

# Unravelling promise of Indian herbal compounds as potential COVID-19 therapeutic agent

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## Research Article

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# Abstract

COVID-19 pandemic, an unprecedented devastation, humanity needs an urgent cure to save the mankind from this deadly disease. Over six million people have been infected worldwide, with 6.3% reported deaths till date. SARS-CoV-2 virus, responsible for Novel Coronavirus (COVID-19) disease has been isolated recently and the vaccine's development is at nascent stage. At present, there are a few anecdotal evidences that anti-viral/anti-inflammatory/anti-malarial drugs can mitigate the disease.

In the present study, we envision the potency of traditional Indian medicinal compounds that can be used as an effective drug. The viral SARS Coronavirus E protein plays a key role in virus life cycle and can be a potential drug target for the development of anti-SARS-CoV-2 drugs. Using the crystal structure of the CoV-E protein, we performed virtual PyRX screening of Indian medicinal compounds which are reported to have efficacy in the treatment of some viral infections. Molecular docking studies were evaluated based on scores analysed by CavityPlus.

The herbal compounds used were found to be more efficient in inhibiting the virus as compared to commercially available drugs. The results showed that  $\beta$ -boswellic acid and Glycyrrhizic acid possessed the best binding as a ligand with target molecule having binding affinity of -9.1 kcal/mol amongst eleven compounds screened.

The study demonstrated that these are found to be strong SARS-CoV-2E protein inhibitors as they revealed compatible, near perfect dock in the overlapping region of functional viral protein pockets. These potential hit compounds can pave a way for designing of anti-viral therapeutics.

## Introduction

### Highlights:

- The viral SARS-CoV-2 E protein can be a potential drug target.
- Screening the potency of Indian medicinal compounds as an effective inhibitor/drug.
- $\beta$ -boswellic acid and Glycyrrhizic acid potential hit bioactives for anti-COVID-19.
- Herbal compounds found to be more efficient as anti-viral therapeutics

Emergence of sudden predawn of highly infectious deadly disease casted a shadow all around the world. Officially known as SARS-CoV-2, it is the third strain of coronavirus found to be inflicting humans tremendously with COVID-19 after Severe Acute Respiratory Syndrome (SARS) and Middle East respiratory syndrome (MERS) (Yang et al. 2020).

Pandemic outburst which began from China's Wuhan, drifted its way to all over the globe. The fear of 2019 novel Coronavirus (2019-nCoV) kept soaring, gushing its path of infection to more than 200

countries. The journey of the virus started on 17<sup>th</sup> November from the city of Wuhan in Hubei province (Davidson 2020). With Thailand emerging as second nation confirming COVID cases, South Korea and Japan also were in the deadly trap by January 20. The next day, the USA with Washington State falling to its prey (Wood et al. 2020). January 24 marked the entry of European nations starting from France, capturing Italy by February 1.

The deadly scare of SARS-CoV-2 virus kept rising, though not taken seriously initially by World Health Organization (WHO), dismissing it as too early to call an emergency, however, later declared a global public health emergency by WHO chair on January 23, 2020. COVID-19 took huge toll on human lives all around the world within no time was announced as a pandemic on March 13, 2020 (Wu and Chiwaya 2020).

The COVID-19 took 111 652 lives amongst the 1 773 084 confirmed cases as on 13 April 2020 according to data compiled by WHO (2020a).

### **Structure of Coronavirus**

The novel coronavirus is of animal origin mutated and then inflicted humans with devastating outcome (Westerbeck and Machamer 2020). The mode of transmission is through droplets of saliva or discharge from the nose of the infected person via coughs or sneezes to get through and attack the respiratory system (WHO 2020b; Cascella et al. 2020). The structure of coronavirus revealed that it carries three to four proteins in their envelopes (Fig.1 created with BioRender.com). The most abundant is type III glycoprotein M, composed of a short amino-terminal ectodomain, three transmembrane domains, and a long carboxy-terminal tail on the inside of the virion (De Haan et al. 2000). The E protein is a minor but essential component of virus. The CoV envelope E protein is an integral and small membrane that form ion channels-viroporin. It is pentameric in nature consisting of A, B, C, D, and E chains. The C-terminal domain is mostly  $\alpha$ -helical. It features a single hydrophobic domain (HD), which is targeted to Golgi membranes for virion release and secretory pathway cascade (Schoeman and Fielding 2019).

