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MOLECULAR DOCKING STUDIES FOR PHYTOCHEMICALS OF *HYGROPHILA SPINOSA* WITH OXYHAEMOGLOBIN PROTEIN

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ABSTRACT

Anaemia in human is a global threat. Traditionally, plant extracts of *Hygrophila spinosa* T. Anders, local name Kulekhara is used to treat anaemic condition. Present *in silico* study aimed to screen the active compound(s) of *H. spinosa*, which play an important role in its oxygen binding capacity as heterotrophic allosteric effectors by molecular docking analysis. The oxyhaemoglobin (receptor) of human was obtained from European Protein Databank (1HHO). Twelve phytoconstituents and one synthetic derivative as L35 (2-[4-({[(3,5-di-chlorophenyl)amino]carbonyl}amino)phenoxy]-2-methylpropa-noic acid) were studied as ligands for docking study. The present results showed the binding modes of these potential heterotropic allosteric effectors with oxyhaemoglobin during molecular docking screening. It is conceivable from the present study on molecular docking that a few phytoconstituents of *H. spinosa* can be developed as lead compound(s) in future for drug designing to prevent anaemia related to oxyhaemoglobin. In addition, in vivo and in vitro study of these lead compounds may confirm suitability of this molecular docking.

KEYWORDS: Oxyhaemoglobin; Molecular docking; *Hygrophila spinosa*; Phytochemicals; Natural compounds; Anaemia control

#Equal contribution as first author.

INTRODUCTION

In human, anaemia is the most common blood disorder that refers to condition the function of haemoglobin is to bind oxygen properly. When the haemoglobin level decreases in blood then the oxygen binding is lower and tissue gets less oxygen.[1] In anaemia, blood is lacking red blood cells and haemoglobin (Hb) during pregnancy and also in menorrhagia condition in women.[2-4] The established synthetic drugs are costly and major people use the extract of an important plant, *Hygrophila spinosa* T. Anders, commonly called as Kulekhara.[5] The researchers have experimented the medicinal properties of *H. spinosa* in rodents as well as human to prevent blood related disorders.[6-11] There are several phytoconstituents found in *H. spinosa*. These bioactive compounds have no side effect as by traditional knowledge.[12]

The oxyhaemoglobin (HbO) is formed when oxygen attached to haemoglobin during transport through blood. The cooperative O_2-binding of Hb has been established to correlate mainly alteration in the quaternary structures of Hb as T(deoxy)- and R(oxy)-quaternary structures, found low and high O_2-affinities, respectively. Heterotropic allosteric effectors have been observed to interact not only with deoxy- but also oxy-Hb, causing significant reduction in their O_2-affinities and the modulation of cooperativity.[13] The heterotropic allosteric effectors have already been documented that these bind to the T-quaternary structure of deoxyhaemoglobin and lower its O_2-affinity as K_T.[14] Subsequently these also bind to the R-quaternary structure of oxyhaemoglobin in a same pathway, also to reduce its O_2-affinity as K_R.[15-20] It was known from experimental studies that the derivatives of benzafibrate as 2-[4-({[(3,5-dichlorophenyl)amino]carbonyl}amino)phenoxy]-2-methyl-propanoic acid (L35) and inositol hexaphosphate (IHP), having potent heterotropic allosteric effects on haemoglobin structurally and functionally.[13; 21-22]

The present study was an attempt to know direct effects of the different phytochemicals present in *H. spinosa* on oxyhaemoglobin to determine allosteric heterotropic effectors in relation to established L35. The binding sites and the affinities of these phytochemicals with the oxyhaemoglobin have been probed by using molecular docking.

