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# Virtual Screening of Phytochemicals for Anti - Tubercular Potential Using Molecular Docking Approach

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**Keywords:** Heat shock protein; Mycobacterium tuberculosis; Phytochemicals; ADME Analysis; Molecular docking; Auto dock vina (1.5.6).

**Abstract**

Tuberculosis (TB) is a curable illness, caused by, Mycobacterium Tuberculosis (Mtb), is still significant. Technological progress in TB treatment has been limited. The major therapeutic technique to treat the condition is first line antituberculosis drugs (A fixed-dose, single-tablet combination of four drugs: Rifampin, isoniazid, pyrazinamide, and ethambutol) with almost an efficacy of 95% in susceptible individuals. But, the usage of these drugs is followed by a series of adverse side-effects. In view of the global TB crisis, new medicines that are relatively inexpensive and with lesser side effects for the treatment of this lethal disease needs to be discovered. Plant-derived products have played a significant role in the manufacture of drugs against infectious disease. Antioxidant activities of herbal medicines are also effective in reducing the toxicities of toxic agents or other drugs. The aim of this study was to screen 302 bioactive compounds from four major phytochemical groups, namely antituberculic, antitubercular, antimicrobial and antibacterial against Mtb Heat Shock Protein (hspX). These mentioned activities of plant extracts have been linked to the presence of some bioactive compounds or secondary metabolites that provide protection to the plants themselves against bacterial, fungal and viral infections. The phytochemicals, which were selected from Dr. Dukes Phytochemical Library, were subjected to ADME analysis based on compliance with the Lipinski's rule of five. Molecular docking analysis was conducted using Auto Dock Vina (1.5.6) using the hspX protein as the receptor. Glycyrrhetinic acid (-11.3 kcal/mol), Asarinin (-9.9 kcal/mol), 1,2,4-Trihydroxyheptadeca-16-ene (-7.7 kcal/mol), Guttiferin (-7.7 kcal/mol), Medicarpin (-7.6 kcal/mol), (+)-Galbacin (-7.3 kcal/mol), 1-Ethyl-Beta-Carboline (-7.1 kcal/mol) were found to potentially bind and inhibit the receptor, in comparison to the other selected inhibitors like Catechin (-6.8 kcal/mol) Gossypetin (-6.8 kcal/mol), Cosmosin (-6.7 kcal/mol), Chelirubine (-6.3 kcal/mol), etc.

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The Swiss ADME Tool was used to recover the bioavailability radars of ligands with the least binding scores. Glycyrrhetinic acid, Asarinin, and Medicarpin resulted to be orally bioavailable. Hence, they represent the opportunity of in vitro biological evaluation and possible application in TB treatment research.

## Introduction

Tuberculosis (TB) is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS) [1]. Despite attempts to eliminate TB, figures about the disease are still appalling. The major cause of the rise in new TB cases in Africa is HIV infection. It has been estimated that 5-10% of infected people are sick in their lifetimes, according to WHO estimates [1].

Recent advances in comprehensive analytical techniques, such as transcriptomics and proteomics, have enabled us to identify proteins associated with active TB in humans. Mtb primarily emerges in the host macrophage upon infection, within an endocytic vacuole called the phagosome. The pathogenic mycobacteria inhibit phagosome-lysosome fusion. Lack of phagosome maturation containing pathogenic Mtb has been generally recognised as a key factor in the survival of mycobacterial pathogens within macrophages [2]. The bacterium reacts to stress conditions through large genome changes in gene expression, including the induction of a transient expression of a well-preserved set of heat shock or heat stress protein encoding genes [3].

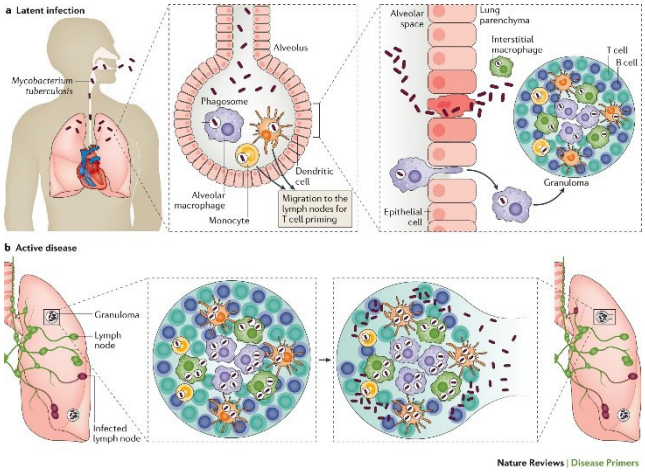



**Figure 1:** Mycobacterium tuberculosis infection: Pathogenesis [4].

a) Infection occurs when Mycobacterium tuberculosis reaches the lungs by inhalation, travels to the alveolar space, and comes into contact with the resident alveolar macrophages. If this first line of defence fails to remove the germs, Mtb penetrates through the lung interstitial tissue, either directly infecting the alveolar epithelium or moving to the lung parenchyma via infected alveolar macrophages. Dendritic cells or inflammatory monocytes then transfer Mtb to pulmonary lymph nodes for T cell priming. This triggers the migration of immune cells, particularly T and B cells, to the lung parenchyma, where they form a granuloma. b) The bacteria multiply within the developing granuloma. If the bacterial burden grows too high, the granuloma will be unable to control the infection, and germs will eventually spread to other organs, including the brain. At this point, the bacteria can reach the bloodstream or re-enter the respiratory system to be discharged; the diseased host is now infectious, symptomatic, and has active tuberculosis illness.

