UNVEILING THE POTENTIAL OF PHYTOCHEMICALS AGAINST LEPROSY AS AN ALTERNATIVE TO RIFAMPIN

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To the

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CANDIDATE'S DECLARATION

I , Anamika, 2K22/MSCBIO/07 hereby certify that the work which is being done presented in the thesis entitled Unveiling the Potential against Leprosy as an Alternative to Rifampin in partial fulfillment of the requirements for the award of the Degree of Master of science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from March 2024 to June 2024 under the supervision of Prof. Asmita Das.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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Certified that **Anamika (2K22/MSCBIO/07)** has carried out their search work presented in this thesis entitled **"Unveiling the Potential of Phytochemicals Against Leprosy as an Alternative to Rifampin"** for the award of **Master of Science** From Department of Biotechnology, Delhi Technological University, , Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University.

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Unveiling the potential of Phytochemicals Against Leprosy as an alternative to Rifampin

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ABSTRACT

Leprosy is a chronic disease caused by *Mycobacterium leprae* and *Mycobacterium lepromatosis* that not only affects skin but also affects nervous system, internal organs and mucous membranes. It has remained a prevalent condition throughout history, that does not account for mortality but causes noticeable deformity which leads to lifelong stigma. Regardless of being designated "Eradicated" as a worldwide public health hazard by the WHO in 2017, it continues to exist around the world, primarily in Asia and Africa. In order to Treat leprosy WHO has advised a Multidrug regimen (Dapsone, Rifampin, Clofazimine) in 1982 and since then this regimen has remained the first line drug for treatment of leprosy. Rifampin is the most effective antibacterial medication used against leprosy. However, it has certain drawbacks, such as causing side effects such as liver dysfunction and thrombocytopenia, as well as inducing the activity of the metabolic enzyme Cyp3A4 in the liver and gut, resulting in a reduction in its half-life as well as that of the drugs taken with it. To address this restriction of Rifampin, this research has conducted in which 35 phytocompounds were docked. Molecular docking is done against 3 different enzymes in order to get the alternatives of Rifampin which has the bactericidal effect along with Cyp3A4 inhibiting property. Docking is performed using the AutoDock Vina tool and Discovery Studio to determine the most efficient drug. Phytochemicals with the highest binding affinity are then chosen for further testing for toxicity, ADME (absorption, distribution, metabolism, and excretion), and drug-likeliness with the help of ProTox 3.0 and SwissADME web tools respectively. After all the analysis and screening only 5 phytochemicals are selected which can be used in place of Rifamycin, these are Tomatidine, Withaferin A, Glabrol, Glycyrrhetic Acid and Glabridin. These selected 5 compounds are shown to be less toxic and most effective comparative to Rifampin based on In-silico studies. However, in vivo investigations are required to gain confidence in the identified phytochemicals.

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List of Abbreviations

PB – Paucibacillary Leprosy

MB – Multibacillary Leprosy

TT – Tuberculoid Leprosy

BT – Borderline Tuberculoid Leprosy

BB – Borderline Leprosy

BL – Borderline Lepromatous Leprosy

LL – Lepromatous Leprosy

WHO – World Health Organisation

ADMET – Absorption, distribution, metabolism, excretion and toxicity

LD50 – Lethal dose 50

PDB – Protei Data Bank

ProTox 3.0 – Prediction of toxicity of Chemicals

BI – Bacteriological Index

AFB – Acid-Fast Bacteria

DDS – Diaminodiphenyl sulfone

MDT – Multidrug Therapy

TPSA – Topological polar surface area

PXR – Pregnane X receptor

RXR – Retinoic Receptor

RNAP – RNA Polymerase

RMSD – Rood mean square Deviation

CHAPTER 1
INTRODUCTION

Leprosy, a neglected, chronic infectious disease caused by Mycobacterium leprae identified by G.H.A. Hansen in 1873 [1]and recently discovered Mycobacterium lepromatous bacteria. It is the acid - fast bacteria that affects the outermost endothelium of skin and Schwann cells in the peripheral nerves that lead to loss of sensation and developmental disabilities. [2] These bacteria use host cell lipid metabolism to make their intracellular survival better and damage Schwann cells by binding itself to alpha 2-laminin and adhesins (on basal lamina) and to alpha-dystroglycan and ErbB2 receptor (on cell surface). [3]On the basis of the number of lesion present and the severity of the disease, it is categorized into five groups Tuberculoid (TT), Borderline Tuberculoid (BT), Borderline (BB), Borderline lepromatous (BL) and Lepromatous (LL), under the classes of paucibacillary and multibacillary. [4]

