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# INTERACTION OF T-STATE HAEMOGLOBIN AND PHYTOCHEMICALS OF *HYGROPHILA SPINOSA* T. ANDERS: AN APPROACH BY MOLECULAR DOCKING

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## ABSTRACT

Anaemia is a condition that develops due to haemoglobin deficiency and is a common blood disorder. Several synthetic drugs have already been used, however, low income group people are often unable to afford the drugs. Use of the plant extract of *Hygrophila spinosa* T. anders, known as Kulekhara for anaemic condition is a traditional practice in Indian subcontinent and China. The present study was aimed to identify the phytochemicals of this plant, which could be potent heterotropic effector for oxygen binding of haemoglobin. The effect of allosteric effectors, such as bezafibrate and/or inositol hexaphosphate, have already been studied on the unliganded T-state haemoglobin using spectroscopy viz. absorption, circular dichroism, Raman and X-ray as well as functional assays. Herein, we document the binding modes of these potential allosteric heterotropic effectors with T-state haemoglobin by using molecular docking approach. It is conceivable from the present study based on molecular docking that a few phytochemicals of *H. spinosa* can be utilized as lead compound(s) in future for drug designing to prevent anaemia and haemoglobin related disorders. Further study should be carried out with R-state haemoglobin and natural

compounds from *H. spinosa*. In addition, functional assay of these lead compounds may confirm suitability of this molecular docking.

**KEYWORDS:** Molecular docking; *Hygrophila spinosa*; Allosteric heterotropic effectors; Phytochemicals; Anaemia prevention.

## INTRODUCTION

Anaemia is diagnosed as condition when body produce insufficient red blood cells and found in human of all age groups worldwide. Sometimes it was observed in young pregnant women and also in menorrhagia condition.[1-3] The people of rural area as well as in low income group are unable to afford medications for the treatment of anaemia and thus the level of haemoglobin remains low. Moreover, human being depends on traditional knowledge to use natural products from plant origin.[4] In this view, an important and easily available herb is *Hygrophila spinosa*, commonly called as Kulekhara found near ponds and ditches in West Bengal, India.

The researchers have documented beneficial properties of *H. spinosa* in rodents.[5-9] It was also reported that the diseases viz. anaemia, arteriovenous malformations, varicose veins and haemorrhages, etc. are prevented by using plant parts in China.[10] The plant, *H. spinosa* contains several phytochemicals viz. 25-oxo-hentriacontanyl acetate, stigmasterol, lupeol, lupenone, betulin, β-carotene, hentriacontane, apigenin-7-O-glucuronide, apigenin-7-O-glucoside, 3-methylnonacosane, asteracanthicine, luteolin-7-rutinoside, methyl-8-n-hexyltetracosanoate, β-sitosterol, 23-ethylcholesta-11(12), 23(24)-dien-3β-ol, luteolin, asteracanthine, histidine, phenylalanine, lysine, ascorbic acid, nicotinic acid, n-triacontane, glucose, mannose, rhamnose, arabinose, xylose, maltose, myristic acid, linoleic acid, oleic acid, palmitic acid, stearic acid, etc. reported by Patra et al.[11]

The oxygen transporting metallo-protein in erythrocytes is known as haemoglobin (Hb), which has four irons ($Fe_{2+}$) containing heme groups attached to the four subunits of globular protein.[12-13] The function of haemoglobin is to carry oxygen and carbon dioxide in tissue as well as blood pH regulation in all vertebrates.[13]

According to Asadi,[14] proteins (receptors) are the main molecular targets to detect drug action easily. Several compounds (ligands) of synthetic or natural origin may bind to the target proteins to show the beneficial or harmful effects, which help in new and efficient drug

designing. It has been documented that heterotropic effectors regulate the haemoglobin functions by interacting with its deoxy and oxy-state.[15] From the experimental studies it was observed that the derivatives of benzafibrate such as L35 (2-[4-({[(3,5-dichlorophenyl) amino]carbonyl}amino)phenoxy]-2-methylpropa-noic acid) and inositol hexaphosphate (IHP), show potent heterotropic allosteric effects on haemoglobin in relation to structure and function. The heterotropic allosteric effectors have been reported to interact not only with deoxy- but also oxy-haemoglobins causing significant reduction in their oxygen-affinities and the modulation of cooperativity.

In general, L35 compound is an established potent heterotropic effector that binds to the T-state haemoglobin and reduces its oxygen affinity.[16] The effect of the combined use of benzafibrate/L35 and IHP on the oxygen-affinity and the T to R-state-quaternary transition has been studied by using several spectroscopic methods.[17]

The objective of the present study is to explore direct effects of the different phytochemicals present in *H. spinosa* on T-state haemoglobin as heterotropic effectors. The binding sites and the affinities of these phytochemicals with the T-state haemoglobin have been probed by using computational approach.