Mtb Heat Shock Proteins (hspX) had gained a lot of attention because it appears to be one of the main immunologically active mycobacterial antigens after infection and is expressed abundantly during intracellular survival adaptation [5,6] A high degree of hspX conservation suggests the fundamental role played by these proteins in cellular processes [7]. The expression of hspX is significantly increased under stress conditions such as hypoxia, starvation of nutrients and oxygen radical's hspX have cytoprotective functions and maintain cellular Organisation and homeostasis under stress conditions in both eukaryotic as well as prokaryotic cells. In previous studies, different scientists have shown that it is the most abundant protein found in the latent stage of Mtb infection using in vivo models.

In vitro survival of Mtb under oxidative stress is a crucial feature of its pathogenesis. HspX are necessary molecular chaperones for the proper functioning of cells. Mycobacterium pathogenicity is reliant on the expression of several genes that are expressed inside macrophages. HspX are implicated in antigen presentation, lymphocyte activation, and macrophage and macrophage cell activation in TB infection, according to several studies. HspX may potentially have a direct role in Mtb pathogenicity [8]. Despite significant progress in understanding the structure and function of hspX, the biological importance of these proteins in bacterial survival inside macrophages remains unknown. These proteins appear to serve a critical function in shielding bacteria from the outside world. For the development of novel anti-tuberculosis medicines and vaccines, a better knowledge of hspX is necessary. Hence, this protein was targeted [9].

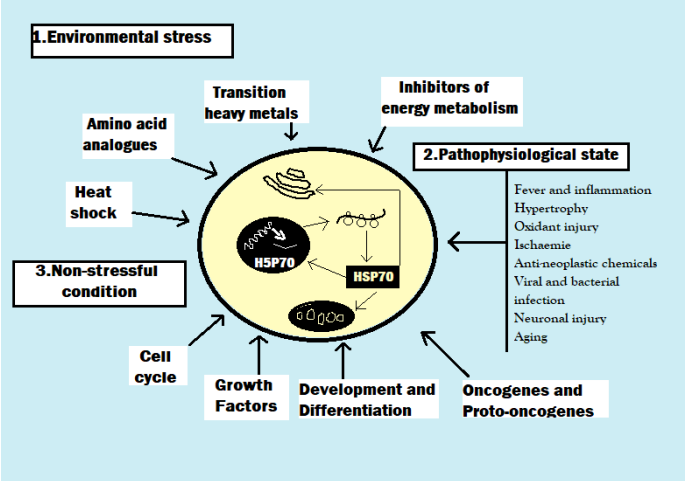



**Figure 2:** Factors inducing expression of heat shock protein in humans [10].

The use of herbal medicinal products and supplements has increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare this is particularly true of many rural communities in Africa, parts of Asia, Central and South America, with accessible and inexpensive plants and knowledge of their traditional uses [11]. The use of anti-TB drugs causes side effects like Allergic reactions, fever, rash, vasculitis, nausea, vomiting, hepatotoxicity, hepatocellular inflammation, peripheral neuropathy and others drugs derived from medicinal plants are advantageous over the synthetic drugs used, for obvious reasons, like the side effect. The use of herbal medicines and phytonutrients or nutraceuticals continues to expand rapidly

across the world with many people now resorting to these products for treatment of various health challenges like TB in different national healthcare settings [12].

Drug discovery is an important field where computational biology and data science have lately gained attraction. Computational approaches have the power to screen hits from a huge database and design novel small molecules. Some computer models thus fairly constitute valid alternatives to experiments. Molecular docking is one such computational methodology, which is a process that predicts the preferred orientation of one molecule to another when bound together to form a stable complex. There are two main types of docking (molecular docking) in practice: small molecule-protein (called "ligand-protein docking") and protein-protein docking. Herewith, the AutoDock Vina (1.5.6) [13] software was used for this study for ligand- protein docking. Awareness of the preferred orientation, in turn, can be used to predict the strength of the interaction or binding affinity between two molecules using score functions [14]. In AutoDock Vina (1.5.6)an empirical scoring function calculates the affinity, or fitness, of protein-ligand binding by summing up the contributions of a number of individual terms Here, we have used the SwissADME web tool, which provides free access to a pool of fast yet reliable predictive models for ADME analysis, and also displays bioavailability radar for a rapid appraisal of drug-likeness [15].

Hence using the mentioned tools, the present research was conducted to discover the potential phytochemicals against TB hspX protein.

## Materials and methods

### Protein and ligand retrieval

HspX21 protein sequence was recovered from the Tuberculist Database (http://genolist.pasteur.fr/TubercuList/) the PDB structure of the same was built using the Swiss-Model Tool (PDB ID: 5NMS).