Initially drugs like dapsone, Rifampin, clofazimine like drugs were used individually but these drugs as monotherapy result in the resistance to these drugs. Considering this situation and high prevalence rate in 20s WHO declared to use the multidrug regimen against leprosy.[5] After the treatment with multidrug regiment it was seen that the prevalence rate for this disease decreased significantly along with this the period of treatment has also decreased. According to National Leprosy Eradication Programme prevalence below 0.1 per 10,000 will be considered as eradicated. It should not be confused with complete elimination of the disease but it show that it transmission rate will decrease.[6] It was observed that even after the use of multidrug regimen the occurrence of new leprosy cases was not decreased or stopped. Because the actual Transmission method for this Disease is not discovered, it is said that this disease spread when a healthy individual remains with the infected person with long time by the bacterium expelled in the air or by the sputum of the infected person [7]. It is also believed that it transmits by the contact of wound of healthy people with the ulcers of infected individuals. According to the Global Health observatory data repository of World Health Organisation Report, there are still thousands of leprosy cases occur in the various parts of the world in 2022. This data showed that 174059 new cases of leprosy occurred in year 2022. This data tells that India, Brazil, Indonesia are countries having highest number of new leprosy cases reported in year 2024 as 103819, 19635 and 12441 respectively.

This whole situation regarding leprosy in the whole world has compelled me to conduct this study. In this study an effort has been done in order to get the best phytochemicals as an alternate of Rifampin so that we can tackle the harmful effects of it and to reduce the treatment period for leprosy by giving it on more frequent bases. The alternative phytochemicals are found by the docking method using AutoDock vina 1.5.6. firstly 33 antibacterial phytochemical structures were taken from the PubChem

[8]. To get best alternative of Rifampin the selected phytochemicals were docked against three enzymes, DNA-dependent RNA polymerase beta subunit enzyme, Cyp3A4 enzyme and nuclear receptor PXR receptor in the order as it is written above. Phytochemicals with high binding affinity with DNA-dependent RNA polymerase beta subunit enzyme compared to Rifampin considered best because these phytochemicals bind more tightly and hence can more effectively inhibit beta subunit and stops the transcription. Then these phytochemicals were docked against Cyp3A4 enzyme, a metabolizing enzyme [9]. Phytochemicals having more binding affinity against Cyp3A4 considered best because this will inhibit it and prevent the metabolism of Drugs using along with this. After this all, all phytochemicals were docked against the nuclear receptor PXR receptor, phytochemical with the least binding affinity is considered best because this will not enhance the production of Cyp3A4 enzymes [10]. Phytochemicals selected on the basis of binding affinity against the selected three enzymes were taken for the ADMET analysis in order to check the water solubility, Gastric Absorption, Drug-likeliness, Blood Brain Barrier permeation, Bioavailability score, Lipinski Rule, lipophilicity, LD50 value, Toxicity (mutagenicity, carcinogenicity, nephrotoxicity, cardiotoxicity, Hepatotoxicity, Immunotoxicity etc.) in order to get the idea about Pharmacokinetic for each individual phytochemical. On considering all the points discussed above, it was found that only 5 phytochemicals fall in the selected criteria out of 33 phytochemicals [11]. These are Tomatidine, Glabridin, Glycyrrhetic acid, Withaferin A and Glabrol these can prove to be much better Drugs than that of Rifampin.