## MATERIALS AND METHODS

### Selection of protein

The structural information T-state haemoglobin (receptor) of human was obtained from European Protein Data Base (http://www.ebi.ac.uk/pdbe/node/1). The crystal structure of T-state human haemoglobin complexed with three L35 molecules (ID: 2d5z) was selected according to the wwPDB validation report.[18] The file was obtained as. ent extension, which was converted to. pdbqt format for docking using Auto Dock Tools developed by The Scripps Research Institute.[19] In this process, the water molecules and existing ligands were deleted and the polar hydrogens were added (Fig. 1).

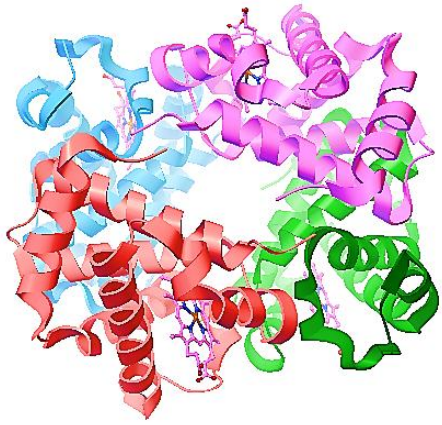
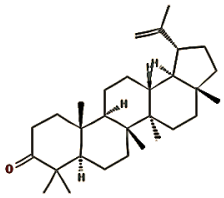


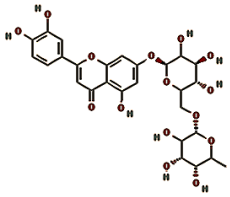
**Fig. 1. Cartoon representation of the crystal structure of T-state human haemoglobin. The four subunits are shown in different colours. Heme groups are shown in stick representation. The central cavity of the T-state haemoglobin is visible in this orientation**

**Selection of compounds**

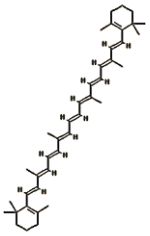
There were 18 compounds (ligands) reported in literature as active ingredients found in *H. spinosa*. These compounds were lupeol, lupenone, 25-oxo-hentriacontanyl acetate, stigmasterol, betulin, β-carotene, hentriacontane, apigenin-7-O-glucuronide, apigenin-7-O-glucoside, 3-methylnonacosane, 23-ethylcholesta-11(12), 23(24)-dien-3β-ol, luteolin, asteracanthine, asteracanthicine, luteolin-7-rutinoside, methyl-8-n-hexyltetracosanoate and β-sitosterol. Out of these, the detail information for only 12 compounds could be obtained from in PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/compound/). The two-dimensional (2-D) structure of all the compounds are depicted in Fig. 2. The Canonical SMILES string for each chemical was taken from Pub Chem (https://pubchem.ncbi.nlm.nih.gov/compound/) and converted to the. pdb file by using CORINA online software (http://www.mol-net.de). The protein-ligand binding was studied for each of the 12 above mentioned phytochemicals viz. lupeol, lupenone, betulin, luteolin, luteolin-7-rutinoside, β-sitosterol, β-carotene, apigenin-7-O-glucuronide, apigenin-7-O-glucoside, 3-methylnonacosane, stigmasterol and hentriacontane. All the compounds were processed to. pdbqt format prior to molecular docking study.



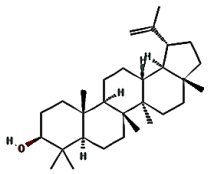
**a. Lupenone**



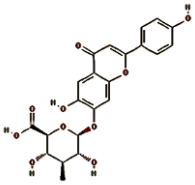
**b. Luteolin-7-rutinoside**



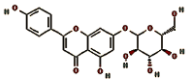
**c. β-carotene**



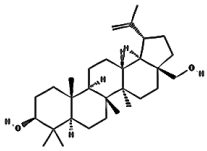
**d. Lupeol**



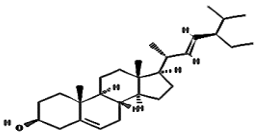
**e. Apigenin-7-O-glucuronide**



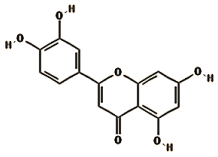
**f. Apigenin-7-O-glucoside**



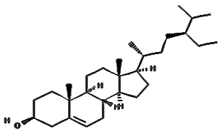
**g. Betulin**



**h. Stigmasterol**



**i. Luteolin**

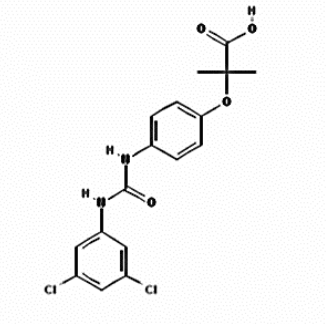


**j. β-sitosterol**

**k. 3-methylnonacosane**



**l. Hentriacontane**



**m. L35**

**Fig. 2 (a-m). Two-dimensional structure of studied compounds (Source: https://pubchem.ncbi.nlm.nih.gov/compound/)**

**Molecular docking**

The molecular docking was carried out by using Auto Dock Vina software, developed by Morris et al. (1998)[19] and the results were rendered by using MGL Tools. Auto Dock Vina is a docking program based on scan interaction site algorithm for predicting protein-ligand

