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IN SILICO ANTIMICROBIAL ACTIVITY OF ACTIVE PHYTOCOMPOUNDS FROM THE LEAF EXTRACT OF *VITEX NEGUNDO* LINN. AGAINST GLUCOSAMINE 6 PHASPHATE SYNTHASE

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ABSTRACT

Phytocompounds play an important role in the field of drug development. In recent years, a great significant attention has been drawn to phytocompounds due to their fewer side effects or without side effects in therapeutic. The main aim of the study is to identify the phytocompounds with antimicrobial properties from the ethanol leaf extract of *Vitex negundo* and also to find the inhibitors of Glucosamine 6 phosphate synthase enzyme through molecular docking. GCMS was performed for the ethanol leaf extract of *Vitex negundo* Linn. Various phytochemical compounds were identified through GCMS. These compounds were *in silico* screened against Glucosamine 6 phosphate synthase as a target protein for the antimicrobial activity through docking studies by using Schrödinger software. The docking results enhanced the activity of new phytocompounds as promising antimicrobial agents. The binding energy is evaluated through docking studies of the ligand with the target protein. The interactions of the phytocompound with the amino acid residues of the Glucosamine 6 phosphate synthase enzyme showed high affinity with in the active site binding pocket. These Phytochemical compounds have a high docking score and glide energy. Results of our study suggested that these phytochemical compounds can be considered as strong inhibitors for Glucosamine 6 phosphate synthase and possess potential medicinal values with anti-microbial properties.

KEYWORDS: Phytocompounds, anti-microbial, Docking studies, inhibitors.

INTRODUCTION

Fungal and bacterial infections constitute large proportion of the infectious diseases resulting in 13 million deaths each year worldwide.[1] In the recent decades microbes are getting more antibiotic-resistant due to the wide spread use of medicines and it is a challenge to develop more powerful antibiotics with less side effects.[2] Glucosamine-6-phosphate synthase (GlcN-6-P synthase) is present in all kinds of cells. The enzyme glucosamine-6-phosphate synthase (GlcN-6-P synthase) is a target for antibacterials and antifungal.[3] Plants are good source of biologically active compounds. In recent years, plant products play a dominant role in the discovery of phytodrugs for treating human diseases. People show their interest towards natural compounds from plant materials for treatment of various ailments. There is a worldwide interest in searching for safe, less effective and new phytochemical drugs. *Vitex negundo* Linn (Verbenaceae) is commonly known as five-leaved chaste tree or Monk's pepper. It grows in waste lands and is also planted as a hedge plant. All parts of *Vitex negundo* are used as medicine in the indigenous system of medicine. All parts of the plant are used for treatment of a wide spectrum of health disorders in traditional and folk medicine.[4] *Vitex negundo* have certain bioactive molecules and many of the drugs are based on these bio active molecules. *Vitex negundo* is one of the medicinal plants used in traditional medicine and reported to have many pharmacological activities such as anti-inflammatory activity[5] anti-bacterial activity[6,7] and anti-fungal activity.[8] The leaves are the most potent part of the plant for medicinal purposes. The main aim of this present study is to the identification and of new structural classes of antibacterial phytocompound agents.

METHODS AND MATERIALS

Collection Plant material

The fresh mature plant leaves of *Vitex negundo* Linn. was collected from Maruthamalai hill area in Coimbatore district. The collected plant materials were identified authentically by Dr.G.V.Murthy. Scientist 'F' from Botanical Survey of India, Southern Circle Coimbatore, Tamil Nadu, India.

PREPARATION OF THE EXTRACT

The mature fresh leaves of *Vitex negundo* were washed in fresh water thoroughly 2-3 times and once finally with sterile water to remove adhering dust. The leaves were dried on sterile blotter under shade and then powdered in a mixture grinder. The air dried powder of leaf was packed in small thimble. Leaf extracts were successively prepared with different solvents

such as petroleum ether, chloroform, ethyl acetate, and ethanol in the order of polarity by using soxhlet apparatus. Each time before extracting with the next solvent, the material was dried at 40^{o}C. The different solvent extracts were concentrated by rotary evaporator and then air dried. The extracts were freeze dried and stored in desiccators until further analysis.