The trimeric spike (S) protein forms the characteristic viral peplomers which are responsible for cell entry via virus-cell attachment and fusion. A subset of coronaviruses comprises a hemagglutinin-esterase (HE) protein, which exists as a disulfide-linked homodimer (De Haan et al. 2000; Schoeman and Fielding 2019; Collins 2020).

### **Mechanism of transmission of Coronavirus**

The coronavirus replication cycle is depicted in Fig. 2 (created with BioRender.com). The replication cycle begins with virus entering the host cell via membrane fusion endocytosis. Release of viral genome occurs for multiplying and make copies. Viral structural proteins gets translated to lead way for maturation of virions pathway at the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). The secretory pathway is regulated by the pH gradient, the cargo efflux is mediated by exocytosis. The main driving

force, which gives the dramatic effects on this secretory pathway is regulated by CoV E proteins-cationic activity of viroporins (Westerbeck and Machamer 2020; Schoeman and Fielding 2019).

The significance of coronavirus envelope proteins is that it carries the structure of a conserved Golgi complex-targeting signal in it, it is a conserved region. It is essential for critical virus life cycle aspects-from production to maturation (Schoeman and Fielding 2019). The process of assembly, budding, envelope formation, and pathogenesis are being regulated by E proteins (Westerbeck and Machamer 2020). The E protein is region where interactions with other CoV proteins and host cell proteins occurs. Hence, it stands as a very crucial link in COVID-19 transmission.

To contain spread of highly contagious COVID-19, the researchers are looking for a solution to treat the COVID infection. Unfortunately, till date, no medications or vaccines exist to cure COVID-19. Even a small promise of the therapeutic agent will be a boon, which is being sought after by scientists. Reports of usage of antiviral drugs, hypertensive medicines or even anti-inflammatory drugs or ACE inhibitors along with angiotensin-receptor blockers (ARBs) on account of preconceived notion and experience gained from SARS and MERS are still not recommended by any recognised regulatory body anywhere in the world that include the WHO or Centers for Disease Control and Prevention (CDC) (Collins 2020; Stebbing et al. 2020; Day 2020).

Without any clinical trials in humans, the approval of these medications without any testing is not advisable by clinicians, as they could lead to unintended and detrimental consequences. Hence, it would be too early to support or refute these claims.

The need of the hour is to dig into the basic fundamental aspects of virus structure, mechanism underlying its devastating spread, which would be vital in guiding, designing and developing effective drugs that can stop the pandemic which has become a global health crisis.

Based on studies on SARS, MERS and Ebola outbreaks, scientists are predicting that the conventional anti-viral drugs could be of benefit in curing the COVID-19 as well.

There are a few principal inhibitors which are also considered as promise for disease control for angiotensin converting enzyme 2 (ACE2), a human cell surface proteins which is the place of entry of virus via spike protein binding to that receptors (Collins 2020).

The urgency is to come up with an alternative solution to knockdown the level of COVID-19 grip. The present study deals with screening of the promising antiviral drugs from traditional Indian herbal compounds which can become the next drug to cure the COVID-19. The molecular docking insights would give a deep insight that will provide a basis for the design and development of therapeutics that specifically target this critical interaction of ligand (drug)-target (COVID proteins). Hence, this study proves to give a promising approach for prevention of COVID, and design of vaccines targeting the critical envelope (E) protein of coronavirus.

Understanding the role of the CoV E protein in virus is thus a paramount prerequisite for potential vaccines as well as in identifying novel antiviral therapeutics.

## Materials And Methods

### Indian Herbal reservoirs

Plant compounds were searched from library of Indian plants named IMPPAT database and the compounds, their structures were taken from PubChem (Mohanraj et al, 2018). All the structures were converted from Smiles strings to Pdb file format using Chimera software tool 12.1.

