and initiate wound repair. Inflammation is local response of living tissues to injury due to foreign matter. It is a body defense reaction to prevent the spread of injurious agent. Inflammation is the growth of fluid, leukocytes and inflammatory mediators such as cytokines. In the third stage (Proliferative stage), the wound surface is enclosed with a new epithelium (Epithelialization) and granulation tissues with new vascularization are formed to repair the injured tissue. In last stage (Maturation) the collagen is remolded and wound fully closes. The cells which used in wound healing are no longer. They are removed by apoptosis. *Euphorbia tithymaloides* commonly known as Lady's Slipper Flower and scientifically known as *Pedilanthus tithymaloides* *Euphorbia tithymaloides* a medicinal plant applied to wounds for rapid healing. *Euphorbia Tithymaloid* (ET) is a perennial succulent spurge is native to tropical and sub-tropical North America and Central America⁷. These shrubs are 6-8 feet long and

1.8-2.4 inch wide. ET grows in fertilized sandy soil rich in metal concentration like boron, copper, iron, manganese, molybdenum and zinc. Their leaves are alternate, sessile, glabrous and acuminate in shape. It is a carcinogenic plant thus has the ability to grow in toxic soil very easily and die.

Sometimes, ET is also used to remediate soil and can be used as border of garden. In this study Ribose from the leaves of ET were characterized in terms of their structural properties. Ribose is the sugar of the pentose class which occurs naturally as a chemical constituent in *Euphorbia tithymaloides* which have ability of wound healing after binding to the specific receptor. Ribose-aurine (Rib-T) put off the growth of inflammatory mediators. (Rib-T) shows anti-inflammatory action by inhibiting nuclear translocation of nuclear factor-kappa B (NF- κ B).

MATERIALS AND METHODS

Molecular docking study

Hardware and Software used: All the computational studies were executed by the PC windows 7 ultimate with Intel Core i5 microprocessor, 4 GB memory and 32 Bit operating system. Biological databases were used such as PubChem, PDB (Protein Data Bank) and Vlife QSAR BioPredict tool software used for molecular dockings and this software study were performed in Rajarambapu College of Pharmacy, Kasegaon in month of Jun, 2019. Thus the structure of Sucrose, D-Maltose, D-Mannose, D-Galactose, D-Glucose, D-Ribose, D-Xylose, Sorbitol, D-Fructose and D-Arabinose was confirmed by spectrometric techniques⁸. Further they are evaluated for *in silico* molecular docking to investigate its anti-inflammatory potential i.e., wound healing activity.

In silico molecular docking of *Euphorbia tithymaloides*

constituent against leukotriene and NF κ B proteins files selection: Molecular docking studies were performed by using

Vlife MDS 4.6.1 version. Where the protein were used for this

study are of two different types which are responsible to wound healing and the receptors searched such as structural basis of the pro-inflammatory signaling complex mediated by TSLP PDB ID: 4NN5 second is Crystal structure of the type-I interleukin-1 receptor complexed with interleukin-1beta PDB ID: 1ITB and NFkB two receptor which are found as Cryptic glucocorticoid receptor-binding sites pervade genomic NF- κ B response elements PDB ID: 5E69 and Structure-function analyses of the bacterial zinc metalloprotease effectors protein GtgA uncover key residues required for deactivating NF-B PDB ID: 6GGR were obtained from the Protein Data Bank (www.rcsb.com). The receptor extracted by X-ray diffraction method with having resolution less than 2.5Å°. These structures contain four complexes were determined with molecular replacement method using AMoRe. The coordinates of native C-lobe (Protein Data Bank code: 1NKX) were used as the search model. The rotation and translation search functions were calculated with data between the resolution ranges in between of 12.0-4.0 Å^o¹³.

Ligand preparation: Ligand of Sucrose, D-Maltose, D-Mannose, D-Galactose, D-Glucose, D-Ribose, D-Xylose, Sorbitol, D-Fructose and D-Arabinose isolated from *Euphorbia tithymaloide* plant constituents were drawn¹⁴. 2D structure of ligand was prepared and converted to 3D by Chem Draw Ultra 8.0 software. Then farther these 3D structures were constructed to energy minimization process by using batch optimization for a set of molecules. MMFF is used for molecular mechanics. For that process the parameter set as 10,000 as Maximum number of cycles, 0.01 as Convergence Criteria, 1.0 as Constant (medium's dielectric constant which is 1 for in vacuo). Select MMFF from Force Field drop down list. For MMFF Force Field, the MMFF atomic charges get automatically selected. Set the value 20 and 10 respectively for electrostatic and vdW interaction. Conformers of the compounds were generated by systematic search method. The docking results were ranked according to the decreasing docking energies of the different possible conformers for each of the ligands¹⁵.

Docking methodology

Parameters for scoring functions: For the molecular docking study the Parameters set as mode of dock process running is exhaustive and input Rotation Angle step size of 30° by which the ligand will be rotated for different poses. Input Number of Placements as 30 and Ligand wise Results as 5 to obtain 5 top poses for each ligand. The docking scoring function, Escore, is defined by the following energy terms:

$$\text{Escore} = \text{Einter} + \text{Eintra}$$

where, Einter is the ligand-protein interaction energy, Eintra is the internal energy of the ligand¹⁵.