Dr. Duke's Phytochemical and Ethnobotanical database (U.S. Department of Agriculture, Agricultural Research Service. 1992-2016) was chosen to retrieve the list of several drug chemical compounds. These compounds have already been recognized for its Antitubercular, antituberculic, antimicrobial and antibacterial activities respectively. The three-dimensional structure of these compounds were collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Using the SwissADME software [15] the canonical smiles for all the compounds were also retrieved.

### ADME analysis

SwissADME (http://www.swissadme.ch/) 15], a web-based tool, was used for initial screening purposes. The Lipinski (Pfizer) filter was the pioneer rule-of-five implemented. This section provides access to these five distinct rule-based filters that cover a wide variety of characteristics and determine if a molecule is drug-like.

### Protein and ligand preparation

Protein: The PDB Structure of hspX21 was downloaded from Swiss Model. Using the Auto Dock Vina (1.5.6), the water molecules of the protein were removed; the Kollman charges were added; and followed by the addition of polar hydrogen atoms. This protein structure was then saved in PDBQT format for further analysis.

Ligand: The native SDF format structures of all the test ligands were downloaded from PubChem. Using the PyMol software [16], the ligands were converted to its PDB format. The ligand structures were then saved in PDBQT format using Auto Dock Vina (1.5.6) for further analysis.

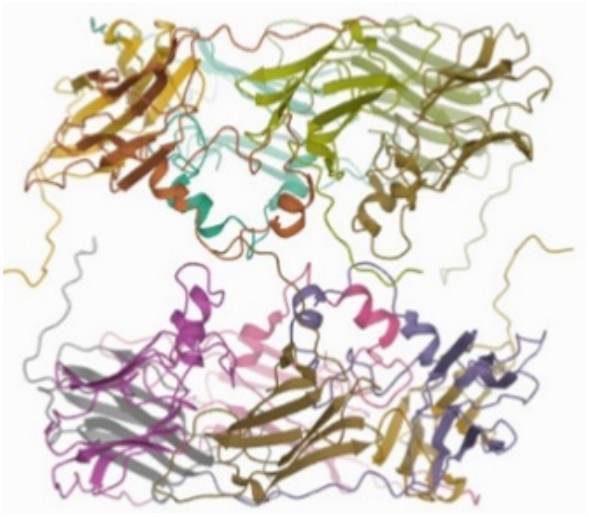


**Figure 3:** Structure of hspX21 (PDB ID: 5NMS).

### Molecular docking

Molecular docking was done using Auto Dock Vina (1.5.6) from the Auto Dock 4 package. AutoDock Vina (1.5.6) calculates the grid maps automatically and collects the data in a concise manner for the user.

Based on their binding position and potential interactions with the main residues, the high-ranking small molecules determined with the binding energy values in the Auto Dock software were considered. After the docking process, the conformations with the lowest connecting affinities (less than -7.0 kcal/mol) were selected to further monitor interacting residues. Using Biovia Discovery Studio Visualizer [17] and Chimera [18] the docking poses were evaluated.

### Bioavailability radar

Out of the 290 ligands bioactives with 2 or more violations of Lipinski's rule of 5 were dropped 8 ligands that portrayed affinity scores less than -7.0 kcal/mol were subjected to bioavailability radar analysis. Six physicochemical properties are taken into account: Lipophilicity, size, polarity, solubility, flexibility and saturation. A physicochemical range on each axis are defined-descriptors and depicted as a pink area [15].

**QR Code Scanning:** The QR code could be scanned to view and synchronize information of the above-mentioned methods, and an internet connection is not required to operate the system. This method has intended to reduce the data load in the article.

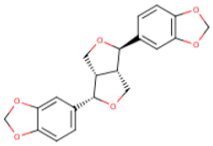
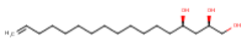
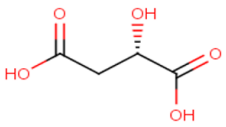
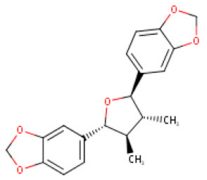
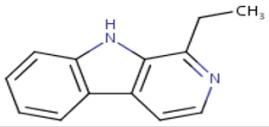
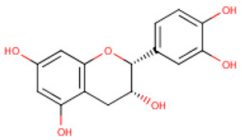
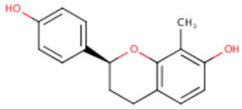
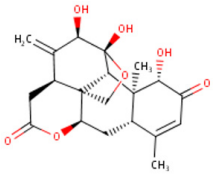
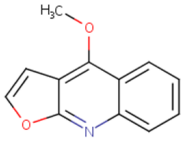
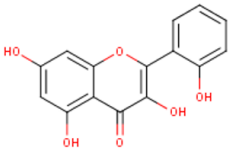
**Figure 4:** How to scan: open, aim + tap.