GC-MS

Preparation of extract

One µL of the ethanol leaf extract of *Vitex negundo* was employed for GC/MS analysis.

Instruments and chromatographic conditions

GC-MS analysis was carried out on a DB 5 - MS capillary standard non - polar column and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30×0.25 mm i.d. × 1 EM df, composed of 100% dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 mL/min.The oven temperature was programmed from 80^{o}C (isothermal for 2 min), with an increase of 8^{o}C/min, to 250^{o}C/min. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Identification of phytochemical compound

The mass spectrums of the unknown components were compared with the spectrum of the known components stored in the Wiley9 library. The name, molecular weight and structure of the components of the test materials were ascertained.

Molecular Docking Studies

Molecular docking studies have been carried out by using *GLIDE* (*Grid-based Ligand Docking with Energetics*) software v5.5 developed by Schrödinger running on Red Hat Enterprise Linux 5 (RHEL5) workstation. *Maestro* v8.5 Graphical User Interface (GUI) workspace was used for all the steps involved in ligand preparation, protein preparation and Docking.

Ligand Preparation

The structures of the phytocompounds obtained from the result of GCMS, Wiley9 library and the structures were drawn by using *Chem Sketch* (ACDLABS 12.0) and converted into 3D structure with help of a 3D optimization tool of ACD Labs Software and the structures were

saved in the MOL format. The structure glucosamine 6 phosphate was obtained from Drug Bank. The ligands used in this study were prepared using *LigPrep*[9] module of v2.3 of Schrödinger Suite 2009. Using the Impact module of glide the ligands were minimized with 1000 cycles of steepest gradient and 5000 cycles of conjugate gradient.

Retrieval of Target Sequence

The *FASTA* format of *E.Coli* GlcN-6-P synthase with Accession number 2VF5 was retrieved from the NCBI protein database. The protein sequence contains 608 amino acid residues.

Glucosamine 6 phosphate synthase enzyme protein structure analysis

The target X- ray crystal structure GlcN-6-P synthase GlcN-6-P synthase enzyme protein having the resolution of 2.9Å was retrieved from Protein Data Bank (PDB) with ID 2VF5. The 2VF5 is a complex of GlcN-6-P synthase with an inhibitor Glucosamine 6 phosphate.[10]

Preparation of 2VF5protein

The X - ray crystal structure of GlcN-6-P synthase (PDB id: 2VF5) retrieved from PDB is a monomeric structure. It consists of 608 amino acids along with an inhibitor Glucosamine 6 phosphate molecule. The raw PDB protein structure could not be used for molecular docking studies. PDB structure consists only of heavy atoms, waters, cofactors, metal ions and can be of multimeric. These structures do not have the information about bond orders, topologies or formal atomic charges. The terminal amide groups may be misaligned because the X-ray structure analysis cannot distinguish between O and NH_2 Ionization and tautomeric states are also unassigned. So, the raw PDB structure retrieved from PDB should be prepared in a suitable manner for docking. *Protein Preparation* Wizard of *GLIDE* software was used to process and prepare the protein. This Wizard allows one to properly prepare a protein for docking. This also follows the Optimized Potential for Liquid Simulations. All Atoms (OPLS-AA) force fields for energy minimization. The X – ray crystal structure of 2VF5 protein was prepared by removing all the water molecules present in the structure. Since the raw data do not contain any hydrogen in it, the implicit hydrogen atoms were added to the atoms to satisfy their appropriate valencies. Then the structure was optimized by assigning the bond orders, bond angles and topology. The formal atomic charges were fixed for the amino acid residues. The optimized structure was then energy minimized to remove the steric clashes between the atoms. The energy minimization was done till it reached a Root Mean Square Deviation (RMSD) cut-off of 0.18 A^o and the resulting structure was used for docking.