Re-ranking score function: Re-ranking score used was computationally more expensive than the scoring function used during the docking simulation but it is generally better than the docking score function at determining the best pose among several poses originating from the same ligand. Whereas the re-rank score in Vlife calculate approximately strength of the interaction, it was not calibrated in chemical units and it didn't get complex contributions (such as entropy) into report. The free energy of binding (FEB) was defined as the sum of final intermolecular energies (Van Der Waals+Hydrogen bond+Desolvation energy), final total internal energy, torsional free energy and unbound system's energy¹⁶.

Interpretation of results: Lastly, the analysis starts by measuring the minimum dock score of ligand and interaction between the atoms present in ligand (i.e., atoms present in different plant phytochemical constituents) and amino acid isolated by docking technique from receptors. In this system correlation of bond interaction present on atoms and receptors, four forms of interaction were mainly observed such as Van Dar Waals interaction, charged interaction, pi-stacking interaction and hydrogen bonding (as in the study the maximum number of hydrogen bonding observed was considered as the ligand and protein interaction is stronger) so this binding score and interaction will succeed Predict wound healing activity of the respective plants¹⁷.

RESULTS

The molecular activity values, based on the predictive ability of the molecules with different receptors coupled with the wound healing cycle. The ligand 2D and 3D structure of Sucrose, D-Maltose, D-Mannose, D-Galactose, D-Glucose, D-Ribose, D-Xylose, Sorbitol, D-Fructose and D-Arabinose was completed by spectrometric validation methods shown in Fig. 1 and 2. Thus the drug-receptor binding interpretation is determined on the basis of the docking results which are shown in (Table 1 and 2). Table 1 summarizes the findings of the docking study of 10 bioactive components of *Euphorbia tithymaloide* with the receptor as structural basis of the pro-inflammatory signaling complex regulated by TSLP PDB ID: 4NN5 and second is Type-I interleukin-1 receptor complex