## Result and discussion

### ADME analysis

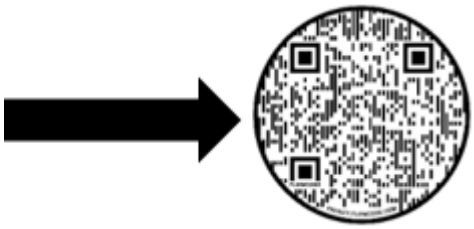
The fact that ligands bind to proteins in an antagonistic manner does not mean they are desirable as drug candidates. Drug development involves assessment of Absorption, Distribution, Metabolism and Excretion (ADME) increasingly earlier in the discovery process. The pioneering study of Lipinski beheld orally active chemicals to determine physicochemical ranges with a high likelihood of being an oral medication (i.e. the drug-likeness). The connection between pharmacokinetic and physicochemical characteristics was defined by the Rule-of-five. As a consequence, ADME is drug-like testing, which assists in making reasonable decisions on whether ligands can be introduced into a living system [19]. It's dependent on four simple physiochemical parameter ranges: The Molecular Weight (MW) to be lower than 500 g/mol, the Lipophilicity (Log P) to be lower than 5, and the number of donors and acceptors of hydrogen bonds to be lower than 5 and 10 [20,21]. Bioactives showing only 0 to 2 violations were considered hence; around 96% of the bioactives were shortlisted, as enlisted in Table 1.

**Table 1:** ADME Analysis Data for 10 representatives (out of 302 ligands).

| Name | PubChem ID | Structure | Molecular Weight | H-Bond Acceptors | H-Bond Donor | I Log P | Lipinski Violations |
|---|---|---|---|---|---|---|---|
| Asarinin | 11869417 |  | 354.35 | 6 | 0 | 3.43 | 0 |
| 1,2,4-Trihydroxyheptadeca-16-ene | 158573 |  | 286.45 | 3 | 3 | 3.72 | 0 |
| Medicarpin | 336327 |  | 134.09 | 5 | 3 | -0.01 | 0 |
| (+)-Galbacin | 442873 |  | 340.37 | 5 | 0 | 3.69 | 0 |
| 1-Ethyl-Beta-Carboline | 5324325 |  | 196.25 | 1 | 1 | 1.96 | 0 |
| Catechin | 9064 |  | 290.27 | 6 | 5 | 1.47 | 0 |
| 7,4'-Dihydroxy-8-Methylflavan | 442361 |  | 256.3 | 3 | 2 | 2.38 | 0 |
| Ailanthone | 72965 |  | 376.4 | 7 | 3 | 1.56 | 0 |
| Dictamnine | 68085 |  | 199.21 | 3 | 0 | 2.38 | 0 |
| Datiscetin | 5281610 |  | 286.24 | 6 | 4 | 1.85 | 0 |

iLogP: Lipophilicity.

(Please scan to view the ADME Analysis all the phytochemicals selected).



**Molecular docking**

The Auto Dock Vina (1.5.6) software performs the prediction of bound conformation based on free binding energies, which is calculated on the basis of the empirical force field. Vina calculates its own grid maps quickly and automatically [13]. It also clusters and ranks the results. Since molecular docking helps to assess the conformation of ligands within the binding site with a high degree of accuracy, it has been an important method in drug predictions [22].

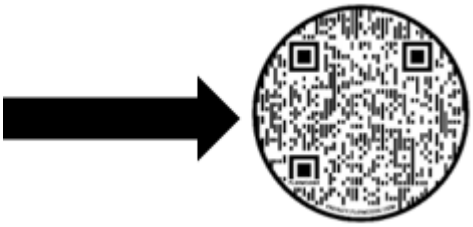
Using Auto Dock Vina (1.5.6), docking of 290 ligands that satisfied the conditions in the ADME Analysis was done. The binding affinity score replicates the potential energy change, when the protein and ligand come together [23]. This indicates that a highly negative score relates to a strong binding and a less negative or even positive score refers to weak or non-existing binding.

Hence, the ligands with the least binding affinity scores (here, less than 7.0 kcal/mol) were considered for further study (Table 2).

**Table 2:** Binding Affinity Scores of 6 representative bioactives.

| Sr No. | Ligands | Binding Energy (ΔG) (kcal/mol) |
|---|---|---|
| 1 | Glycyrrhetinic acid | -11.3 |
| 2 | Asarinin | -9.9 |
| 3 | 1,2,4-Trihydroxyheptadeca-16-ene | -7.7 |
| 4 | Guttiferin | -7.7 |
| 5 | Medicarpin | -7.6 |
| 6 | (+)-Galbacin | -7.3 |

(Please scan to view Binding Affinity Scores of all the Ligands with less than 2 Lipinski Violations).



Glycyrrhetinic acid, which has known antibacterial properties exhibited the least binding affinity score of all the molecules (-11.3 kcal/mol) (Figure 5). It is the triterpenoid aglycone of glycyrrhizin, the principal licorice component [24]. It is used topically for allergic or infectious skin inflammation and orally for its aldosterone effects in electrolyte regulation [25].