Docking protocol

Docking of the prepared ligands with the prepared proteins was performed using Glide *Docking* protocol of *GLIDE* v5.5 from Schrödinger Suite 2009.[11] Docking combines GLIDE and Prime refinement modules. Prime accurately predicts the ligand binding modes and concomitant structural changes in the receptor. Systematic and Simulation methods are adopted by glide for searching poses and ligand flexibility. Incremental construction for searching is employed by the systematic method, with Glide score (G-score) being the empirical scoring function. In docking, both the ligand and the receptor are enables to dock the ligand at the receptor's binding site to generate multiple poses of the receptor-ligand complex, each including unique structural conformations of the receptor to fit the ligand pose and ranks them by Glide score (G-score) to find the best structure of the docked complex. G-score takes into account a number of parameters like hydrogen bonds (H-bond), hydrophobic contacts (Lipo), van der-Waals (vdW), columbic (Coul), polar interactions in the binding site (Site), metalbinding term (Metal) and penalty for buried polar group (BuryP) and freezing rotatable bonds (RotB). The calculation of GScore in Kcal/mol is: G-Score = H bond + Lipo+ Metal + Site + 0.130 Coul + 0.065vdW – Bury P – RotB. Where Hbond = Hydrogen bonds, Lipo = hydrophobic interactions, Metal – metal binding term, Site = Polar interactions in the binding site, vdW = Vander Waals forces, Coul = coulombic forces, Bury P = penalty for buried polar group, RotB = freezinf rotatable bonds. The prepared protein was docked with the minimized ligands. The active sites in the protein 2VF5 were selected to be docked with the ligand. The prepared structure of 2VF5was used for induced fit docking simulations. Docking was performed and best conformations were selected based on Docking score, Glide energy, and Glide emodel scores. In docking , both the ligand and the receptor are enables to dock the ligand at the receptor's binding site to generate multiple poses of the receptor-ligand complex, each including unique structural conformations of the receptor to fit the ligand pose and ranks them by docking score (D-score) to find the best structure of the docked complex. Initially a receptor grid, where the ligand has to be docked with the receptor was set by picking the centroid of the co-crystallized inhibitor (2VF5) present at the active site. It creates a grid box and the size of the grid box was limited to 20Å. The generation of different conformations of the docked complexes (poses) was set to a maximum of 20. The poses generated were ranked based on D-score. The pose that made the maximum hydrogen bond (H-bond) interactions from phytocompounds docked complexes were considered for further analysis and the results are compared.

Visualization and Analysis

The *PyMol* Molecular Graphics System[12] was used to analyze the protein structure, the hydrogen bond interactions with the active site residues and preparation of high resolution images.

RESULT AND DISCUSSION

GCMS was performed for identification of phytocompounds. The ethanolic leaf extract of *Vitex negundo* was taken for GCMS study. The GCMS method determined that the ethanolic extract of vitex negundo Linn showed the presence of phyto compounds (Table1). The structure conformation of the identified phytocompounds was accomplished by comparing the mass spectra obtained to their commercial Wiley9 library. GCMS result concludes that the number of phyto compounds were present in the ethanolic leaf extract of vitex negundo linn.

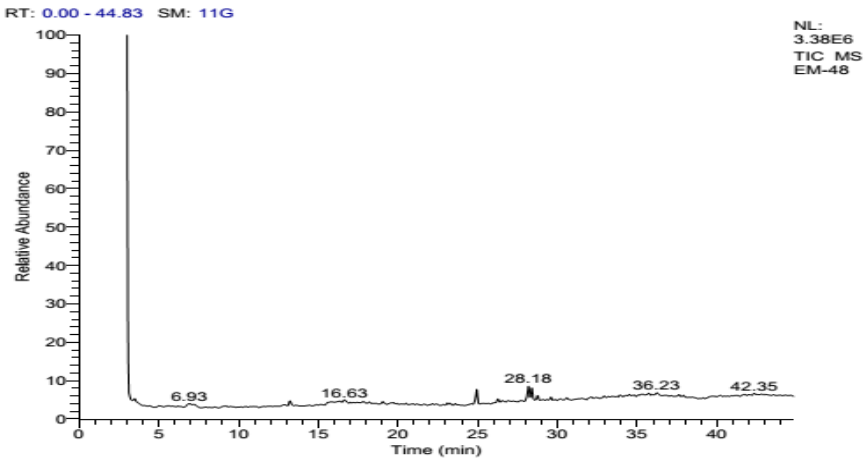


Figure 1. GCMS result for ethanol leaf extract of *V. negundo* Linn.