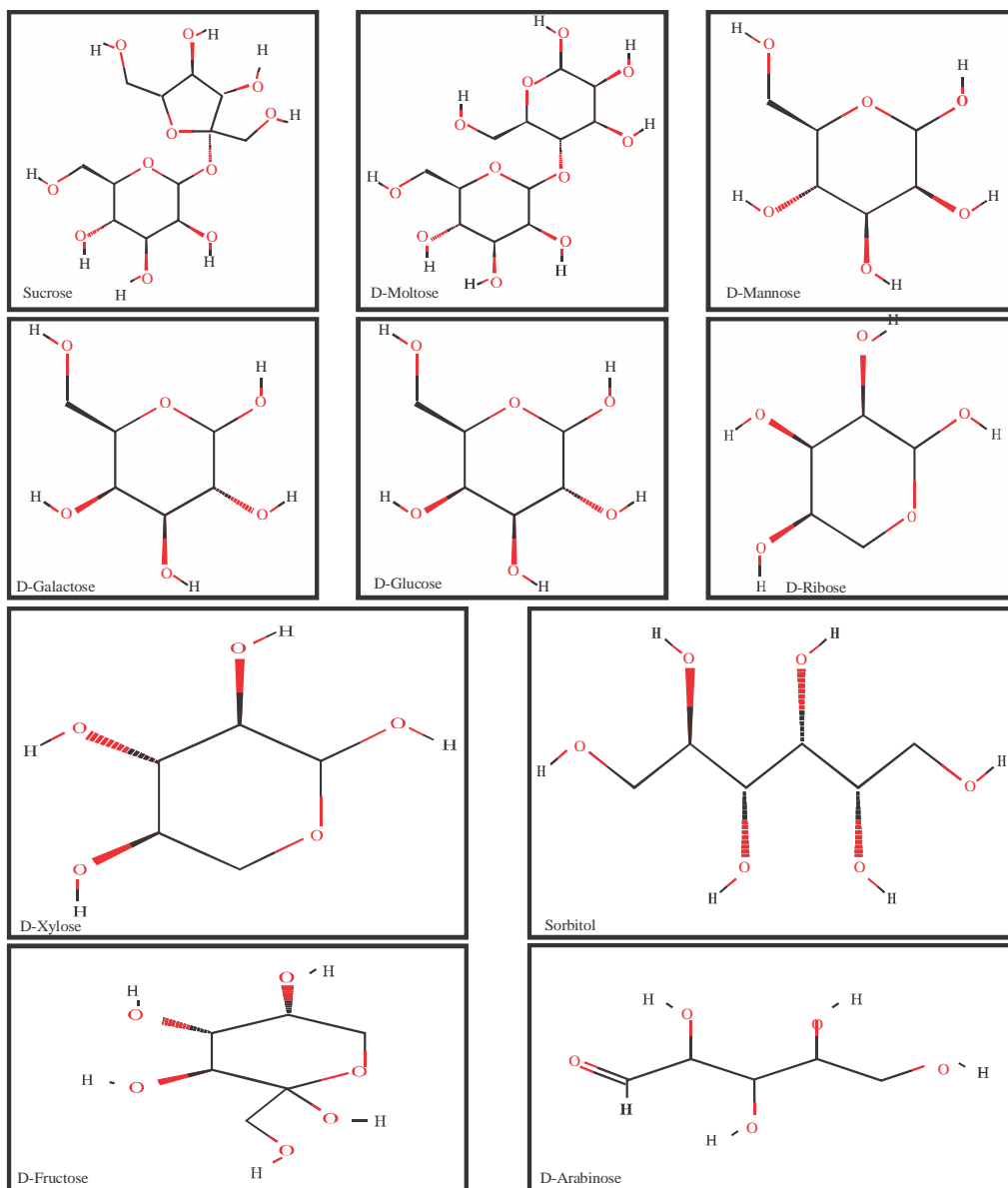


Fig. 1: Structures of bioactive compound present in *Euphorbia tithymaloide*

Table 1: Molecular docking results of bioactive constituents present in *Euphorbia tithymaloide* with leukotriene receptors

Molecule	Final energy	Final GRMS value	Dock score	
			4NN5	1ITB
Sucrose	117.0440	0.8750	-27.89	-51.85
D-Maltose	90.4100	0.6642	-43.23	-56.38
D-Mannose	47.7400	0.8775	-26.44	-38.01
D-Galactose	45.0880	0.7957	-25.61	-39.57
D-Glucose	44.7461	0.9157	-28.52	-38.95
D-Ribose	-37.9008	0.9161	-51.51	-75.56
D-Xylose	31.5016	0.7505	-19.24	-32.32
Sorbitol	45.1167	0.8750	-26.89	-40.88
D-Fructose	37.8695	0.5960	-29.67	-43.13
D-Arabinose	30.0214	0.8955	-26.62	-38.62

GRMS*: Conjugate gradient optimization until a root mean squared deviation of the gradient (GRMS) of 0.01 kcal mol⁻¹.Å