interactions. Docking of 12 phytochemicals with haemoglobin (PDB ID: 2d5z) was analysed following the docking of ligands and the receptor to know the probable receptor ligand interactions. Before docking study each compound was converted to. pdbqt format. The present software creates protein-compound interaction profile by obtaining energy value for each test compound. Finally, all the 12 test compounds were analysed by comparing with previously established L35 compound to detect similarities on binding position and energy value.[18] The resultant structural complexes of the individual phytochemical and with haemoglobin were finally analyzed by using the Lig Plot software, Ver. 1.4 to determine some specific contacts between the atoms of the ligand and receptor.[20]

## RESULTS AND DISCUSSION

The docking results indicate that the interaction of the phytochemicals of *Hygrophila spinosa* with the target protein haemoglobin was energetically favourable. Table 1 showed the energy values for two compounds viz. hentriacontane (-4.1) and 3-methylnonacosane (-4.9) as highest value while lowest values for another two compounds namely lupenone (-10.4) and luteolin-7-rutinoside (-10.2) were obtained, followed by β-carotene (-9.7), lupeol (-9.6), apigenin-7-O-glucuronide (-9.5), betulin and apigenin-7-O-glucoside (-9.0), stigmasterol (-8.2) and β-sitosterol (-7.9) when compared to established compound L35 drug (-7.8). All figures were obtained through Auto Dock Tool interface.[21]

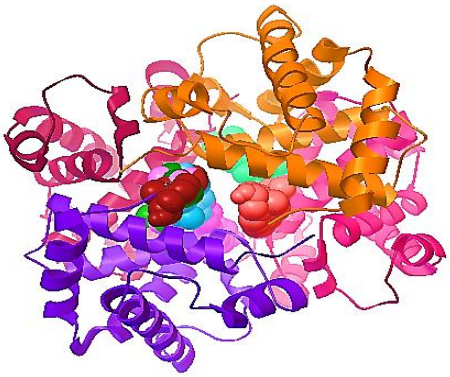
**Table 1. Binding energies of the compounds of *H. spinosa* with T-state haemoglobin**

| Sl. No. | Compounds | Binding energy (Kcal/mol) |
|---|---|---|
| 1. | Lupenone | -10.4 |
| 2. | Luteolin-7-rutinoside | -10.2 |
| 3. | β-carotene | -9.7 |
| 4. | Lupeol | -9.6 |
| 5. | Apigenin-7-O-glucuronide | -9.5 |
| 6. | Apigenin-7-O-glucoside | -9.0 |
| 7. | Betulin | -9.0 |
| 8. | Stigmasterol | -8.2 |
| 9. | Luteolin | -8.0 |
| 10. | β-sitosterol | -7.9 |
| 11. | L35 | -7.8 |
| 12. | 3-methylnonacosane | -4.9 |
| 13. | Hentriacontane | -4.1 |

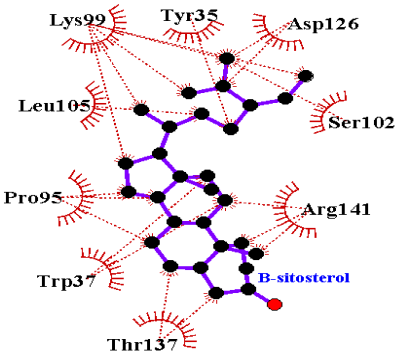
In Fig. 3 (A, C, E, G, I, K, M, O, Q, S, U and W) the docking results for each phytochemical was obtained on the basis of binding positions that few compounds viz. β-carotene, β-

sitosterol, stigmasterol, Luteolin-7-rutinoside, Apigenin-7-O-glucuronide and Apigenin-7-O-glucoside closely related to previously reported L35 compound[16-17] and Fig. 3 (B, D, F, H, J, L, N, P, R, T, V and X) showed binding interactions through schematic representation for each phytochemical.