MATERIALS AND METHODS

Selection of protein

The structural information oxyhaemoglobin (receptor) of human was obtained from European Protein Data Bank (http://www.ebi.ac.uk/pdbe/node/1). Three-dimensional X-ray crystallized structure of human oxyhaemoglobin (PDB ID: 1hho) with a resolution of 2.1 Å, was

downloaded from the protein data bank.[23] This protein is contained two chains, haemoglobin A (oxy and alpha chain) with 141 residues and haemoglobin A (oxy and beta chain) with 146 residues of amino acids.[24]

It was established that the 1hho structure contains only one dimer and the second was constructed by symmetry to obtain tetrameric R-HbA.[24] The crystal structure of human oxyhaemoglobin complexed with three PO4 molecules (ID: 1hho) was selected according to the wwPDB validation report.[23;25] The file was obtained as .ent extension, which was converted to .pdb format for docking using AutoDockTools developed by The Scripps Research Institute.[26] The crystallized structure of oxyhaemoglobin was depicted in Fig. 1.

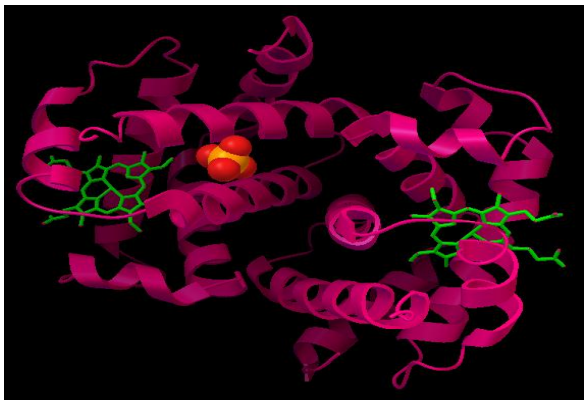


Fig. 1. Ribbon representation of the crystal structure of human oxyhaemoglobin (ID: 1hho). The two chains as ribbon structure are shown in pink colours (α and β chain). Heme groups are shown in stick representation of yellow colour. The red and yellow balls are PO4142. The central cavity of the oxyhaemoglobin is visible in this orientation

Selection of compounds

All the test compounds were studied as per details information from PubChem, a chemicals database (https://pubchem.ncbi.nlm.nih.gov/compound/). In the present work, 12 phytochemicals and 1 chemical derivative were selected and the structure as pdb extension were individually taken from PubChem. For all the test compounds as two-dimensional structures had exhibited in previous docking study.[27] The Canonical SMILES string for each chemical was taken from PubChem and incorporated in CORINA online software (http://www.mol-net.de) and finally downloaded the pdb extension. The protein-ligand binding was studied for each of the 12 above mentioned phytochemicals viz. β-carotene, luteolin-7-rutinoside, lupenone, luteolin, lupeol, apigenin-7-O-glucoside, stigmasterol, betulin, apigenin-7-O-glucuronide, β-sitosterol, hentriacontane and 3-methylnonacosane and synthetic derivative as L35.

Molecular docking

The molecular docking was carried out by using PyRx software, Version 0.8 [28] and validated in AutoDock Vina software, developed by Morris et al.[26] and the results were rendered by using MGL Tools. The protein was loaded in PyRx software for the conversion of pdbqt file that contains a protein structure with polar hydrogens. The protein and test compounds were converted pdbqt file through PyRx software. All bonds of ligands were set to be rotatable. The docking site on this oxyhaemoglobin target was expressed by forming a grid box with the dimensions of X: 41.5565 Y: 56.9246 Z: 57.1943 Å, with a grid spacing of 0.375 Å, centered on X: 13.1922 Y:0.3351 Z: 0.1358 Å. The best conformation was selected as lowest energy value after the docking search was finished for 12 phytochemicals and 1 synthetic derivative. Docking of 12 phytochemicals and 1 synthetic derivative (L35) with oxyhaemoglobin (PDB ID: 1hho) was analysed following the docking of ligands and the receptor to know the probable receptor ligand interactions. These present softwares create protein-compound interaction interface by obtaining higher to lower energy value for each test compound. Finally, all the 12 ligands were compared with previously established L35 compound to determine similarities on binding position and energy value.[18;27] The protein-ligand interactions as individual phytochemical and synthetic derivative with oxyhaemoglobin were finally analysed by using the LigPlot software (Version 1.4) to understand some specific contacts between residues of the receptor and the atoms of the ligand.[29]