### Structure of SARS Corona virus Envelope protein

Input file was searched from Protein Data Bank (PDB) with PDB Id 5X29. It is envelope protein of SARS Coronavirus. Input file for ligands were constructed using UCSF Chimera software 12.1 (Pettersen et al. 2004). Computational studies for cavity and the mechanism of attachment with residues binding of the ligand on proteins were done using software tool CavityPlus (Xu et al. 2018).

Docking studies of target molecules with envelope proteins of coronavirus was performed using PyRX virtual screening tool (Dallakyan and Olson 2015). The input file format was pdb file. Binding affinity of the ligand with target proteins were studied and compounds with better binding affinity were determined.

## Results And Discussion

In our study, medicinal compounds were selected from traditional herbal plants mainly prominent in India and Indian sub-continent. These compounds were screened on basis of their indigenous antiviral and anti-inflammatory properties. As most of the herbal plants possess anti-inflammatory properties which undoubtedly fits into the cause to target corona proteins. These compounds are reported to be effective against asthma and bronchitis, thus, they can be promising anti-viral drugs for curing corona patients. Owing to fact that these compounds are used in the treatment of various respiratory diseases which are the primary target in COVID-19 infections. The conventionally used commercial anti-viral drugs in the clinics were also taken into accounts to provide a relevance to the present study by comparing the natural versus synthesised drug's efficacy. To get insights into the structure of the envelope proteins, the cavity/pocket required for catalytic sites were studied using CavityPlus software. The target protein had two cavity/pocket which were found to be essential for catalysis. Ligand binding site was observed on the site present on the cavity. The drug target binding affinity prediction was calculated in pKd terms where values more than 6.0, had been termed as druggable. Values near or lesser than 6.0 determined the poor drug affinity to the target. Fig 3 shows the cavities and pocket of the proteins which could be targeted by the ligands. Further to it, studies were carried out to predict the orthosteric or allosteric site present in the target molecules. As per our findings, there was no allosteric pockets detected and thus, confirming that the binding had taken place at the orthosteric site as reflected in Fig 4. Binding at orthosteric site gives information about the nature of the ligand binding. For orthosteric binding, ligand

will be used at lower doses and tends to have better binding at the active site of the protein (Nussinov and Tsai 2012). Although, all the compounds screened were taken from herbal plants which are considered safe for medicinal use. The study also revealed that the covalently modified Cystine residues played the most important role in the active site binding. It displayed lower pKa values and higher solvent exposure. However, there were no covalently modified Cystine residues found in the catalytic residues of the protein. But certain unmodified cysteine residues were present at active site binding shown in supplementary material (Fig. S1).

We analysed the docking of ligand with target molecules using Autodock Vina to get better overview of binding affinity when outcome was established along with Root Square Mean deviation values (Trott and Olson 2010). The binding affinity should be higher and more than 6.0. Contrary to expressing high values by binding affinity, RMSD values need to be lowest which proves the best fit result. Table 1 shows the binding affinity of the selected plant compounds. Docking and binding affinity of the compounds with target protein were shown in Fig 5.  $\beta$ -boswellic acid present in Indian plant- *Boswellia serrata* was found to be the best anti-viral compound among screened ligand for drug targeting of the protein and it was also studied for cavity binding of the protein as shown in Fig 6. The results showed that this ligand molecules could be druggable and best suitable. Apart from these compounds, commercially available drugs like Azithromycin and Hydroxychloroquine which are proposed to be effective remedy against COVID-19 disease were also taken up as reference/control, screened and compared with medicinal herbal compounds.

The present study showed that medicinal compounds were having greater efficacy and far better binding affinity than these drugs for inhibiting coronavirus. While binding affinity of proposed drugs; Azithromycin denoted -5.9 kcal/mol and Hydroxychloroquine had -6.4 kcal/mol values, whereas our potential best antiviral compounds - 9.1 kcal/mol, performed far way better in docking studies.