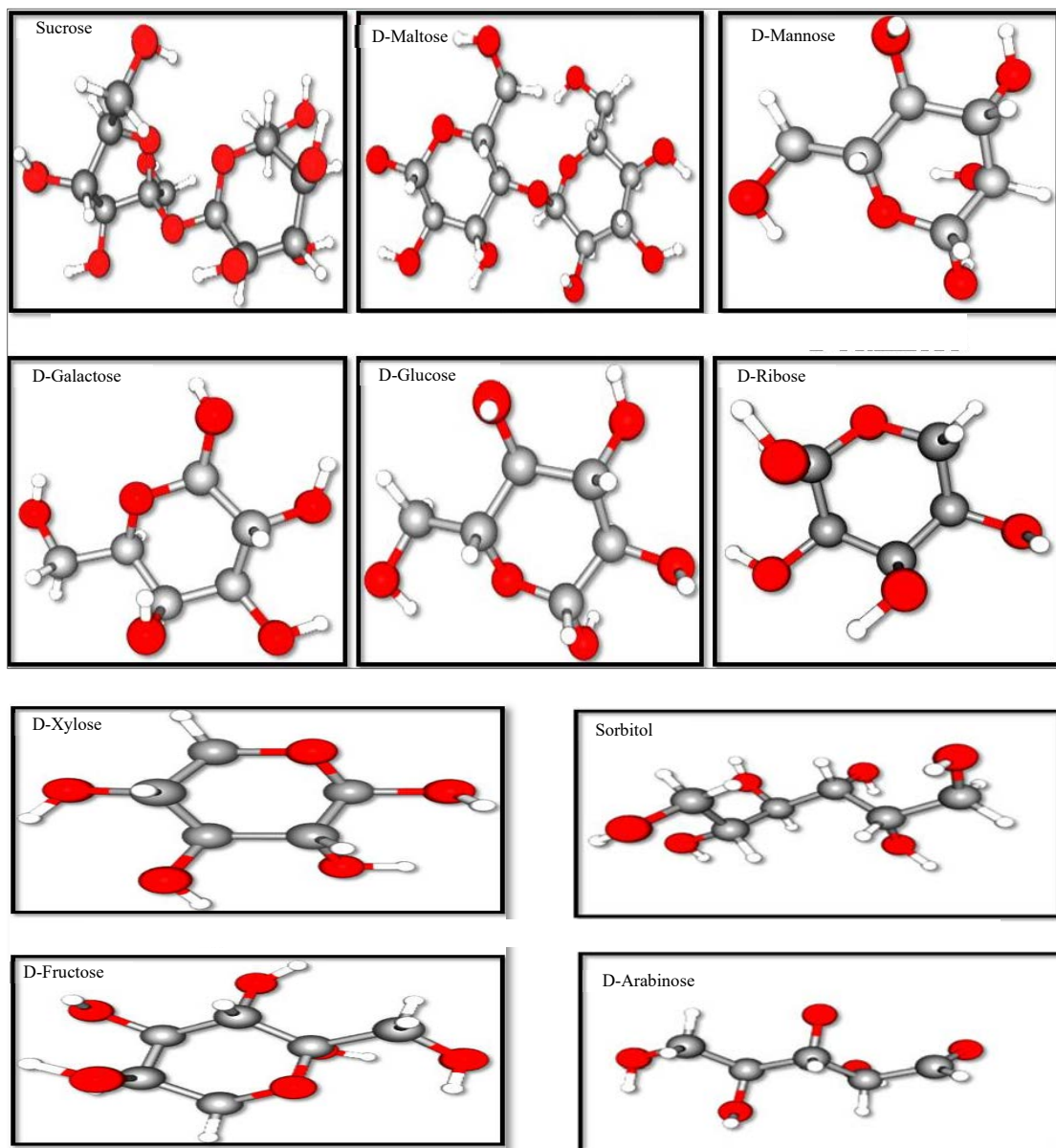


Fig. 2: 3D Structure of ligands molecules present in *Euphorbia tithymaloide*

Table 2: Molecular docking results of bioactive constituents present in *Euphorbia tithymaloide* with NF- κ B receptors

Molecule	Final energy	Final GRMS value	Dock score	
			4NN5	1ITB
Sucrose	117.0440	0.8750	-49.97	-49.35
D-Maltose	90.4100	0.6642	-51.68	-55.48
D-Mannose	47.7400	0.8775	-29.64	-26.07
D-Galactose	45.0880	0.7957	-31.36	-25.86
D-Glucose	44.7461	0.9157	-31.43	-28.02
D-Ribose	-37.9008	0.9161	-76.42	-64.69
D-Xylose	31.5016	0.7505	-25.42	-20.47
Sorbitol	45.1167	0.8750	-30.39	-30.39
D-Fructose	37.8695	0.5960	-33.27	-32.74
D-Arabinose	30.0214	0.8955	-30.14	-24.24

GRMS*: Conjugate gradient optimization until a root mean squared deviation of the gradient (GRMS) of 0.01 kcal mol⁻¹.Å