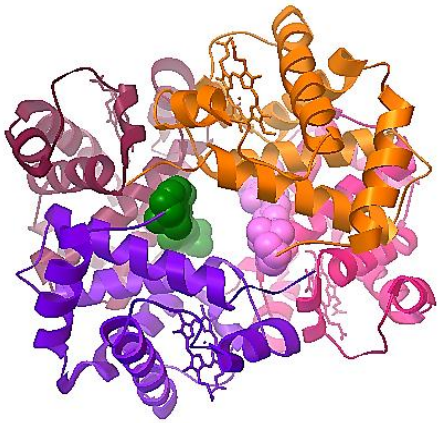
The compounds viz. β-sitosterol, β-carotene, stigmasterol, lupenone and 3-methylnonacosane have showed binding inside the central cavity of T-state haemoglobin and mainly hydrophobic in nature. The residues such as Asp 126, Tyr35, Lys99, Leu105, Pro95, Trp37, Thr137, Arg141 and Ser102 for β-sitosterol; Tyr140, Tyr35, Lys99, Leu100, Trp37, Thr137, Ala130, Arg141, Asn108, Arg104, Ala135, Gln131 and Phe36 for β-carotene; Asn108, Asp126, Lys99, Tyr35, Trp37, Arg141, Pro95, Thr137 and Ala130 for stigmasterol; Lys99, Ser102, Tyr35, Asp126, Ala130, Trp37, Arg141, Thr137, Lys127, Tyr140 and Ser131 for lupenone; Tyr35, Asp126, Ser133, Ser102, Lys99, Ala130, Lys127, Thr134, Thr137, Arg141, Ser131, Trp37, Ser138 and Pro95 for 3-methylnonacosane as residues of haemoglobin were found to form hydrophobic contacts.