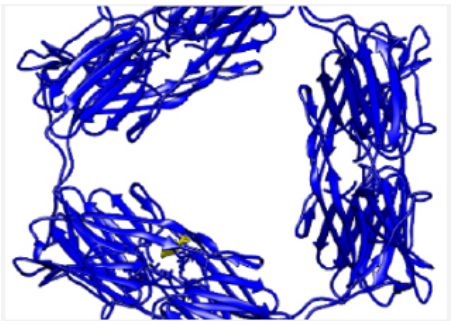
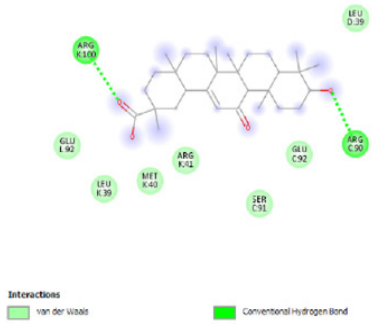
Asarinin (-9.9 kcal/mol) is an antibacterial Diterpenoid, found in Asarum species [26]. Asarinin displays quite a lot of biological activities such as decreasing cholesterol levels, exhibiting antihypertensive and antiangiogenic properties and providing immunosuppressive, hepatoprotective activities (Figure 6) [27].

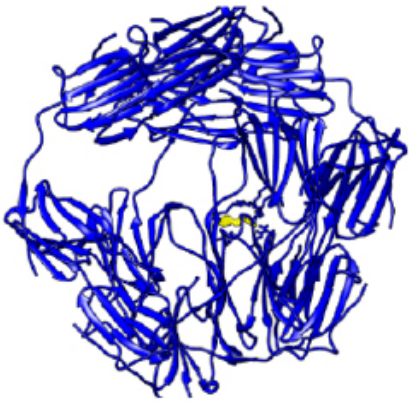
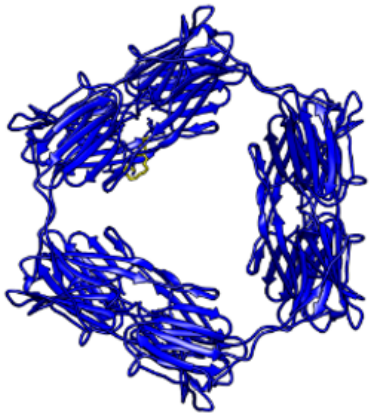
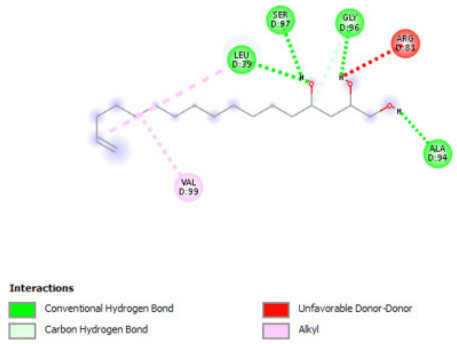
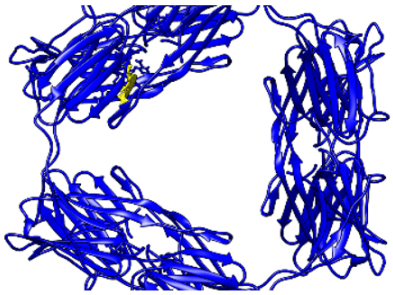
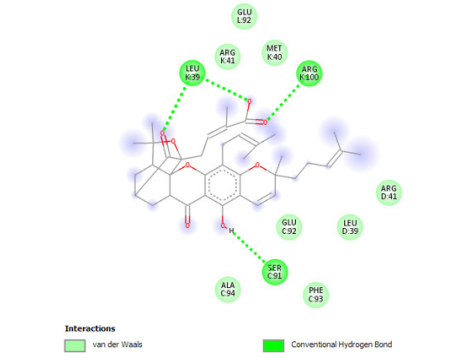
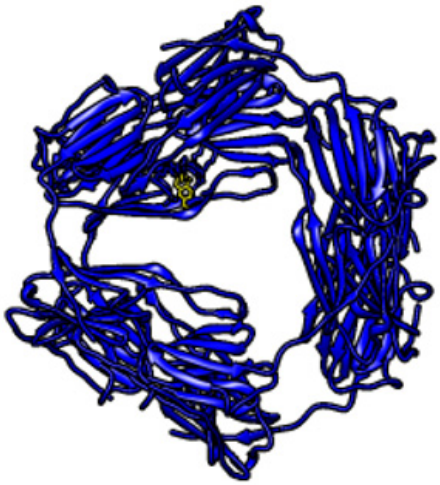
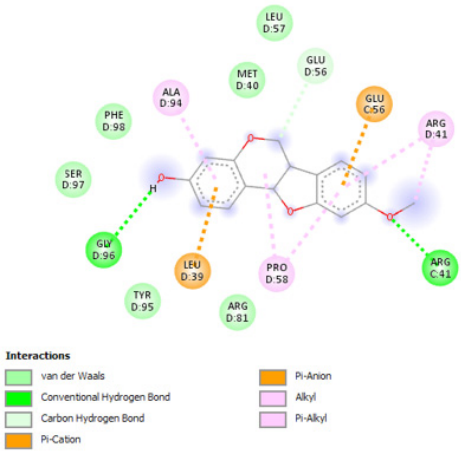
Persea americana (Avocado) contains 1,2,4-Trihydroxyheptadeca-16-ene (-7.7 kcal/mol) [28]. Avocado fruit extracts are known to exhibit antibacterial as well as antimicrobial properties (Figure 7).