In our study, we selected phenolic and flavonoid compounds. The name, molecular weight and structure of the components of the test materials were ascertained given in the table 3.9. GCMS result concludes that the more number of phenolic compounds were present in the ELE of *V. negundo.*

Various researchers studied only *in vitro* and *in vivo* studies for anti-microbial and anti-inflammatory activity of *V. negundo*. To the best of our knowledge, for first time we reported *in silico* anti-microbial and anti-inflammatory study of ELE of *V.negundo* and reported isolated compounds of the leaf extract of *V.negundo*.

Table 1 List of phytocompounds identified through GCMS in the ELE of *V. negundo*

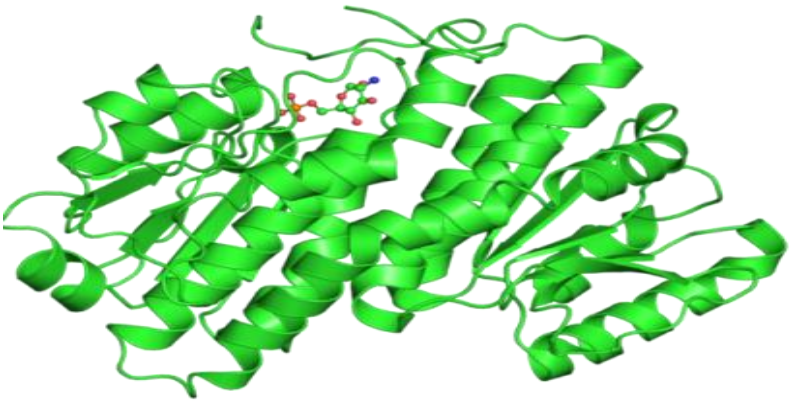
S.No	Phytocompound Name	Molecular Weight	RT	Area (%)
1.	2-[(4'-Methoxyphenyl)carbonyl]-5-(4''-methoxyphenyl)oxazole	309	3.49	2.77
2.	4-(4-Methoxybenzoylamino)benzopyran-2-one	309	3.49	2.77
3.	S,S)-3-(1-tert-Butyloxycarbonylamino-2-methylbutyl)-4-tert-butyloxycarbonyl-5-methylisoxazole	368	9.21	2.33
4.	(4S)-2-Methyl-2-phenylpentane-1,4-diol	194	15.59	2.99
5.	(3R,4S)-4-(methylamino)-1-phenylpent-1-en-3-ol	191	16.65	3.38
6.	anti-1-Cyclohexyl-1-hydroxybut-3-en-2-yl Methyl Carbonate	228	19.04	2.15
7.	3-(tert-Butoxycarbonyl)-6-(3-benzoylprop-2-yl)phenol	340	26.27	3.04
8.	(-)-(2S,1'S)-1-Benzyl-2-[1'-(dibenzylamino)ethyl]aziridine	356	32.12	2.97
9.	7-Methoxy-2,3-dihydro-2-phenyl-4-quinolone	253	33.91	1.42
10.	N-(t-Butyl)-2-benzoylbenzamide	281	35.72	1.91
11.	5-(Benzyloxy)-3-(ethoxycarbonyl)-6-methoxy-1-(phenyl-amino)isoquinoline	428	37.50	1.65
12.	3-(Cyclohexylamino)-1,5-dihydro-5-ethylidene-4-(n-propyl)-2H-pyrrol-2-one	248	39.74	3.56

Glucosamine 6 phosphate synthase enzyme protein structure analysis

Glucosamine 6 phosphate synthase enzyme protein with glucosamine-6-phosphate. GlcN-6-P synthase as obtained from PDB with ID 2VF5 which would be considered as the best accurate active region as it is solved by experimental crystallographic data.[13] Crystal structure of 2VF5 was determined by using X-Ray diffraction at the resolution of 2.9Å. The PDB entry 2VF5 contains 608 amino acids. It is Monomeric. GlcN-6-P synthase (2VF5) protein was taken from the source of *E. Coli*. The target protein bounded with the ligand Glucosamine-6-Phosphate. The native ligand interact with the active site residues are, THR 302, SER 303, SER 347, GLN 348, SER 349, THR 352, VAL 399, and ALA 602.