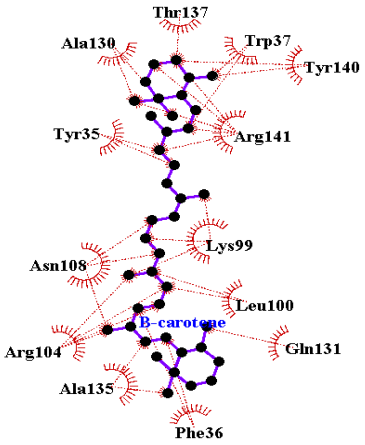
**A. β-sitosterol docking**



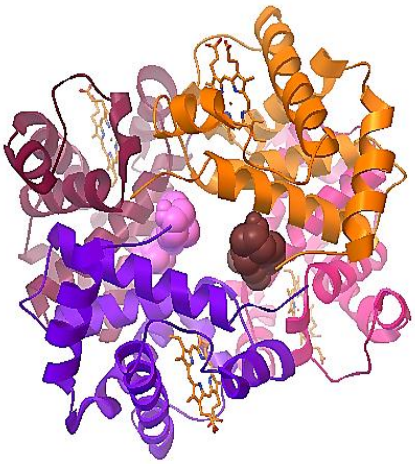

**B. Binding interaction**



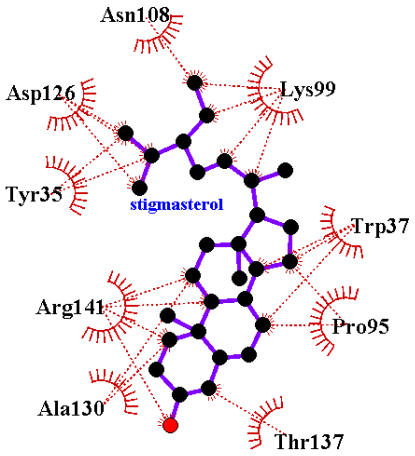
**C. β-carotene docking**



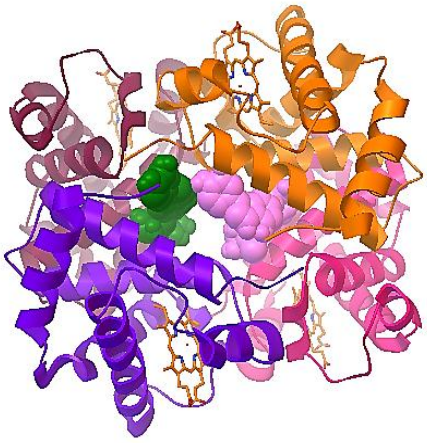

**D. Binding interaction**



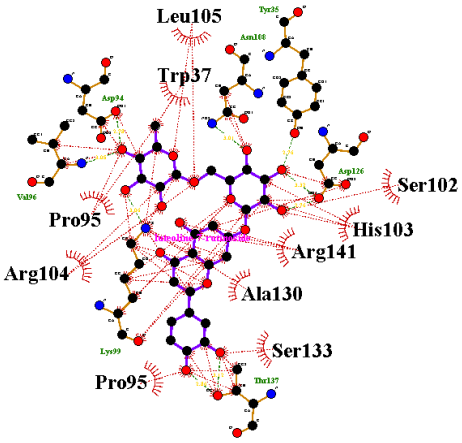
**E. Stigmasterol docking**



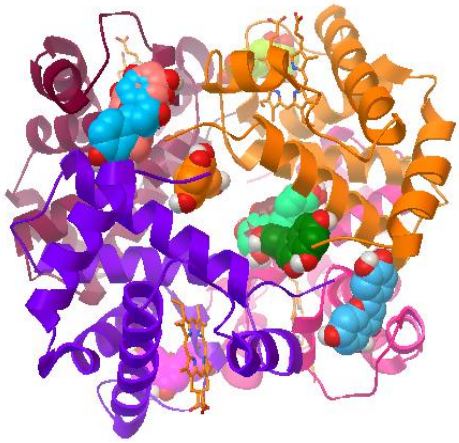

**F. Binding interaction**



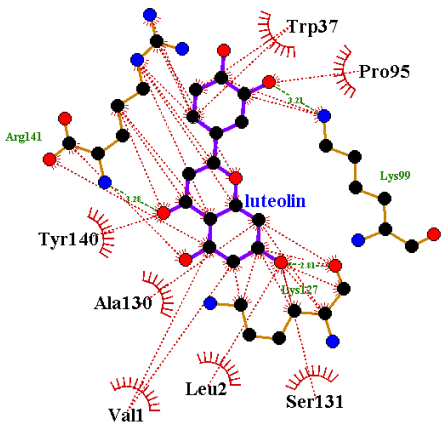
**G. Luteolin-7-rutinoside docking**




**H. Binding interaction**



**I. Luteolin docking**

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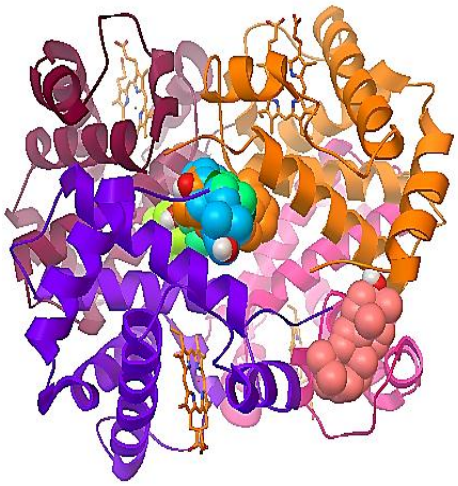
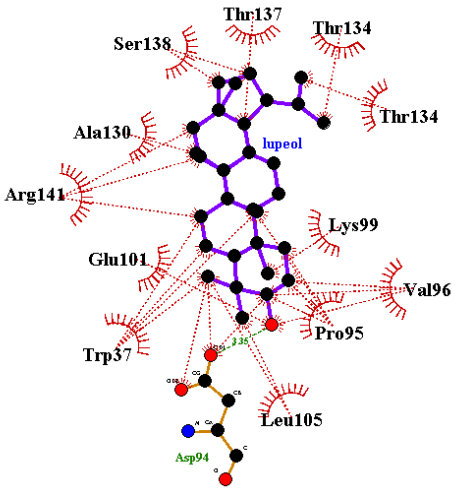
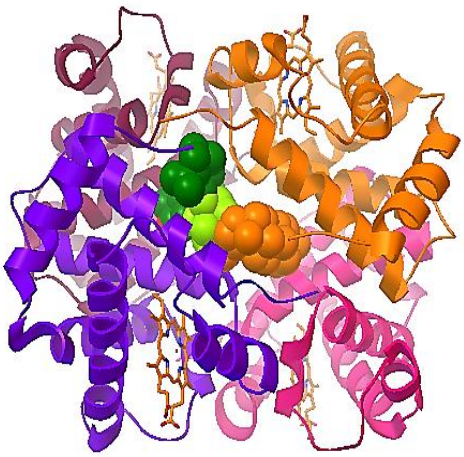