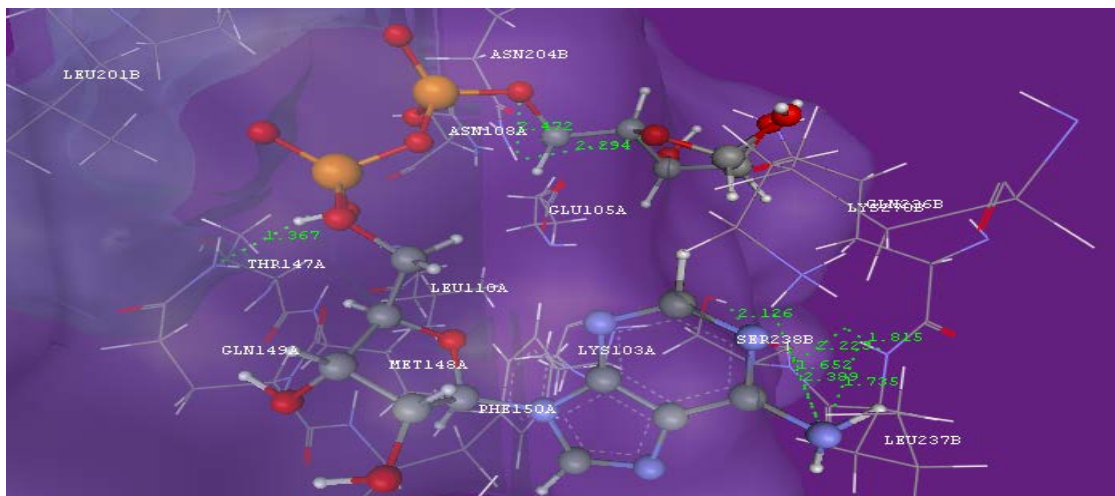


Fig. 3: 3D Dock poses of D-Ribose with leukotriene receptor having PDB ID: 1ITB

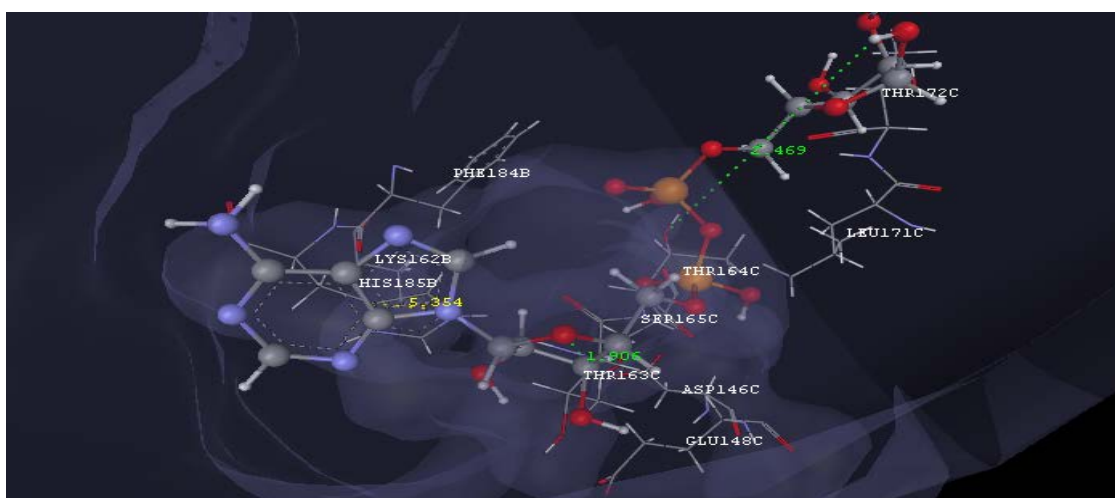


Fig. 4: 3D Dock poses of D-Ribose with leukotriene receptor having PDB ID: 4NN5

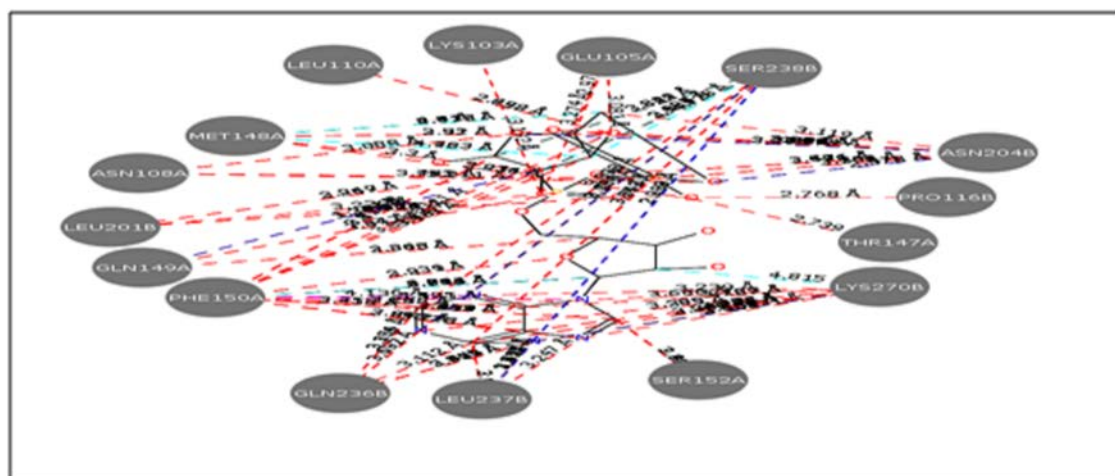


Fig. 5: 2D Interactions pose of D-Ribose with leukotriene receptor having PDB ID: 1ITB

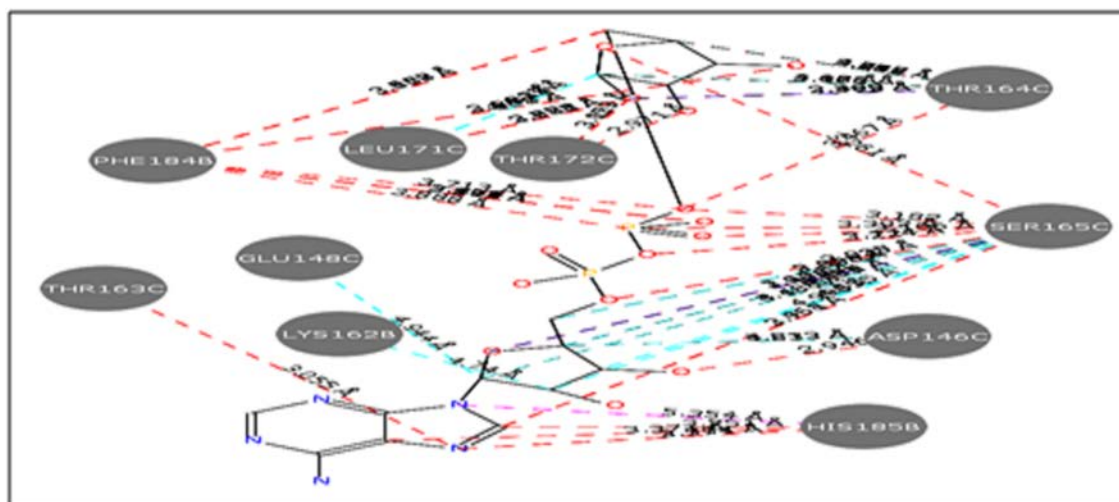


Fig. 6: 2D Interactions pose of D-Ribose with leukotriene receptor having PDB ID: 4NN5