Structure validation of 2VF5

Validation of the model 2VF5 protein by PROCHECK presented a Ramachandran plot. The result provides that the 2VF5 protein is a good model. The analysis rendering 80.7% residues in the most favoured regions, 18.4% in the additionally allowed regions and 0.6% generously allowed regions. All the amino acids are in allowed region. The results confirm that 2VF5 is a good quality protein. PSIPRED (Figure 2).



Glucosamine-6-phosphate synthase in complex with glucosamine-6-phosphate

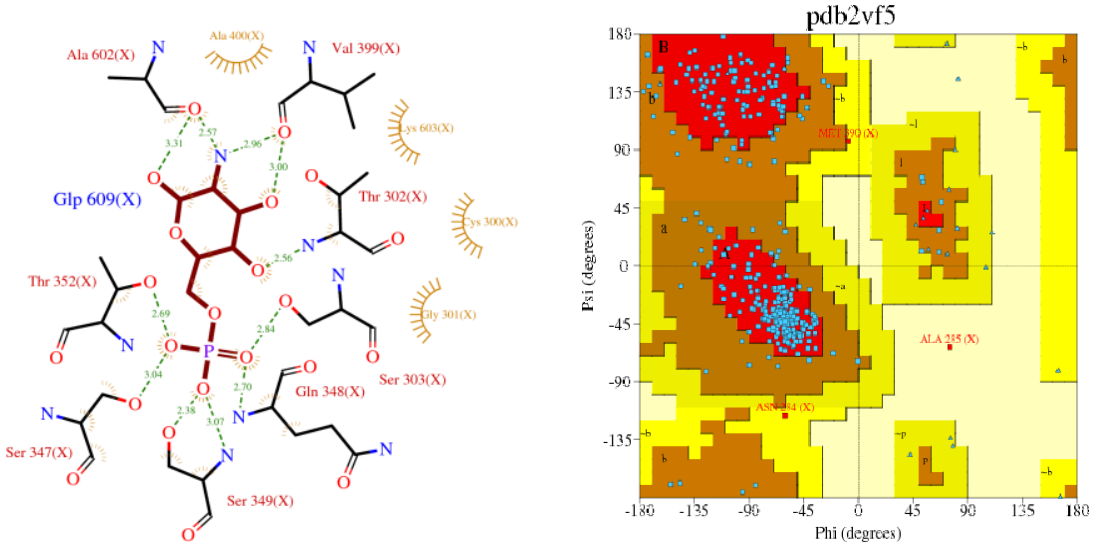


Figure 2 Crystal structure, LIGPLOT and Ramachandran plot of 2VF5

Molecular docking study with GlcN-6-P synthase

A docking software program can be used to identify different orientation poses by minimizing the energy of intermolecular interactions (Yang and Chen 2004).[14] The docking process is useful for predicting the ligand conformation as well as its position and orientation. Vijesh[15] revealed that 2VF5 with six new compounds, have minimum binding energy and have good affinity towards the active pocket of 2VF5 and GlcN-6-P synthase which was considered as a good target for antimicrobial studies. Molecular docking studies showed the potential of the phytocompounds of ELE of *V. negundo* against GlcN-6-P synthase enzyme. The phytocompounds bind to the active sites of the target protein 2VF5 and have a good docking score and glide energy. The binding poses, interactions and distances were analysed by *Glide*.

The docking result obtained by Schrodinger was saved in PDB format and the interactions were visualized in the LIGPLOT software. The docking of receptor GlcN-6-P with ligands exhibited well established hydrogen bonds with one or more amino acids in the receptor active pocket. The interaction energy between the 2VF5 and phytocompounds interactions were computed as glide score and glide energy. The native ligand Glucosamine 6 phosphate is bound in a hydrophobic cavity formed by THR302, SER303, SER347, GLN348, SER349, THR352, ASN375, VAL399 and ALS602 residues and formed hydrogen interaction with the target protein. Docking studies were done by using 12 compounds, among 12 compounds five compounds have high potential to bind with the active site (Table 2 & 3 and Figure. 3). The glide score and glide energy for the native ligand Glucosamine 6 phosphate were –7.65 and -39.86 Kcal/ mol. The glide score and glide energy for the Compound_1 (4S)-2-Methyl-2-phenylpentane-1,4-diol were -5.95 and -36.53 Kcal/ mol.