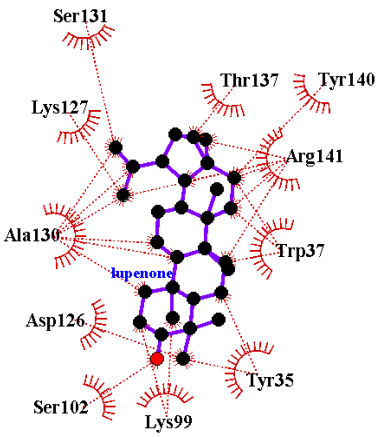
**K. Lupeol docking**



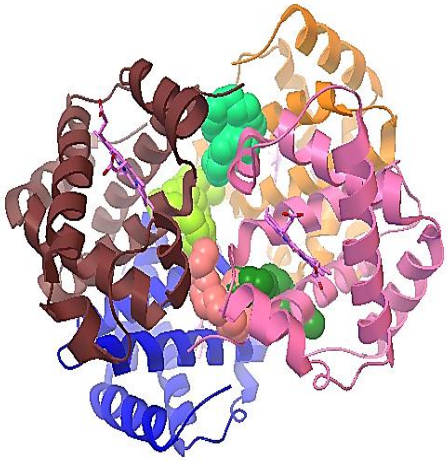

**L. Binding interaction**



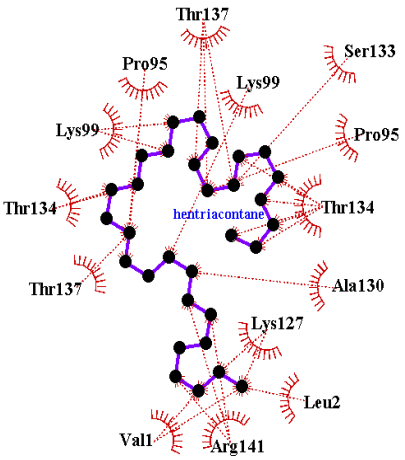
**M. Lupenone docking**



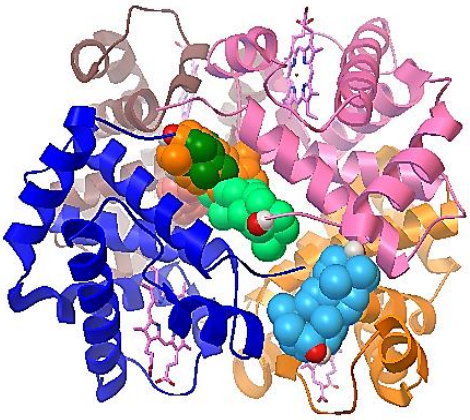

**N. Binding interaction**



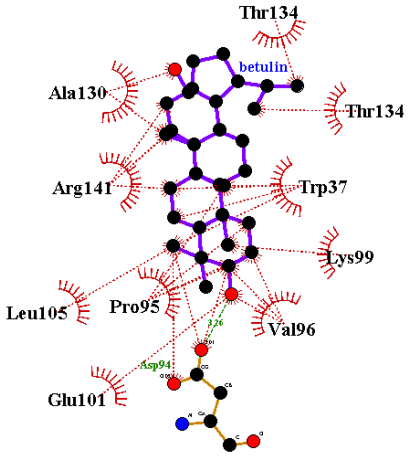
**O. Hentriacontane docking**



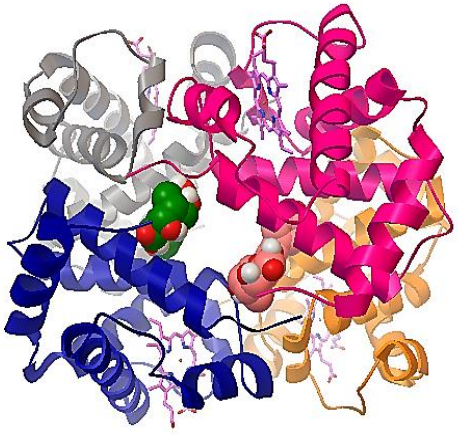

**P. Binding interaction**



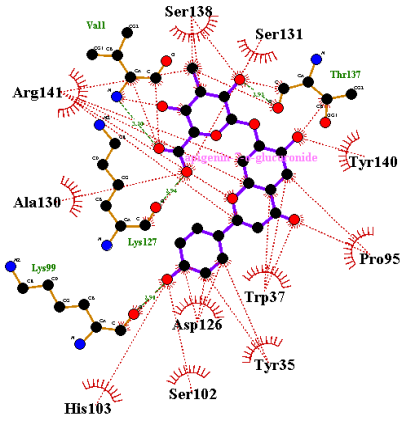
**Q. Betulin docking**



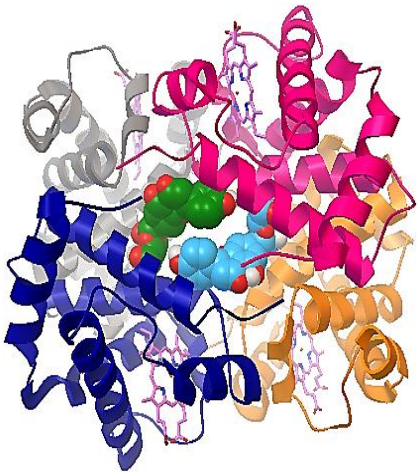

**R. Binding interaction**



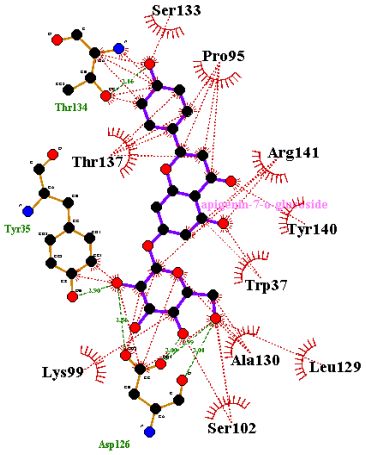
**S. Apigenin-7-O-glucuronide docking**



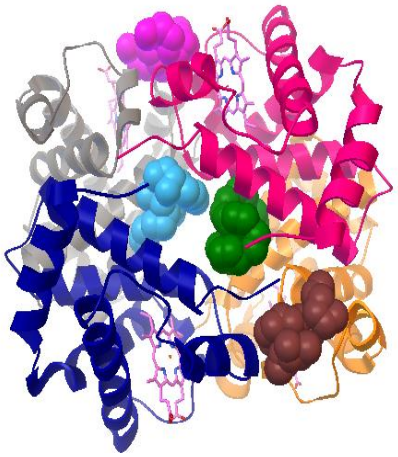

**T. Binding interaction**



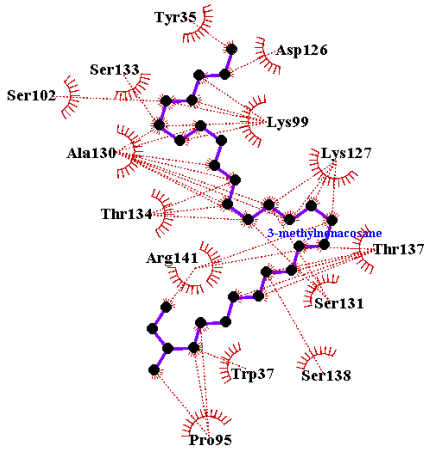
**U. Apigenin-7-O-glucoside docking**




**V. Binding interaction**



W. 3-methylnonacosane docking




X. Binding interaction

**Fig. 3. (A, C, E, G, I, K, M, O, Q, S, U and W) Docking of individual phytochemical (ligand) binding with T-state haemoglobin (receptor) and (B, D, F, H, J, L, N, P, R, T, V and X) 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)**

In case of other compounds viz. lupeol, luteolin, luteolin-7-rutinoside, apigenin-7-O-glucuronide and apigenin-7-O-glucoside have showed inside the central cavity same as benzafibrate binding with T-state haemoglobin. It was found luteolin, luteolin-7-rutinoside, lupeol, betulin, apigenin-7-O-glucuronide and apigenin-7-O-glucoside have observed 3, 9, 1, 1, 4, 6 nos. respectively of