Hence, the study proved that conventional medicinal compounds could be the most promising anti-viral therapeutics rather than relying on available anti-viral drugs.

## Conclusions

The pandemic outbreak of deadly viral attack globally by SARS-CoV-2 has apparently no drugs for its treatment and cure. The present study was carried out to look for potential anti-viral therapeutic agents to combat COVID-19. Molecular docking studies were conducted to find out from traditional Indian medicinal herbal compounds. Out of eleven compounds,  $\beta$ -boswellic acid (*Boswellia serrata*) was found to be best suitable along with Glycyrrhizic acid (*Glycyrrhiza glabra*). Being medicinal compounds, their efficacy was found to be higher than conventionally available anti-viral, anti-inflammatory and anti-malarial drugs. With the advantage of being natural source, it features no harmful side effects, these novel compounds make a great choice to be used for the treatment of COVID patients. These drugs present an outlook and promising vision which can be applied for developing effective therapeutics.

Hence, further investigations on their clinical use must be undertaken to validate the curative effects of these medicinal drugs to deal the pandemic of SARS-CoV-2.

## Declarations

### Acknowledgements

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### Author contributions

SWF conceived of the presented idea and designed the experimental study along with SA. Execution of computational study was done by SA. SWF and SA both did the analysis and interpretation of data along with writing the manuscript. SKK supervised the findings of this work. All authors read and approved the final manuscript.

### Compliance with ethical standards

**Conflict of interest:** The authors declare that they have no conflict of interest.

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## Table 1

Table 1 shows bioactive compounds with binding affinity with Coronavirus envelope protein

## Figures

### Coronavirus Structure and Protein Visualization

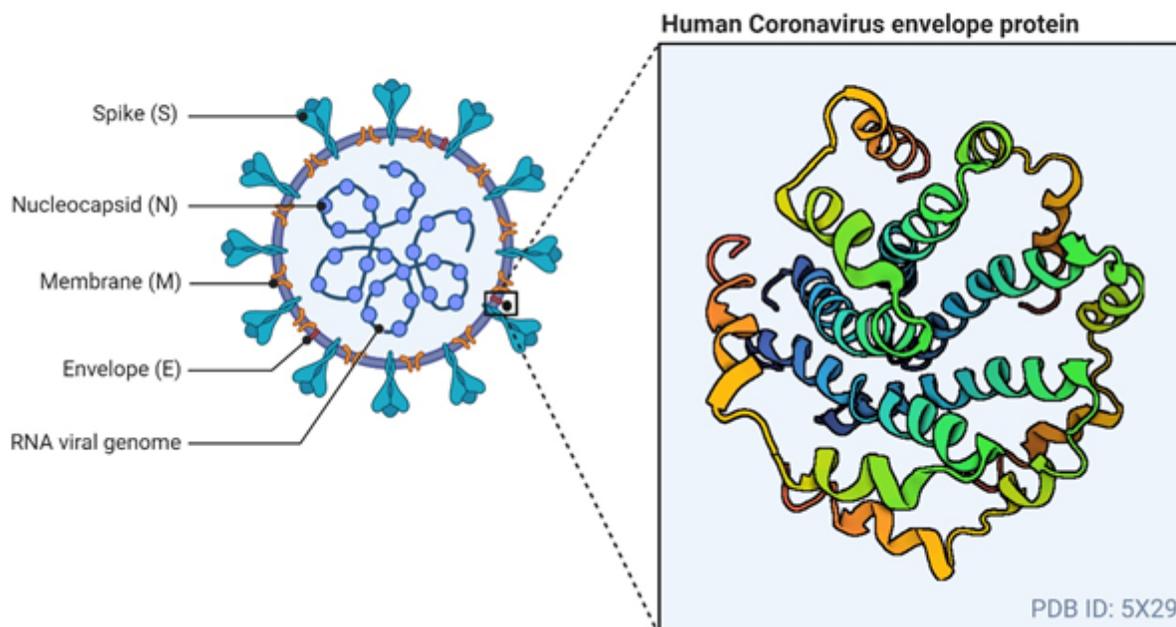
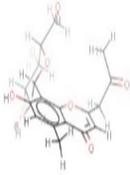
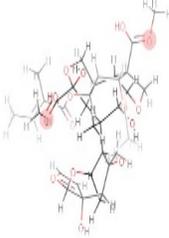
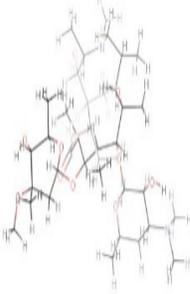
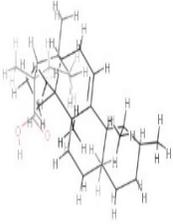
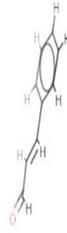