Table 2 List of phytocompounds having high affinity to 2VF5

S.No	Phytocompound Name
1.	Compound_1 (4S)-2-Methyl-2-phenylpentane-1,4-diol
2.	Compound_2 7-Methoxy-2,3-dihydro-2-phenyl-4-quinolone
3.	Compound_3 3-(tert-Butoxycarbonyl)-6-(3-benzoylprop-2-yl)phenol
4.	Compound_4 (3R,4S)-4-(methylamino)-1-phenylpent-1-en-3-ol
5.	Compound_5 (-)-(2S,1'S)-1-Benzyl-2-[1'-(dibenzylamino) ethyl]aziridine

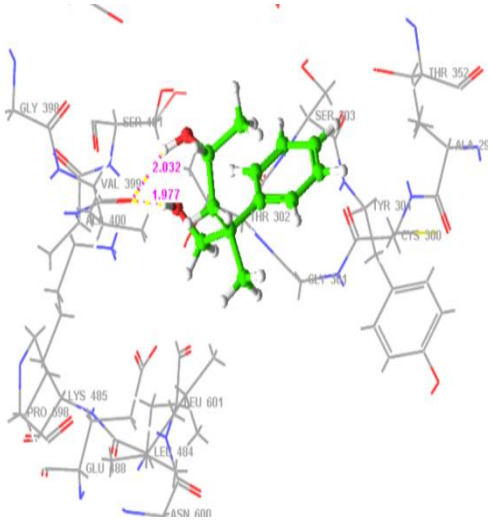
Table Error! No text of specified style in document.**: Hydrogen bond / coordination bond interactions of phytocompound of *V. negundo* with the amino acids of GlcN-6-P synthase.**

S. No	Compound	H-bond/Coordination Interactions	Distance (Å)	Glide Score	Glide energy (Kcal/mol)
1.	Compound_1	VAL399[O-H....O] VAL399[O-H....O]	1.99 2.03	-5.95	-36.53
2.	Compound_2	GLN448[O-H....O] THR352 [O-H....O]	2.18 1.86	-5.30	-33.57
3.	Compound_3	THR302 [0-H....O] [O-H....O] GLU396	1.96 1.80	-5.12	-35.90
4.	Compound_4	[0-H....O] GLN396 [0-H....O] GLY398	1.65 1.84	-5.88	-33.88
5.	Compound_5	[0-H....O]GLY398 [0-H....O] GLN396	2.00 1.78	-4.26	-37.65

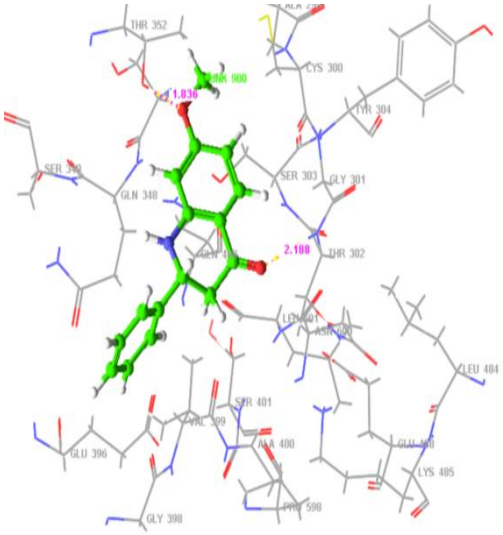
All the five compounds have favourable binding potential with the active site residues. The phytocompounds 1 and 4 shows the highest glide score than other three compounds. The hydrogen bond makes an important contribution to the interaction between the ligand and the

protein. When the binding energy of both the phytocompounds was compared to native ligand, the glide score and glide energy for the native ligand should be more are less same in the phytocompounds of ELE of *V. negundo*. The glide score and glide energy for the compound 1 were -5.95 and -36.53 Kcal/ mol. The glide score and glide energy for the compound 4 were -5.88and -33.88 Kcal/ mol. The phytocompounds1 and 4 shows the highest glide score than other compounds. It was very much satisfactory and it supports its anti-microbial activity. By these studies it can be concluded that among the twelve compounds given in table 3 five compounds showed higher activity and it could act as antimicrobial compound.