hydrogen bonds with haemoglobin during interaction. It was observed from schematic diagram, the hydrogen bonds formation involved with particular residues for each phytochemical, viz. luteolin: Arg141, Lys99 and Lys127, luteolin-7-rutinoside: Thr137, Asp126, Asp168, Asp94, Val96 and Lys99, lupeol and betulin: Asp94, apigenin-7-O-glucuronide: Thr137, Val1, Lys127 and Lys99 and apigenin-7-O-glucoside: Asp126, Tyr35 and Thr134 respectively. The hydrophobic interactions play a vital role in binding with haemoglobin for all the studied compounds. The residues were obtained for hydrophobic interactions in each compound Leu105, Trp37, Ser105, Arg141, Ala130, Arg104, Pro95, His103 and Ser133 for luteolin-7-rutinoside, Trp37, Tyr140, Ala130, Pro95, Val1, Leu2 and Ser131 for luteolin, Thr137, Ala130, Arg141, Glu101, Lys99, Val96, Trp37 and Leu105 for lupeol, Thr134, Ala130, Arg141, Glu101, Lys99, Val96, Trp37, Pro95 and Leu105 for betulin, Ser138, Ser131, Ser102, His103, Tyr140, Tyr35, Ala130, Asp126, Arg141, Trp37 and Pro95 for apigenin-7-O-glucuronide and Ser133, Thr137, Ser102,

Tyr140, Ala130, Lys99, Leu129, Arg141, Trp37 and Pro95 for apigenin-7-O-glucoside respectively.

It was also studied that L35 compound and hydrophobic interactions were also noted in central cavity of haemoglobin. The hydrogen bonds were obtained 4 nos. and connected with residues such as Asp94, Val96 and Lys99. The residues were observed for hydrophobic interaction as Pro95, Leu105, Arg141 and Trp37 respectively (Fig. 4a and b).