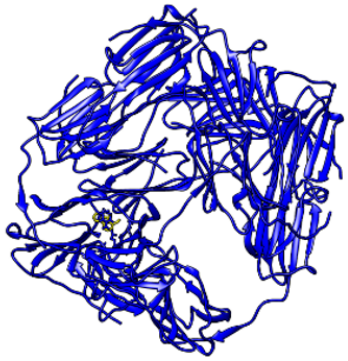
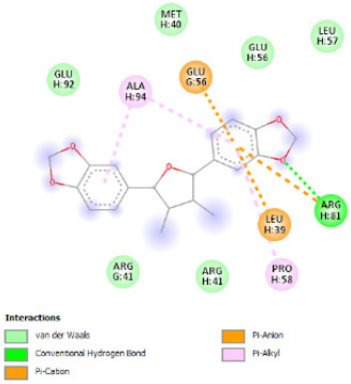
Garcinia morella Desr has its Seed coat with content of Guttiferin (-7.7 kcal/mol) is a class of Coumarins [29]. Gum-resin-hydragogue, cathartic, anthelmintic are used in dropy and amenorrhoea. The gum contains the antibacterial- beta-guttiferin and alphaguttiferin and their derivatives (Figure 8).

Medicarpin (-7.6 kcal/mol) is an antitubercular pterocarpan, is a derivative of isoflavonoids is found in Medicago truncatula and Swartzia madagascariensis. Medicarpin, a legume phytoalexin has excellent oral bioavailability and potent antiproliferative activity against breast cancer and Acute Myeloid Leukemia (AML) cells (Figure 9).

Figure 10 (A&B) Galbacin (-7.3 kcal/mol) is a natural lignan found in the Australian Endemic Plant Austrobaileya scandens. Galbacin an antibacterial, is active against L. amazonensis, showing a moderated growth inhibition (Figure 10) [31].

| Fig No. | 3-D interaction Diagram | 2-D interaction diagram | Amino Acids |
|---|---|---|---|
| 5 |  A. 3D interaction diagram of Glycyrrhetinic acid docked with hspX21 visualized using Chimera |  B. 2D interaction diagram of Glycyrrhetinic acid docked with hspX21visualized using Discovery Studio 2020 | ARG 100 and ARG 90 amino acid residues were seen to interact with the ligand through Conventional Hydrogen bonds. GLU 92, LEU 39, MET 40, ARG 41, SER 91, GLU 92, LEU 39 residues were found to interact with the ligand via Van der Waals forces. |