RESULTS AND DISCUSSION

The docking results indicate that the interaction of the phytochemicals of *Hygrophila spinosa* with the target protein oxyhaemoglobin was energetically favourable. Table 1 showed the energy values for two phytoconstituents viz. hentriacontane (-3.7) and 3-methylnonacosane (-3.7) as highest value while lowest values for another two phytochemicals namely β-carotene (-9.0) and luteolin-7-rutinoside (-9.0) were obtained, followed by lupenone (-8.7), apigenin-7-O-glucoside (-8.3), lupeol (-8.3) and luteolin (-8.3), stigmasterol (-8.0), betulin (-7.8), apigenin-7-O-glucuronide (-7.6) and β-sitosterol (-6.7). The energy value for L35 compound was -7.1. All figures were obtained through AutoDockTool interface and depicted in Fig 2A-L.[21] It has already been established that when dock scoring values lower, then the binding affinities higher.[30]

Fig 2. (A-L) shows each phytochemical (ligand) binding position with oxyhaemoglobin (receptor) and (a-l) shows binding interaction through schematic representation for same phytoconstituent. The compounds viz. β-carotene, lupeol, hentriacontane and 3-methylnonacosane have showed binding inside the central cavity of oxyhaemoglobin and mainly hydrophobic in nature. The residues such as Lys90, Tyr35, Trp37, Glu101, Ala130, Asp126, Leu105, Asn108, Arg104, Phe36, Gln131, Ala135, Lys132, His2 and Po4142 for β-carotene; Ala135, Gln131, Val134, Arg104, Phe36, Ser35, Lys132 and Pro37 for lupeol; Gln131, Lys132, Ala135, Ser35, Phe36, Pro37, Val96, Thr38, Glu101, Arg104, Asn139 and Asn108 for hentriacontane and Val96, Arg104, Glu101, Lys99, Leu100, Asn108, Phe36, Gln131, Ala135, Thr38, Pro37 and Ser35 for 3-methylnonacosane as residues of oxyhaemoglobin were found to form hydrophobic contacts.

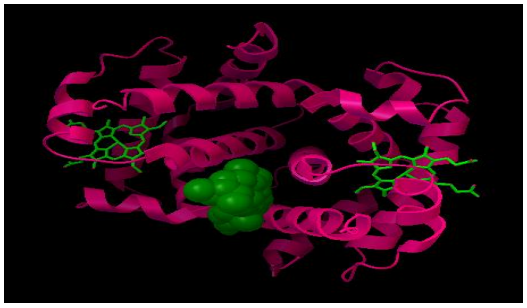
Table 1. Binding energies of the ligand with oxyhaemoglobin

Sl. No.	Ligands name	Binding energy (Kcal/mol)
Phytoconstituents		
1.	β-carotene	-9.0
2.	Luteolin-7-rutinoside	-9.0
3.	Lupenone	-8.7
4.	Luteolin	-8.3
5.	Lupeol	-8.3
6.	Apigenin-7-O-glucoside	-8.3
7.	Stigmasterol	-8.0
8.	Betulin	-7.8
9.	Apigenin-7-O-glucuronide	-7.6
10.	β-sitosterol	-6.7
11.	Hentriacontane	-3.7
12.	3-methylnonacosane	-3.7
Synthetic derivative		
13.	L35	-7.1

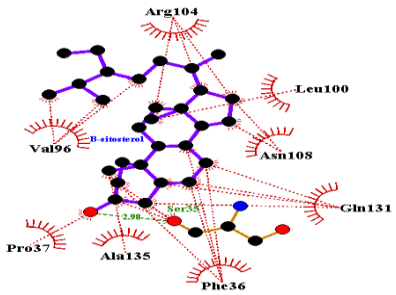
In case of other compounds viz. β-sitosterol, stigmasterol, lupenone, betulin, luteolin, luteolin-7-rutinoside, apigenin-7-O-glucuronide and apigenin-7-O-glucoside have showed inside the central cavity same as β-carotene, lupeol, hentriacontane and 3-methylnonacosane with oxyhaemoglobin. It was found β-sitosterol, stigmasterol, lupenone, luteolin, betulin, luteolin-7-rutinoside, apigenin-7-O-glucuronide and apigenin-7-O-glucoside have observed 1, 1, 1, 3, 1, 2, 3 and 3 numbers respectively of hydrogen bonds with oxyhaemoglobin during interaction. It was observed from schematic diagram, the hydrogen bonds formation was involved with particular residues for each phytochemical, viz. Ser35 for β-sitosterol, Ser35 for stigmasterol, Lys127 for lupenone, Asn108, Thr134 for luteolin, Arg104 for betulin,