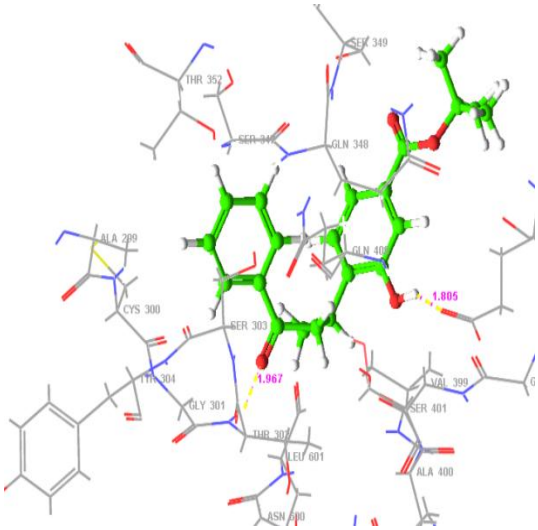
The present study showed that these phytochemical compounds possess potential medicinal values with anti-microbial properties. These phytocompounds could be the potential inhibitory source against microbial protein. The phytocompounds of ELE of *V. negundo* showed a better docking simulation and interaction analysis. No studies reported *in silico* anti-microbial activity of ELE of *V. negundo*. Our *in silico* docking studies strongly recommend ELE of *V. negundo* was found to be more effective against microbes



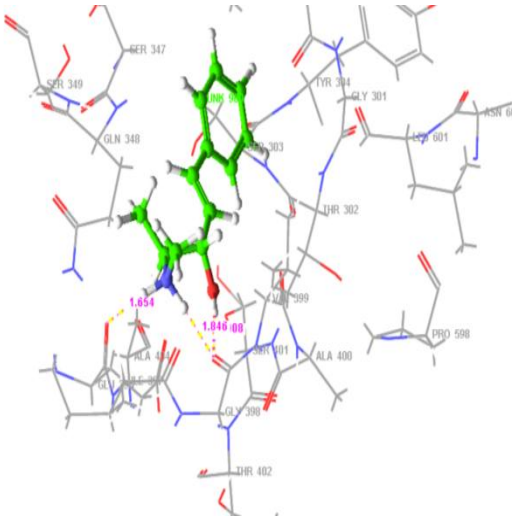
2VF5 with Compound_1



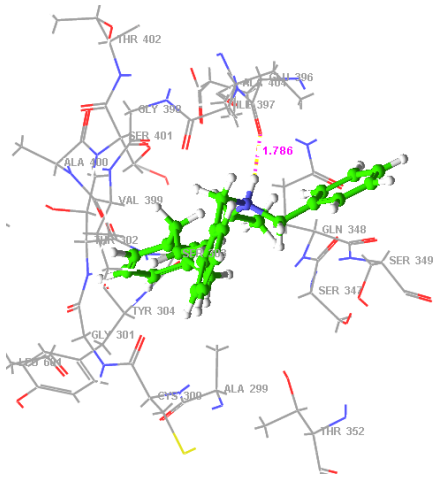
2VF5 with Compound_2



2VF5 with Compound_3



2VF5 with Compound_4



2VF5 with Compound_5

Figure 3 Hydrogen bonding interaction of ELE of *V. negundo*

CONCLUSION

The molecular docking of Glucosamine 6 phosphate synthase enzyme with the phytocompounds of ELE of *V. negundo* Linn. revealed that these identified phytocompounds would be used for antimicrobial agent. The results obtained from this study were useful for understanding the inhibitory mode of *V. negundo* phytochemicals as well as accurately predicting the activities of phytochemical inhibitors on the basis of docking scores and Glide energy. The docking studies result concluded that these five compounds showed higher activity and they could act as antimicrobial compounds. These results of docking study can be useful to identify inhibitors for specific microbial target proteins and thus this is useful to design new drugs. There is need for further studies in order to isolate, identify, characterize and elucidate the structure of these phytocompounds.

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