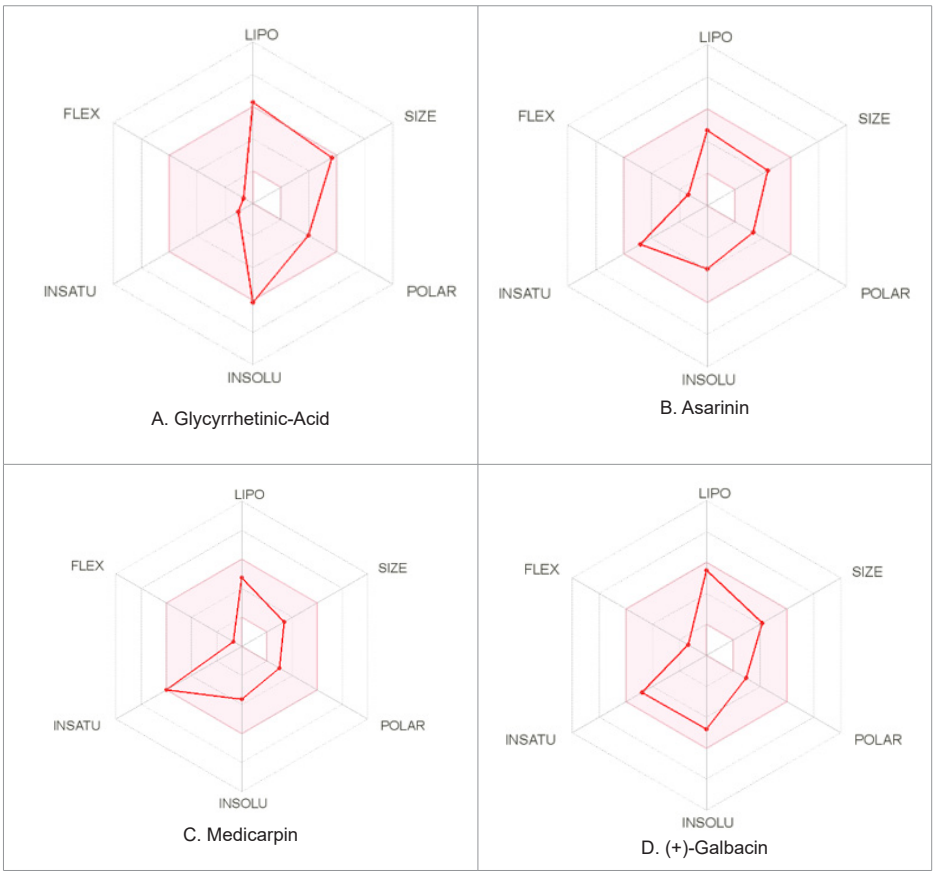
| | | | |
|---|---|---|---|
| 6 |  <br>**A.** 3D interaction diagram of Asarinin docked with hspX21 visualized using Chimera |  <br>**B.** 2D interaction diagram of Asarinin docked with hspX21visualized using Discovery Studio 2020 | Carbon-Hydrogen bond was formed by LEU 39, GLU 56, SETR 97 with the ligand. An unfavourable bond was observed between the ligand and LEU 39 residue of the protein. GLY 96, PHE 98, TYR 95, ARG 81, PRO 58, MET 40, ARG 41, ARG 41VAL 60 and ALA94 were observed to interact through Van der Waals forces with the ligand. |
| 7 |  <br>**A.** 3D interaction diagram of 1,2,4-Trihydroxyheptadeca-16-ene docked with hspX21 visualized using Chimera |  <br>**B.** 2D interaction diagram of 1,2,4-Trihydroxyheptadeca-16-ene docked with hspX21visualized using Discovery Studio 2020 | It formed conventional Hydrogen bonds with LEU 39, SER 97, GLY 96, ALA 94. It also formed Carbon hydrogen bond with GLY 96 amino acid residue. It made Alkyl bonds with VAL 99 residue. An unfavourable bond was observed between the ligand and ARG 81 residue of the protein. Unfavourable bonds affect the stability of the drug. In a receptor-ligand complex, the existence of such unfavourable bonds makes it unstable because it indicates repulsive forces. No amino acid residues were found to interact with the ligand through Van der Waals forces. |
| 8 |  <br>**A.** 3D interaction diagram of Guttiferin docked with hspX21 visualized using Chimera |  <br>**B.** 2D interaction diagram of Guttiferin docked with hspX21visualized using Discovery Studio 2020 | A conventional Hydrogen bond was observed between the ligand and the ARG 100, LEU 39 and SER 91 residues of the protein. GLU 92, ARG 41, MET 40, LEU 39, PHE 93, ALA 94 amino acid residues interacted with the ligand through Van der Waals forces. |
| 9 |  <br>**A.** 3D interaction diagram of Medicarpin docked with hspX21 visualized using Chimera. |  <br>**B.** 2D interaction diagram of Medicarpin docked with hspX21visualized using Discovery Studio 2020. | ARG 41 and GLY 96 amino acid residues were seen to interact with the ligand through Conventional Hydrogen bonds. A carbon Hydrogen bond was also observed between GLU 56 and the ligand. PRO 58, ARG 41, ALA 94 formed a pi-alkyl bond with the ligand. LEU 57, MET 40, PHE 98, SER 97, TYR 95, and ARG 81 residues were observed to interact through Van der Waals forces with the ligand. |

| | | | |
|---|---|---|---|
| 10 |  **A.** 3D interaction diagram of (+)-Galbacin docked with hspX21 visualized using Chimera. |   **B.** 2D interaction diagram of (+)-Galbacin docked with hspX21visualized using Discovery Studio 2020. | ARG 81 amino acid residues were seen to interact with the ligand through Conventional Hydrogen bonds. PRO 58, ALA 94 formed a pi-alkyl bond with the ligand. LEU 57, GLU 56, GLU 92, MET 40, and ARG 41 residues were observed to interact through Van der Waals forces with the ligand. |

**Bioavailability of ligands**

Around 96% of the initially selected phytochemicals satisfied the conditions of Lipinski's law, implying that they function like drugs. Bioavailability radar was used to do more thorough and reliable tests. The parameters are lipophilicity, scale, polarity, solubility, flexibility, and saturation. A molecule's radar plot must fall completely within the pink shaded area to be rendered drug-like. The pink region denotes the appropriate range for each property, such as lipophilicity: XLOGP3 between 0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA (topological polar surface area) between 20 and 1302, solubility: log S not greater than 6, saturation: sp3 hybridization fraction not less than 0.25, and flexibility: no more than 9 rotatable bonds [15,32]. Hence, 4 out of 7 molecules were identified to be bioavailable orally Refer -Table 3.

**Table 3:** Bioavailability Radar Diagrams of ligands with the binding affinity scores less than -7.0 kcal/mol.




A. Glycyrrhetinic-Acid

B. Asarinin

C. Medicarpin

D. (+)-Galbacin

(Please Scan to view Bioavailability Radar of all 9 bioactives).

## Discussion

Despite great technical improvements in drug discovery and medicinal chemistry, pharmaceutical development is still expensive and time-consuming. Using data science to digitally screen chemical libraries to generate drug, leads more efficiently and correctly promises to solve this problem. For small-molecule ligands to macromolecular targets, computational docking can be utilized to predict bound conformations and binding free energies. Virtual screening of ligand libraries containing tens of thousands of molecules is possible because to the techniques. In the development of small-molecule probes and drugs, target identification and mechanism-of-action analyses are critical. Discovering novel protein activities, identifying molecular mechanisms of therapeutic action, and obtaining information for lead compound optimization can all be accomplished by identifying protein targets of bioactive chemicals.