Thr38, Tyr35, Leu28, Leu106, Leu96, Asn102, Leu31, Ala27 for luteolin-7-rutinoside, Glu101, Gln131, Ser35 for apigenin-7-O-glucuronide and His92, Val98, Lys95 along with Hem147 and Oxy150 for apigenin-7-O-glucoside.

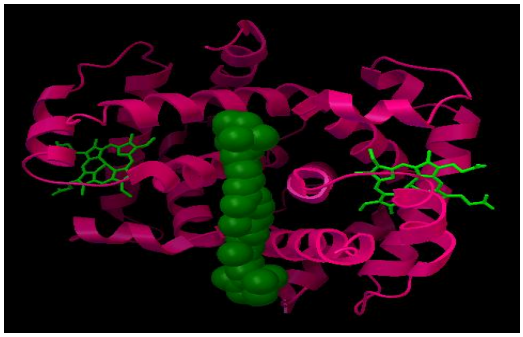
Fig 3. (A) shows for L35 (ligand) binding position with oxyhaemoglobin (receptor) and (a) shows binding interaction through schematic representation for same compound. It was also found that L35 also binds inside the central cavity of oxyhaemoglobin and mainly hydrophobic in nature. The residues such as Leu105, Trp37, Asp126, Tyr35, Lys99, Ser102.