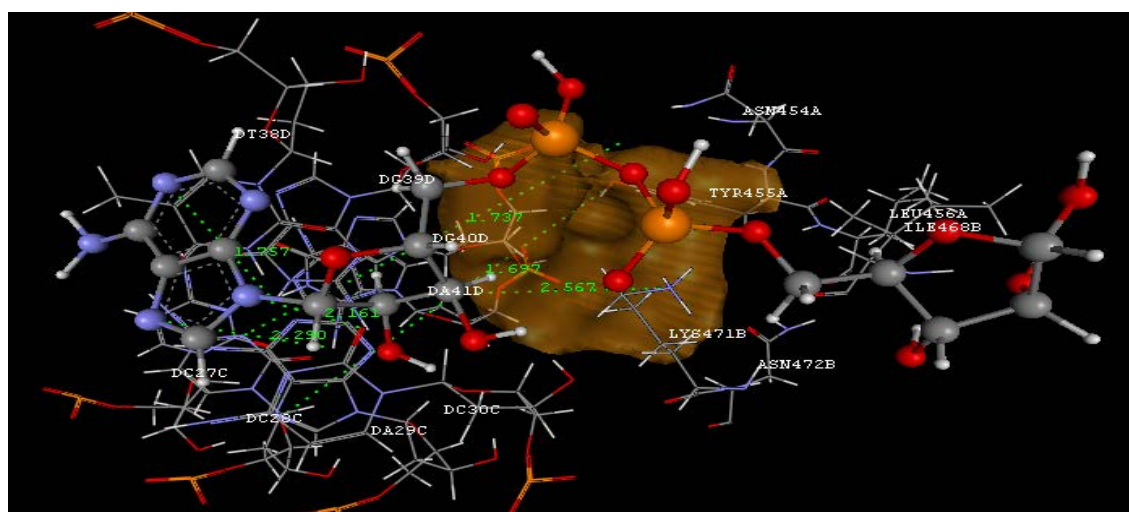


Fig. 7: 3D Dock poses of D-Ribose with NF-κB receptor having PDB ID: 5E69

crystal structure with interleukin-1beta PDB ID: 1ITB. 10 Bioactive components of the *Euphorbia thymaloide* have receptor affinity 1ITB and 4NN5 none of these have reported Pi stacking interactions. The docked ligands scores of D-Ribose shows the best- docking score -51.51 and -75.56 kcal mol⁻¹ with their respective receptors such as 4NN5 and 1ITB, accordingly. The D-Ribose has nine H-bonding interactions with the 1ITB receptor with forming amino acid bond such as GLN149A, ASN204B, ASN204B, LEU237B, LEU237B, SER238B, SER238B and LYS270B, They shows two H-bonding interactions with 4NN5 by bonding the amino acid such as THR164C, SER165C. D-Ribose's best-scoring pose did not exhibit hydrophobic interactions. The 3D visualization of D-Ribose's best-docked pose with 1ITB and 4NN5 is also

illustrated in Fig. 3 and 4, respectively, by the amino acid interaction shown in Fig. 5 and 6.

Table 2 shows the second docking analysis of 10 bioactive components of *Euphorbia thymaloide* with the receptor such as Cryptic glucocorticoid receptor binding sites pervade PDB ID: 5E69 and Structure–function analysis of the bacterial zinc metalloprotease effectors protein GtgA reveal key residues needed to deactivate NF-B PDB ID: 6GGR. *Euphorbia thymaloide*'s bioactive components have an attraction for receptor 5E69 and 6GGR no receptor displays Pi stacking and hydrophobic interactions. The docked ligands scores range from -20 to -76 Kcal mol⁻¹, with the best-scored ligand again is D-Ribose with a docking score of -76.42 and -64.69 Kcal mol⁻¹ with their respective receptors including