HspX are a double-edged sword as a protein target. While hspX play a crucial role in maintaining protein homeostasis in normal cells. In case of cancer cells, that have adapted the function of hspX proteins to help them survive. As a result, hspX have piqued researchers' curiosity as a possible anticancer target.

Induction of the hspX protein has been shown to aid in the recovery of a variety of illnesses, including ischemic heart disease, diabetes, and neurodegeneration. Various studies have shown that hspX is also the most abundant protein found in the latent stage of Mtb infection using in vivo models [9]. The Despite the availability of effective chemotherapy and the moderately protective vaccine, new anti-TB agents are urgently needed to decrease the global incidence of TB development of membrane-interacting drugs that change particular membrane domains and therefore modulate the heat shock response might be extremely beneficial.

Traditional plants contain compounds that might be beneficial in the treatment of illnesses or the manufacture of pharmaceuticals. Secondary metabolites that are found naturally in plants (leaves, stems, barks, and roots), have therapeutic properties and are effective against a variety of illnesses. Existing phytochemical agents known to have antituberculic, antitubercular, antimicrobial and antibacterial activities. Many research in recent years have revealed that phytochemicals exhibit antibacterial activity through a variety of mechanisms, including damage to the bacterial membrane and suppression of virulence factors, such as enzyme and toxin inhibition, and bacterial biofilm formation.

In our present study, 302 phytochemicals from four major categories (namely antituberculic, antitubercular, antimicrobial and antibacterial) were chosen from Dr. Dukes Phytochemicals Library. The phytochemicals were subjected to ADME Analysis, a critical screening of pharmacologically active drugs. On selecting only bioactives with less than 2 violations for further analysis, 96% of them were docked against hspX21. Molecular docking generates a score based on the binding of ligand and receptor. The higher binding energy represents the lower bonding affinity and vice versa [23]. Hence, 7 ligands that exhibited the least binding score (less than 7.0 kcal/mol) namely of Glycyrrhetinic acid (-11.3 kcal/mol), Asarinin (-9.9 kcal/mol), 1,2,4-Trihydroxyheptadeca-16-ene (-7.7 kcal/mol), Guttiferin (-7.7 kcal/mol), Medicarpin (-7.6 kcal/mol), (+)-Galbacin (-7.3 kcal/mol), 1-Ethyl-Beta-Carboline (-7.1 kcal/mol) were subjected to retrieval of their bioavailability radar.

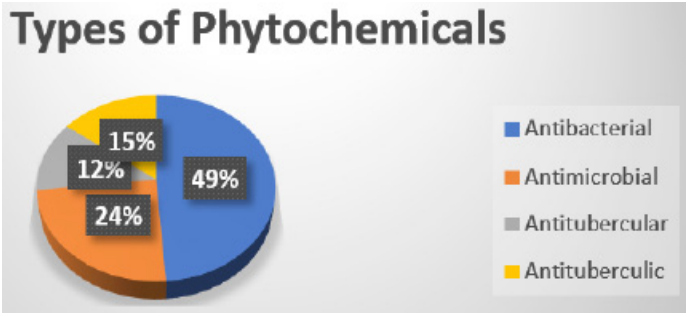



**Figure 11:** Percentage of each phytochemical category subjected to bioavailability radar.

It provided an overview of a molecule's drug-likeness in a pink zone depicting the LIPO (Lipophilicity), SIZE (Molecular Weight), POLAR (Polarity), INSOLU (Insolubility) INSATU (Insaturation) and FLEX (Rotatable Bond Flexibility) parameters. Glycyrrhetinic-Acid, Asarinin, Medicarpin, (+)-Galbacin proved to exhibit a good physiochemical profile because their predicted parameters lied within the limit. The others bioactives might have other routes of administration.

## Conclusion

Tuberculosis disease is transmitted between individuals through the respiratory route and most usually affects the lungs, but it can affect any tissue. Because populations of Mtb differ in metabolic activity, treatment for this bacillus is always extensive. Furthermore, because spontaneous chromosomal variations might lead to drug resistance, the therapy must include a variety of medicines. Allergic reactions, fever, rash, vasculitis, nausea, vomiting, hepatotoxicity, hepatocellular inflammation, peripheral neuropathy and others are some of the common side effects associated with TB treatment. The complementary and alternative treatment, the herbal medicine, has gained more attention and has also become popular. Phytochemical bioactives have decreased toxicity.

Drug development is a time-consuming and exhausting process; nevertheless, statistical methods have come to the rescue and have undoubtedly aided in the process's simplification. In this study, an in-silico screening approach was used to find a possible antagonist against Mtb hspx21. By using the SwissADME tool, the bioactives screened using Lipinski rule of five method, yielding 290 bioactive molecules based on the parameters. These 290 molecules were further screened and were then docked with the enzyme. To find possible inhibitors, the bioactives with the least binding affinity scores were shortlisted (bioactives with binding affinity scores less than 7.0 kcal/mol) orally bioavailable molecules were determined and only four molecules, namely Glycyrrhetinic acid, Asarinin, and Medicarpin, and Galbacin met it. These molecules can be hence represented as potential drugs against Mtb. As a result, in vitro and in vivo wet lab laboratory validations can be conducted to further confirm the efficiency these bioactives.

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