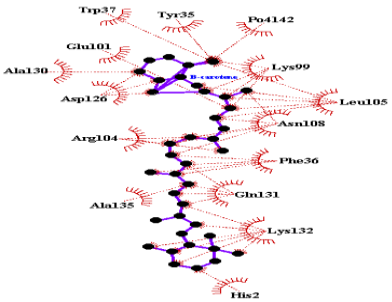
A. β-sitosterol docking



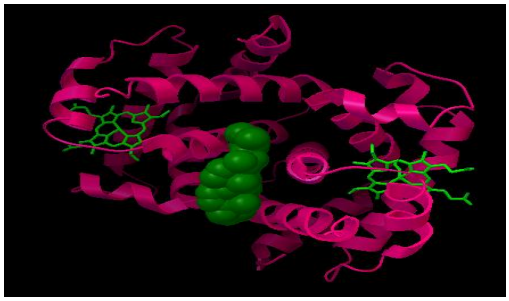
a. 2D Binding interaction



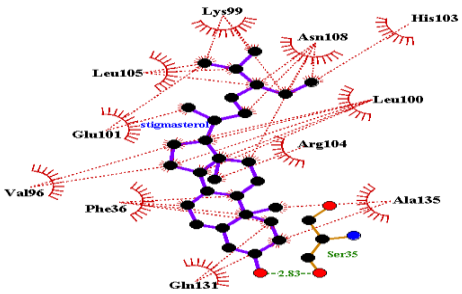
B. β-carotene docking



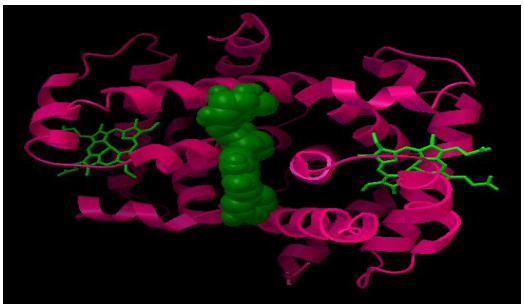
b. 2D Binding interaction



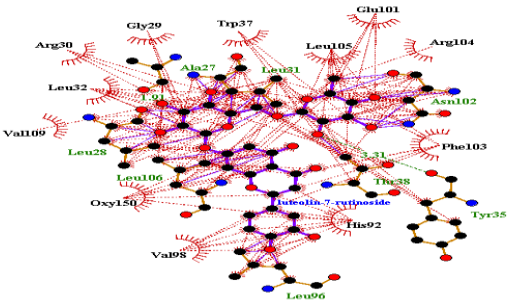
C. Stigmasterol docking



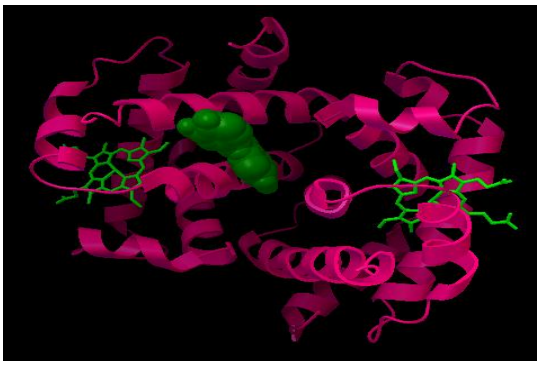
c. 2D Binding interaction



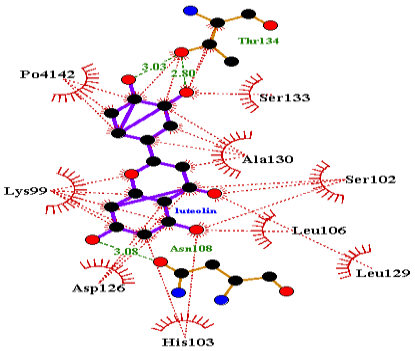
D. Luteolin-7-rutinoside docking



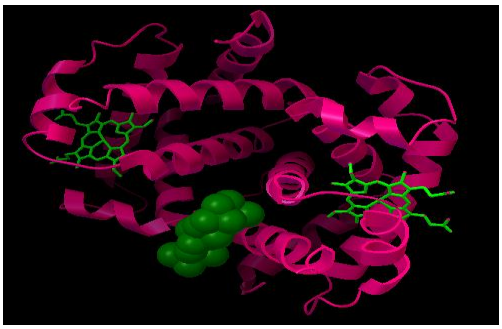
d. 2D Binding interaction



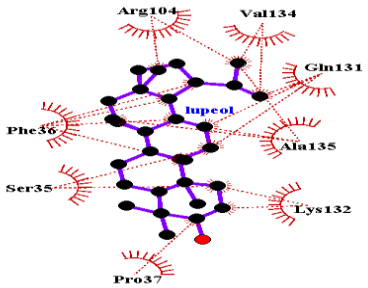
E. Luteolin docking



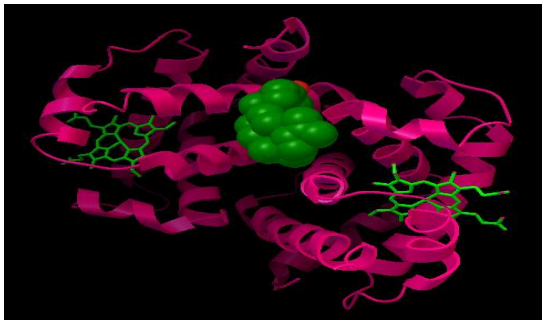
e. 2D Binding interaction



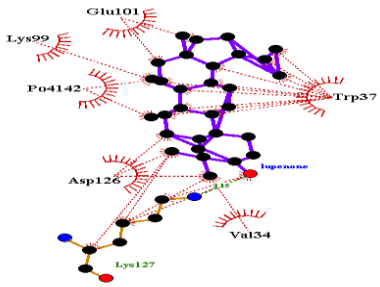
F. Lupeol docking



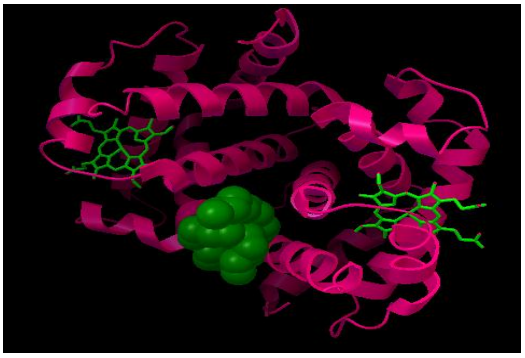
f. 2D Binding interaction