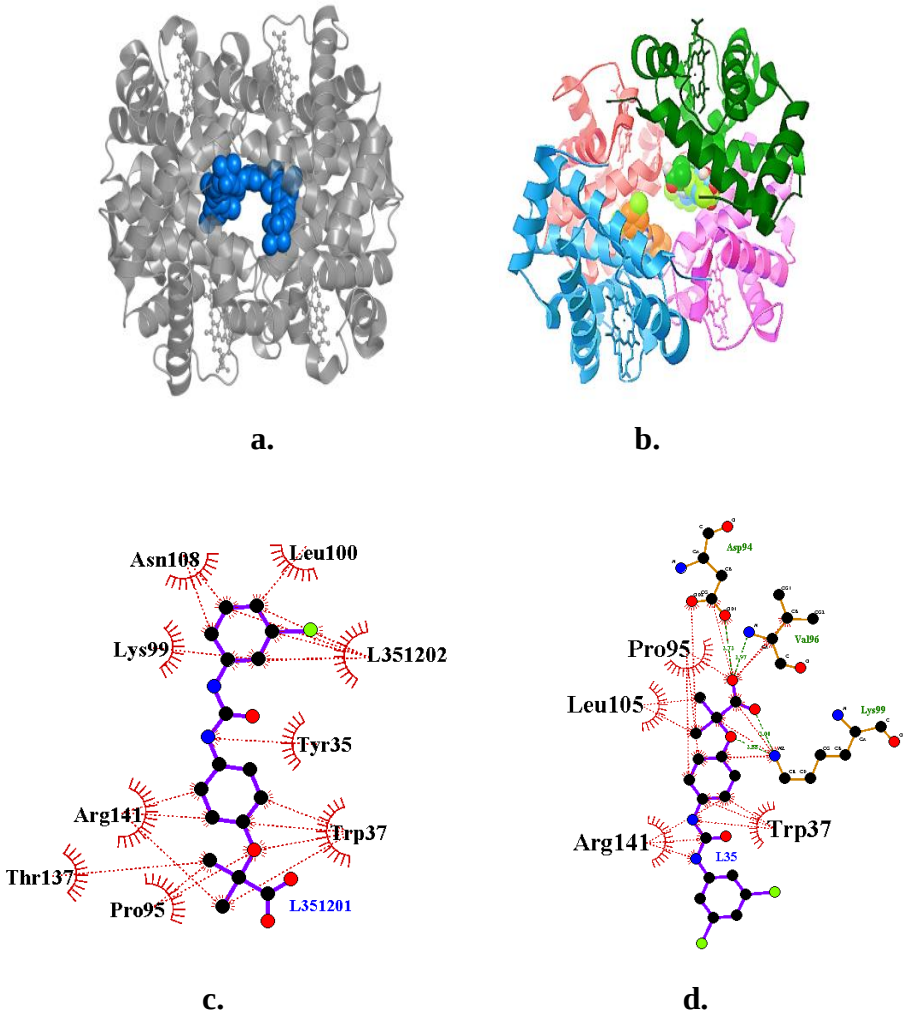



**Fig. 4. Comparison between crystal structure with L35 and L35 docked compound with T-state haemoglobin (a and b) and protein-ligand interaction schematic diagram through LigPlot (c and d) through LigPlot ( = Ligand bond; = Non-ligand bond; = Hydrogen bond with length; = Non-ligand residues involved in hydrophobic contacts; = Corresponding atoms involved in hydrophobic contacts; = hydrophobic connections)**

There were not any major deviation when compared the binding interactions between previously docked crystal structure with L35 binding was retrieved from http://www.ebi.ac.uk/pdbe/entry/pdb/2d5z/bound/L35and freshly docking with L35 compound along with T-state haemoglobin (Fig. 4 a, b, c and d).

Moreover, few phytochemicals viz. flavonoids, phenols and terpenoids of this plant show potent haematopoietic efficiency by haloperidol iron deficiency anaemia in albino rat.[22-23] According to them, ethanolic extract of whole plant has been induced RBC count, haemoglobin, haematocrait, serum iron and protein. These phytochemicals may act as erythropoiesis-inducing agents (EIAs) by showing central cavity binding of T-state haemoglobin in the present work because EIAs help in the management of anaemia clinically.[24] It is well known by several reports that L35 compound individually or combined with IHP exhibit potent heterotropic allosteric effect on the T-state haemoglobin,[16-17] which supported the present observation and these phytochemicals can be further studied for drug designing and development to prevent anaemia and other haematological diseases. Also these compounds may affect the process of haematopoiesis and/or globin/haem biosynthesis.

This is a computer prediction work and the phytochemicals of *H. spinosa* have already been studied experimentally to prevent many diseases[9,10,25,26] and used as folk medicine but no one has attempted before the molecular docking approach that few compounds and/or particular compound can be utilized for drug designing, development and therapeutic efficacies for particular protein target.

Thus, an approach to molecular docking in relation to receptor-ligand binding affinity can be a suitable screening method prior to identify the efficacy of exact compound having allosteric effect. In the present study, it was observed that available phytochemicals from *H. spinosa* can be used in future drug designing and development to prevent anaemia at a low cost therapy. This present work also helps to identify compound for drug as disease specific because *H. spinosa* has contained potent natural ingredients to prevent several diseases.[26]

## CONCLUSION

In conclusion, the present preliminary study on molecular docking indicates important phytochemicals of *H. spinosa* that can be used in future lead compound(s) for drug designing and development to prevent anaemia. The results reveal that specific compounds has heterotropic allosteric effect on the receptor (T-state haemoglobin) when compared to L35

compound, a derivatives of benzafibrate.[16-17] However, further study on the binding of these compounds with R-state haemoglobin and also experimental studies are required to confirm these results of this computational prediction prior to drug design with present phytochemicals of *H. spinosa* because traditionally people use this plant to prevent anaemia.

**Conflict of interest**

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

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