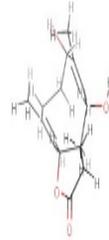
Figure 1

Structure and protein visualization of novel SARS-CoV-2 virus.

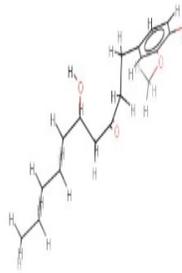
S. No	Name of the compound	Plant Source	Chemical Structure	Binding Affinity (- kcal/mol)	Root Square Mean Deviation (RMSD)
1	Aleosin	<i>Aleo vera</i>		6.4	14.53
2	Azadirachtin	<i>Azadirachta indica</i>		6.8	1.91
3	Azithromycin	-		5.9	1.82
4	$\beta$ - boswellic acid	<i>Boswellia serrata</i>		9.1	1.47
5	Cinnamaldehyde	<i>Cinnamomum verum</i>		6.2	12.18



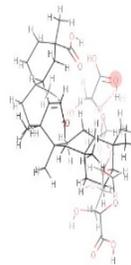
6      Germacranolide      *Artemisia pallens*      6.8      3.14



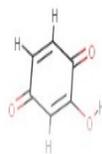
7      Gingerols      *Zingiber officinale*      7.7      1.60



8      Glycyrrhizic acid      *Glycyrrhiza glabra*      9.1      1.85



9      Hydroxychloroquine      -      6.4      4.53



10      Isofraxidin      *acanthopanax*      5.4      2.57



Replication cycle of SARS-CoV-2 virus.