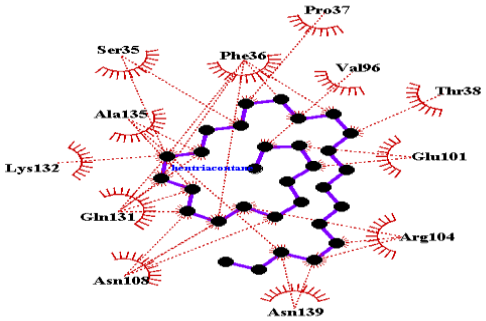
G. Lupenone docking



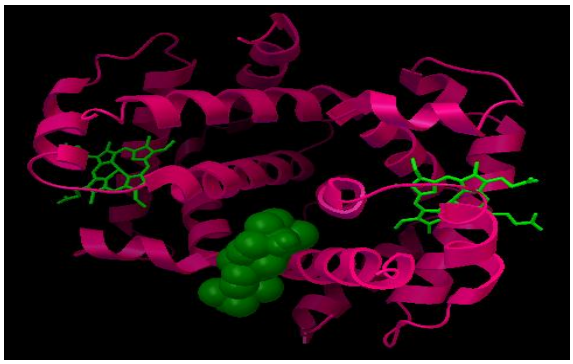
g. 2D Binding interaction



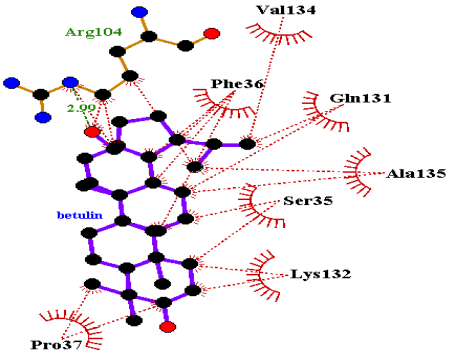
H. Hentriacontane docking



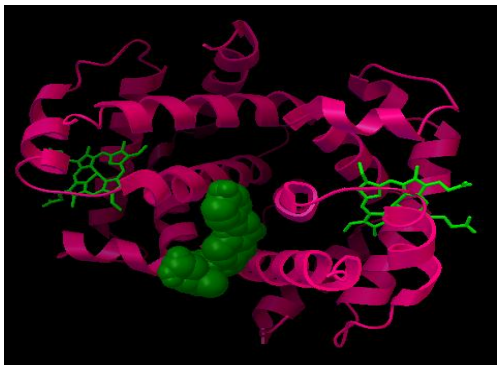
h. 2D Binding interaction



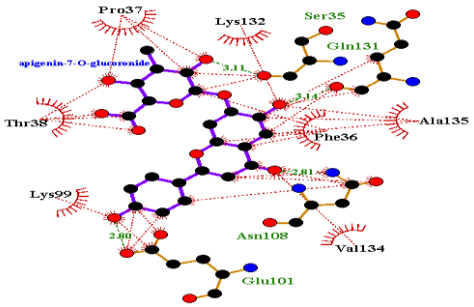
I. Betulin docking



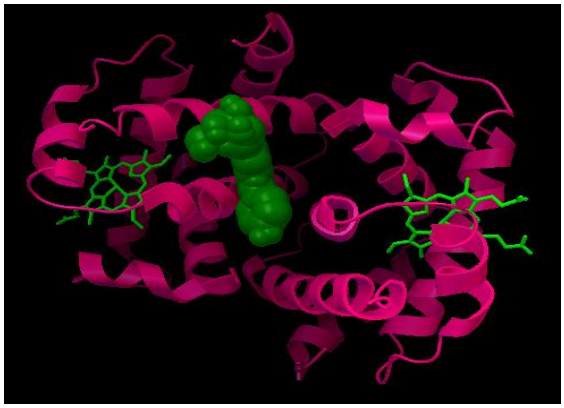
i. 2D Binding interaction



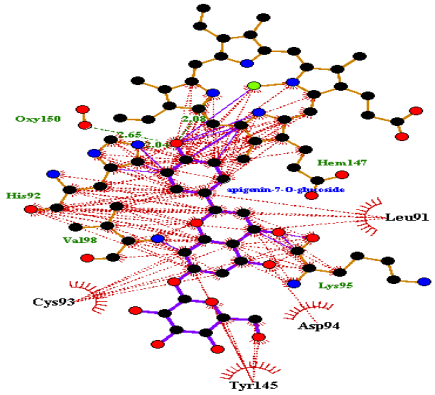
J. Apigenin-7-O-glucuronide docking



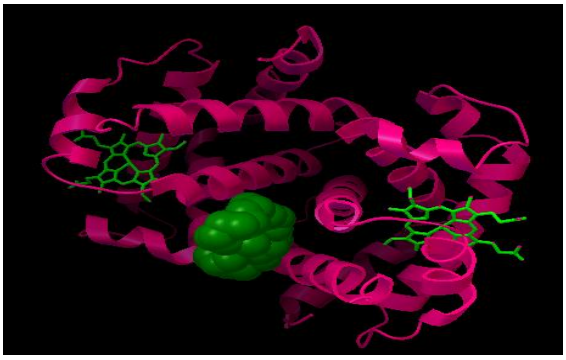
j. 2D Binding interaction



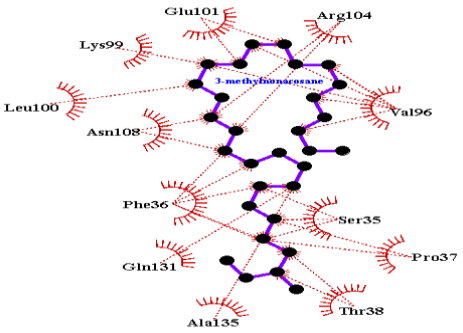
K. Apigenin-7-O-glucoside



k. 2D Binding interaction

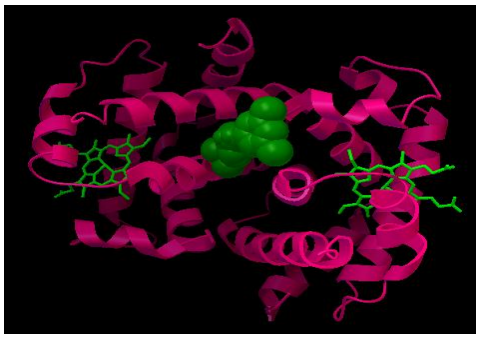


L. 3-methylnonacosane docking

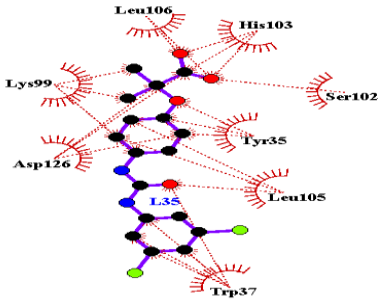


l. 2D Binding interaction

Fig. 2 (A, B, C, D, E, F, I, J, K and L). Docking of individual phytochemical (ligand) binding with oxyhaemoglobin (receptor) and (a, b, c, d, e, f, g, h, I, j and k) protein-ligand interaction schematic diagram through LigPlot (= Ligand bond; = Non-ligand bond; = Hydrogen bond with length; = Non-ligand residues involved in hydrophobic contacts; = Corresponding atoms involved in hydrophobic contacts; = hydrophobic connections)



a. L35 docking



a. 2D Binding interaction

Fig. 3. (A) Docking of L35 (ligand) binding with oxyhaemoglobin (receptor) and (a) protein-ligand interaction schematic diagram through LigPlot (= Ligand bond; = Non-ligand bond; = Hydrogen bond with length; = Non-ligand residues involved in hydrophobic contacts; = Corresponding atoms involved in hydrophobic contacts; = hydrophobic connections)