CHAPTER 2
LITERATURE REVIEW

2.1 Understanding Leprosy Disease and its pathogen

Leprosy often known as Hansen's disease, is caused by infection with *Mycobacterium leprae*, this was first discovered by Gerhard Armauer Hansen of Norway in 1873[12] and *M. lepromatosis*. [13] *M. leprae*, an intracellular, aerobic, rod-shaped and acid-fast bacillus are slow-growing organisms that primarily reproduce in macrophages, endothelial cells, or Schwann cells. They are obligate intracellular creatures that do not thrive in artificial media environments. Their optimal growing temperature ranges from 27 to 33°C. *M. lepromatosis* has recently been recognized as an etiologic agent, despite the fact that its pathological characteristics may be analogous to M. leprae infection.[14]

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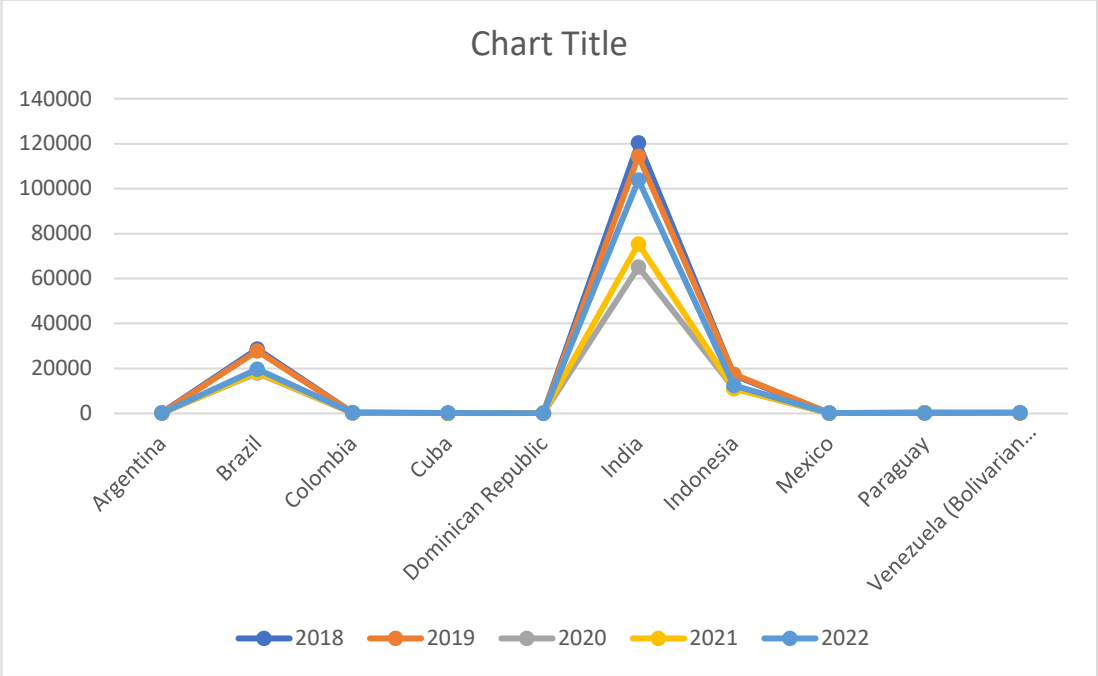

Fig.1 Graph showing countries along with the new leprosy cases occurred in them in last 5 year (Data taken from World Health Organisation's Data Repository)