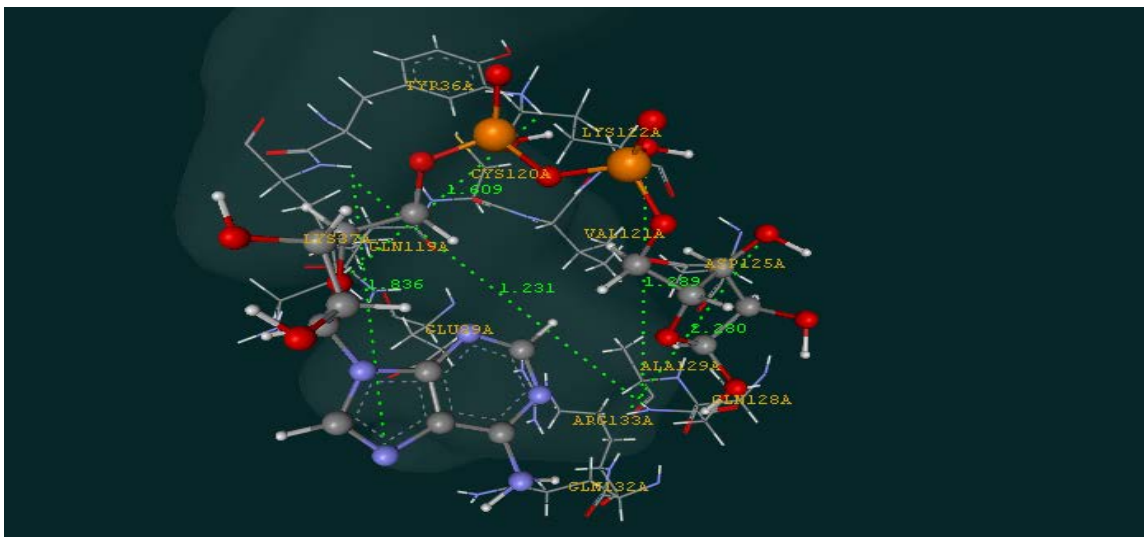


Fig. 8: 3D Dock poses of D-Ribose with NF- κ B receptor having PDB ID: 6GGR

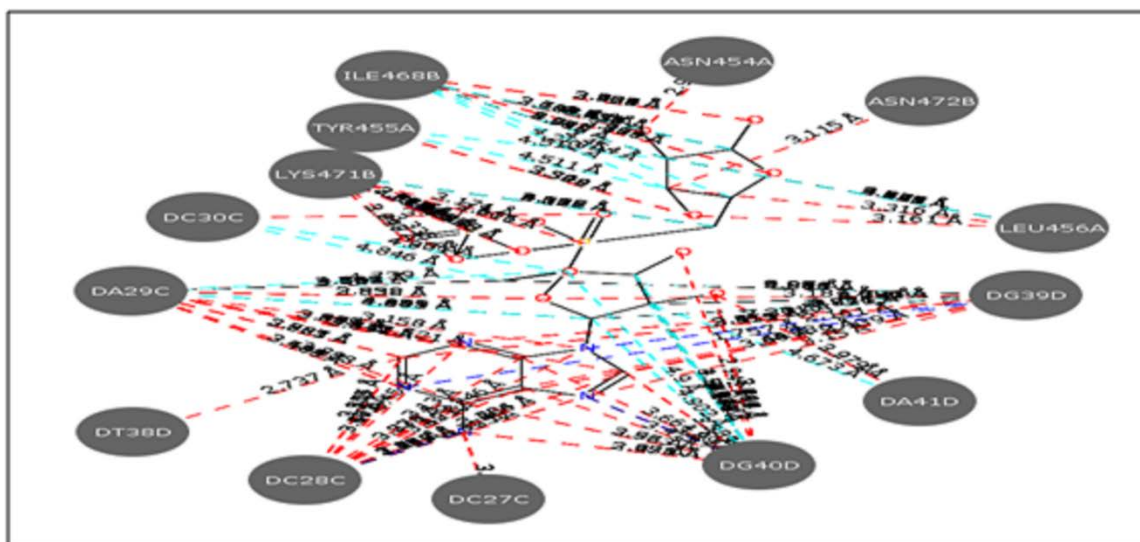


Fig. 9: 2D Interactions pose of D-Ribose with NF- κ B receptor having PDB ID: 5E69

5E69 and 6GGR. The D- Ribose has six H-interactions with the 5E69 receptor with amino acid forming bond such as DC28C, DC28C, DG39D, DG39D, DG40D and LYS471B with forming five H-interactions with 6GGR by bonding the amino acid such as LYS37A, LYS122A, GLN128A, GLN128A and GLN128A. D-Ribose's best-scoring position has demonstrated no hydrophobic interactions. The 3D visualization of D-Ribose's best-docked pose with PDB ID: 5E69 and PDB ID: 6GGR is also illustrated in Fig.7 and 8, respectively, by the amino acid interaction shown in Fig. 9 and 10.

DISCUSSION

All 10 reducing sugar derivatives have been tested for their ability to promote wound healing by analyzing their binding interactions with the various proteins that play specific roles in the different stages of wound healing in the human body; unlike the docked ligand, the compounds were docked into each of the various proteins and their binding interactions were evaluated. For this reason, the 10 bioactive constituents identified in the *Euphorbia tithymaloide* plant were compared with those of the native ligand for the purpose behind

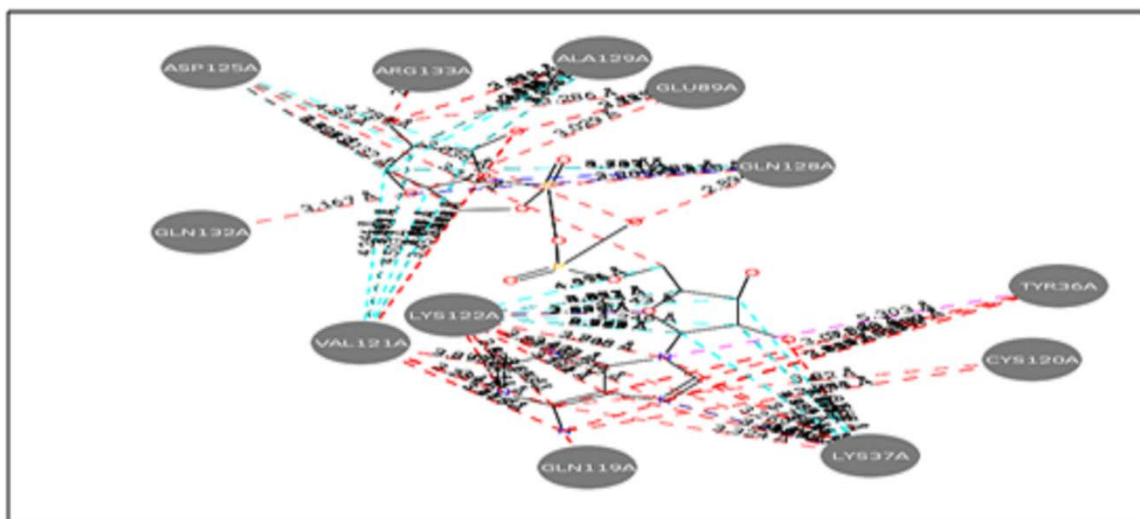


Fig.10: 2D Interactions pose of D-Ribose with NF- κ B receptor having PDB ID: 6GGR