The hydrophobic interactions play a vital role in binding with oxyhaemoglobin for all the studied compounds. The residues were obtained for hydrophobic interactions in each compound such as Val96, Asn108, Leu100, Arg104, Pro37, Ala135, Phe36 and Gln131 for β-sitosterol; Glu101, Leu105, Lys99, Asn108, His103, Leu100, Arg104, Val96, Phe36, Gln131 and Ala135 for stigmasterol; Val34, Asp126, Trp37, Glu101, Lys99 and Po4142 for lupenone; Leu106, Ser102, Lys99, Asp126, His103, Ala130, and Ser133 for luteolin; Ser35,

Ala135, Lys132, Gln131, Phe36, Val134 and Pro37 for betulin; His92, Val98, Phe103, Val109, Leu32, Arg30, Gly29, Trp37, Leu105, Glu101 and Arg104 for luteolin-7-rutinoside; Pro37, Thr38, Lys132, Ala135, Phe36, Lys99 and Val134 for apigenin-7-O-glucuronide and Leu91, Asp94, Tyr145 and Cys93 for apigenin-7-O-glucoside.

On the other hand, experimental study revealed that few phytochemicals such as flavonoids, phenolics and terpenoids of this herb have potent haematopoietic ability during haloperidol iron deficiency anaemia induced albino rat.[31-32] According to these researchers, induction of RBC count, haemoglobin, haematocrait, serum iron and protein by ethanolic extract of whole plant. Among these phytochemicals, few of them may be act as erythropoiesis-inducing agents (EIAs) by showing central cavity binding with oxyhaemoglobin in the present work because EIAs help in the prevention of anaemia by clinical trial.[33] It is well documented in previous studies that L35 compound individually or combined with IHP have potent heterotropic allosteric effect on the T-state haemoglobin,[13;27;34] which supported the present observation in relation to binding energy and these phytochemicals can be further studied for lead compound identification in drug development to prevent anaemia and other haematological diseases, when cause by both oxy and deoxyhamoglobin deficiency. Talapatra et al.[27] have investigated in previous study for binding energy value that phytochemicals viz. lupenone (-10.4) and leuteolin-7-rutinoside (-10.2) for T-state haemoglobin while β-carotene (-9.0) and leuteolin-7-rutinoside (-9.0) for oxyhaemoglobin obtained in the present study. These active phytoconstituents of *H. spinosa* may be the lead compound for haemoglobin maintenance and recover anaemia.

This is a computer simulation work to know active phytochemicals in *H. spinosa* for allosteric effectors for oxyhaemoglobin and the phytochemicals of *H. spinosa* have already been studied experimentally to prevent in haemoglobin deficiency in rodents and human by traditional knowledge.[7-14] Talapatra et al.,[27] have observed same phytoconstituents showed potent allosteric heterotropic effectors with T-state haemoglobin during molecular docking when compared to L35 compound and similar effects also recorded in the present study with oxyhaemoglobin.

Thus, molecular docking can be a suitable screening to know receptor-ligand binding affinity and the phytoconstiteunts of *H. spinosa* can also be used for anaemia prevention because oxyhaemoglobin binding with ligands have allosteric effect. The functional deficiency of

oxyhaemoglobin can be prevented by this herbs extract, which supported by traditional and experimental knowledge.[35]

CONCLUSION

It is concluded from the present computational prediction study, few phytochemicals of *H. spinosa* can be used in future lead compound(s) for drug designing and development to treat anaemia based on oxyhaemoglobin deficiency. The results revealed that specific compounds have heterotropic allosteric effect on the receptor (oxyhaemoglobin) in relation to previous study[27] and other studies with the derivatives of benzafibrate.[13;22;24] However, further study on the binding of these compounds with oxyhaemoglobin in relation to experimental studies to confirm these simulations through computational prediction prior to drug design with present phytochemicals of *H. spinosa* because this herb is highly medicinal potential for anaemia as traditional practice.[12;35]

Conflict of interest

The authors declare that there are no conflicts of interest for the present study.

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