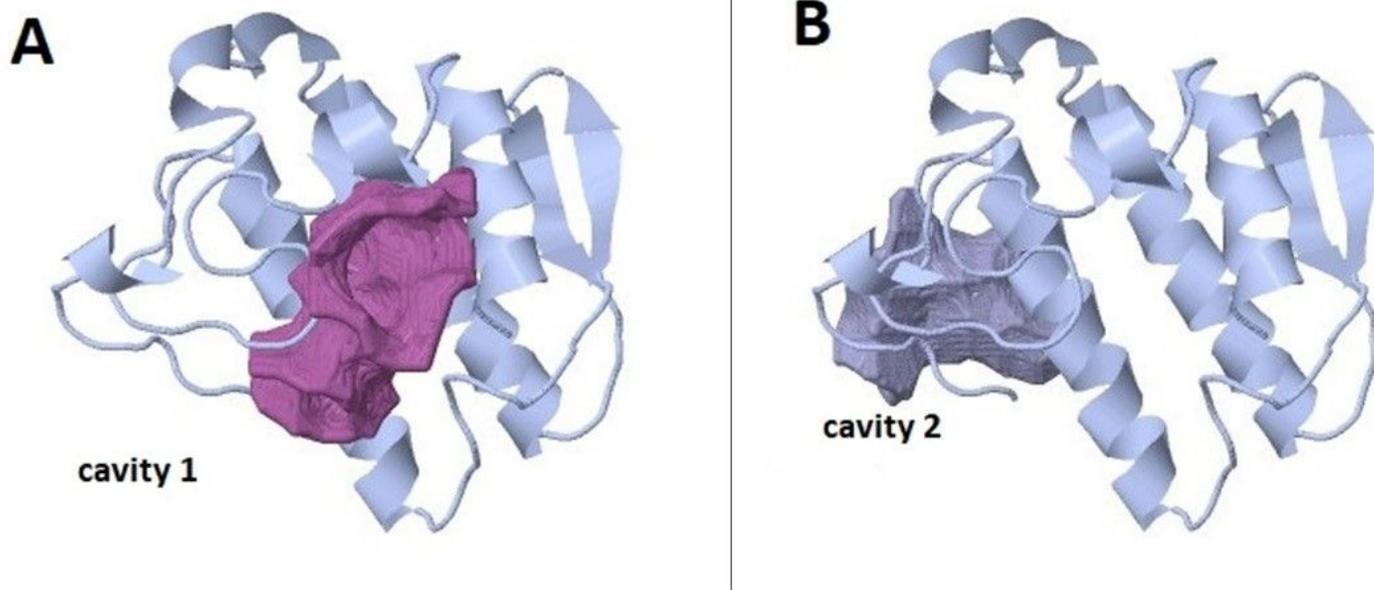


Figure 3

[A] Cavity 1 within protein for ligand binding; [B] Cavity 2 within protein for ligand binding.

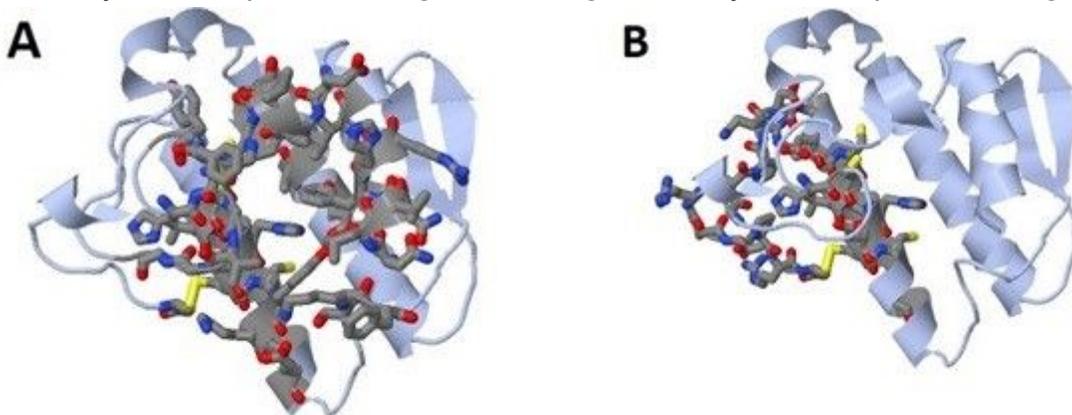


Figure 4

[A] showing orthosteric site of the cavity 1 where most of the drug binding takes place. [B] showing lesser prominent cavity 2 where less ligand binding takes place. It was found that there was no allosteric binding of ligand within cavity 1 and cavity 2.