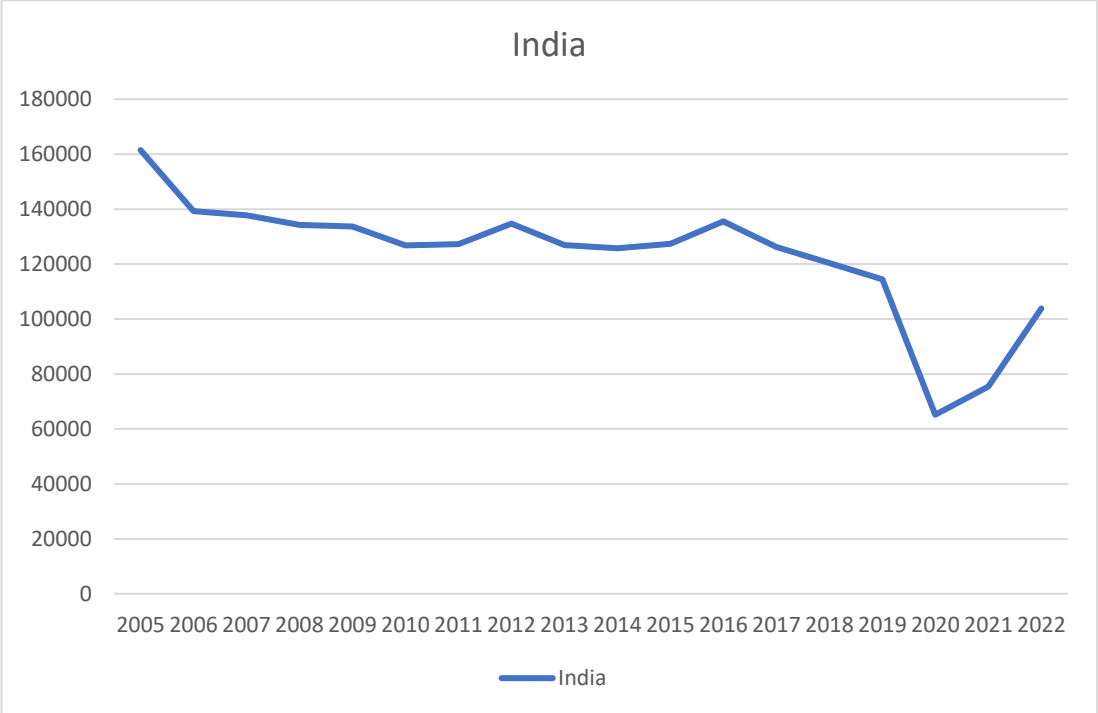

Fig.2 Graph showing new cases of leprosy observed in India in past few years (Data taken from World Health Organisation's Data repository)

2.2 Classification of leprosy

Leprosy has been classified several times throughout history, including the Madrid classification (1953) and the Ridley-Jopling classification (1966). Based on the aforementioned classification systems, WHO developed a new method in 1982, which was amended in 1988 and 1996. According to the amended leprosy classification given in 1996 by WHO there are two classes, first is Paucibacillary (PB) which further includes Tuberculoid Tuberculoid (TT) and Borderline Tuberculoid (BT) leprosy. Second is Multibacillary (MB) which includes Borderline Borderline (BB), Borderline lepromatous (BL) and Lepromatous lepromatous (LL). It also states that Individuals with a Bacteriological Index (BI) of zero with less than or equal to five skin lesions are classified as paucibacillary, whereas those with a Bacteriological Index more than or equal to one with more than five skin lesions are classified as multibacillary.[15]