validation and interactions. The behaviors of the molecules studied were predicted based on their ability to dock into the receptor's active site. Theoretically all the ligand molecules showed encouraging binding energy and docking score. Among them, the docking against receptor 1ITB and 4NN5 with interacted amino acid with minimum docking score and is considered a strong affinity to bind with interleukin-1 receptor complex inhibitor. The outcomes of the docking analysis of bioactive constituents with the receptor such as Type-1 interleukin-1 receptor and TSLP having PDB ID: 1ITB and PDB ID: 4NN5 respectively. The ligand was deeply docked by strong interaction in binding pocket area the dock score ranging from -19 to -75 kcal mol⁻¹. The active compound D-Ribose binds to the receptor at a mol dock score of -51.51 and -75.56 kcal mol⁻¹ with their respective receptors named as PDB.ID: 1ITB and 4NN5 (Table 1). It binds to the PDB.ID:1ITB receptor showing nine H-bond interactions GLN149A, ASN204B, ASN204B, LEU237B, SER238B, SER238B and LYS270B is the amino acid in cavity 2 (Fig. 3, Fig. 5) and has only two hydrogen bond interactions with the receptor PDB.ID: 4NN5 the amino acid is THR164C and SER165C (Fig. 4, Fig. 6). Analysis of the receptor/ligand complex models developed after successful D-Ribose docking was based on parameters such as hydrogen bond interactions compared to the previously mentioned literature¹³. Similarly D-maltose had a mole. Dock score of -43.23 and -56.38 Kcal mol⁻¹ and the van-dar wall bond interactions were low as compared to that of D-Ribose. Compounds like D-Mannose and D-Arabinose display very less Mol. Dock score and thus have a significantly lower affinity to the target. For D-maltose the van-dar wall bond

value was -56.38 Kcal mol⁻¹. There are 9 amino acid residues involved in association with D-maltose from various domains of the inhibitor complex interleukin-1 receptor. A compound D-Ribose shows binding energy -51.51 and -75.56 Kcal mol⁻¹ in these docking studies and it is high as compared to entagenic acid showed minimum binding (-8.79 kJ mol⁻¹) and docking (-8.95 kJ mol⁻¹) in the previous research⁷. Both work findings are therefore expressed for action on wound healing. Thus both research work results are expressed for wound healing activity. Table 2 tabulated second docking analysis of the ligands. The different interaction energies, such as van der Waals energy, intermolecular hydrogen bonding were calculated for each minimized complex. Dock scores for 10 ligands of reducing sugar *Euphorbia tithymaloide* plant range from -20 to -76 Kcal mol⁻¹ to Mysterious glucocorticoid receptor PDB ID: 5E69 and bacterial zinc metalloprotease effectors GtgA PDB ID: 6GGR protein¹⁷. The D-Ribose dock scores docked against Mysterious glucocorticoid shown as -76.42 Kcal mol⁻¹ and dock score against the GtgA protein effectors of receptor bacterial zinc metalloprotease are found to be as -64.69 Kcal mol⁻¹¹⁵. These compounds from the plant *Euphorbia tithymaloide* indicated that the compounds had the highest binding potential when docking against the respective protein structure. Residues DC28C, DC28C, DG39D, DG39D, DG40D and LYS471B present in the protein, PDB ID: 6GGR play an important role in receptor binding interaction with ligands in which all are H-bond interactions were revealed from the conformational analysis of several docked complexes⁴. Five H-Bond interactions with 6GGR are also observed by bonding the amino acids LYS37A, LYS122A,

GLN128A, GLN128A and GLN128A¹. 3D view of best-docked D-Ribose site with PDB ID: 5E69 and PDB ID: 6GGR Fig. 7 and 8 the 2D view is shown in Fig. 9 and 10. D-Ribose is stated to have a wide variety of biological applications for wound healing agents and anti-inflammatory activity. Developing D-Ribose as a possible inhibitor will aid in successful wound treatment with limited or lower toxic effects. Thus the present research has been an attempt to computationally classify compounds that can bind to the essential anti-inflammatory activity targets. The research state that docking scores and their analysis of compound interactions indicate that most of the compounds are capable of binding to multiple targets involved in inflammatory hyperalgesia and regulation of it. With this prediction we go to the experimental evaluation of compounds such as D-Ribose, D-Mannose and D-Arabinose, etc., by practical approaches will lead us to clinically successful molecules for treating various chronic pain disorders.

CONCLUSION

In the present *in silico* research work, the pharmacology involved in between the wound healing process and it was observed that the reported bioactive such reducing sugar constituent's isolated form *Euphorbia tithymaloide* showed effectual interactions with the amino acid residues present in the active binding site of the proteins, leukotriene and NF- κ B. This was shown for the conductively inhibitory effects of these constituents towards the respective receptors. The results thus provide an insight for the structure of the ribose which is in *Euphorbia tithymaloide* constituent's that would assist their activity as wound healing agents. Subsequently, the in house extracted phytochemical constituents can be properly formulated into a pharmaceutical dosage form that can be efficiently used for the treatment of different types of wounds.

SIGNIFICANCE STATEMENT

This study discover the significant approach to the structural requirements of the reducing sugar derivative that would make possible their interaction with the receptors concerned to the wound healing process.

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