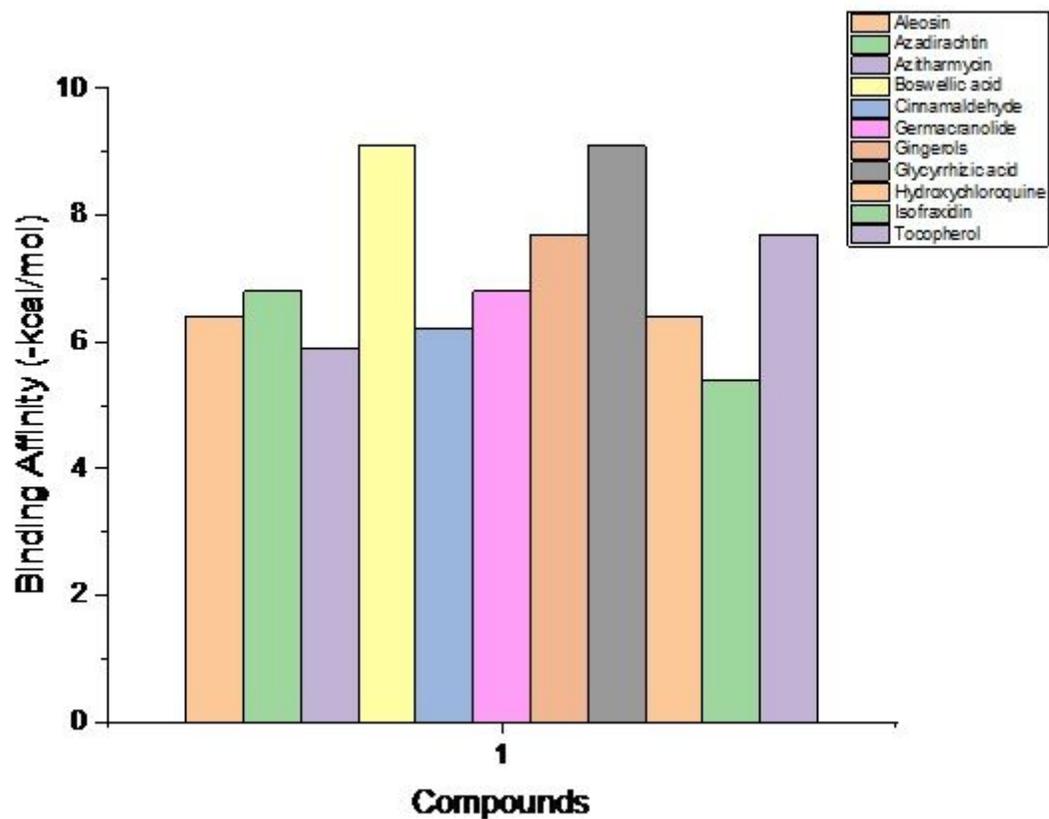


Figure 5

Docking and Binding affinity of the tested compound against envelope protein. B- boswellic acid and Glycyrrhizic acid had best binding with target molecule having binding affinity -9.1 kcal/mol.

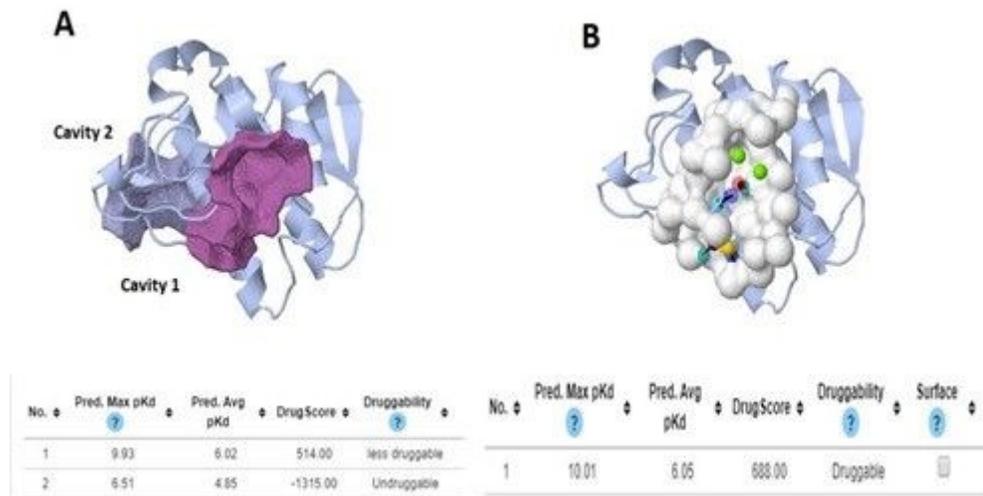


Figure 6

[A] Cavity 1 and 2 of the protein was illustrated in which critical amino acid residues bind with the ligands. Predicted Max and Ave value (pKd) of the molecule without ligand was found to be 9.93 and 6.02 which is less druggable. Cavity 2 in the protein was not suitable for any drug binding; [B] Cavity1 with ligand binding was predicted with suitable compound ( $\beta$  boswellic acid) and found to be druggable with Max and Ave pKd value of 10.01 and 6.05 respectively.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial3B1.docx](#)
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