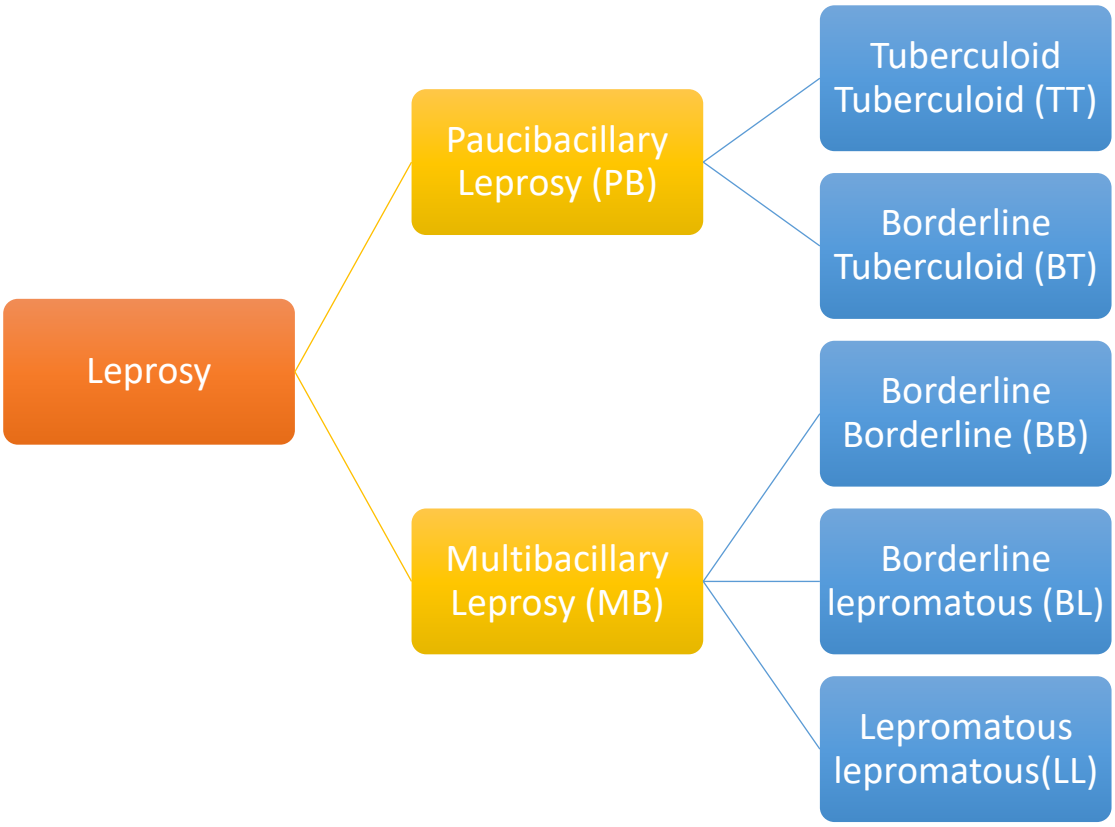

Fig.3 Classification of Leprosy (WHO,1996)

Paucibacillary TT reveals a granulomatous inflammatory infiltration, with epidermal characteristics usually degraded and atrophic. Lymphocytes cover the nerve perineurium; BT has a comparable infiltration to TT, but the number of lesions differs. BT generally has a larger number of lesions than TT. Multibacillary BB features ill-defined granulomas with immature epithelioid cells. Giant cells are absent, although lymphocytes and macrophages are abundant. The epidermis is atrophic and there are no bulging nerves. The BL infiltration is made up of lymphocytes and macrophages (which have foamy cytoplasm), as well as neurons with onion-skin-like characteristics surrounded by lymphocytes. The epidermal layer of LL has foamy macrophages that are evenly dispersed. Epithelioid cells are not present. Acid-fast bacteria (AFBs) exist in the form of globular clusters.[16]

Leprosy classification is critical in determining the severity of the condition and allowing a doctor to propose the optimum therapeutic regimen for a person. Misdiagnosis can cause paucibacillary leprosy to progress to multibacillary leprosy, hence it is critical to identify the kind of leprosy before beginning therapy.

2.3 Treatment of Leprosy

The treatment of leprosy has an excellent history. Initially, **Dapsone** (DDS), a bacteriostatic drug, was utilized to treat this condition. It prevents *M. leprae* from using para-aminobenzoic acid (PABA) to synthesize folic acid.[17] It has a number of adverse effects, including headaches, haemolytic anaemia, and hepatitis, but is generally well tolerated. Pettit and Rees reported the first case of Dapsone resistance in the 1960s, which led to the discontinuation of its usage as a monotherapy.[18]

After this in 1978, Brazil Ministry of health recommended Dapsone, **Rifampin** (RMP) combined therapy for first three months and then with Dapsone alone. Patients with Dapsone Resistance are treated with Rifampin Monotherapy that leads to Resistance to Rifampin as well. The first cases of Rifampin resistance were found in the 1970s.[19] Since 1981, WHO has not advised the combination DDS/RMP due to the development of resistance to both Rifampin and Dapsone.[17]

Clofazimine (CLF) is an iminophenazine dye that has effectiveness equivalent to DDS and anti-inflammatory properties. It creates dark pigmentation, which limits its usage and hence reduces resistance to it. It was discovered that utilizing drugs as a monotherapy result in resistance and low efficacy. In 1982, WHO established the DDS + RMP + CLF drug regimen as the first line of treatment for leprosy. This combination is known as multidrug treatment (MDT) or polychemotherapy (PCT). [17]

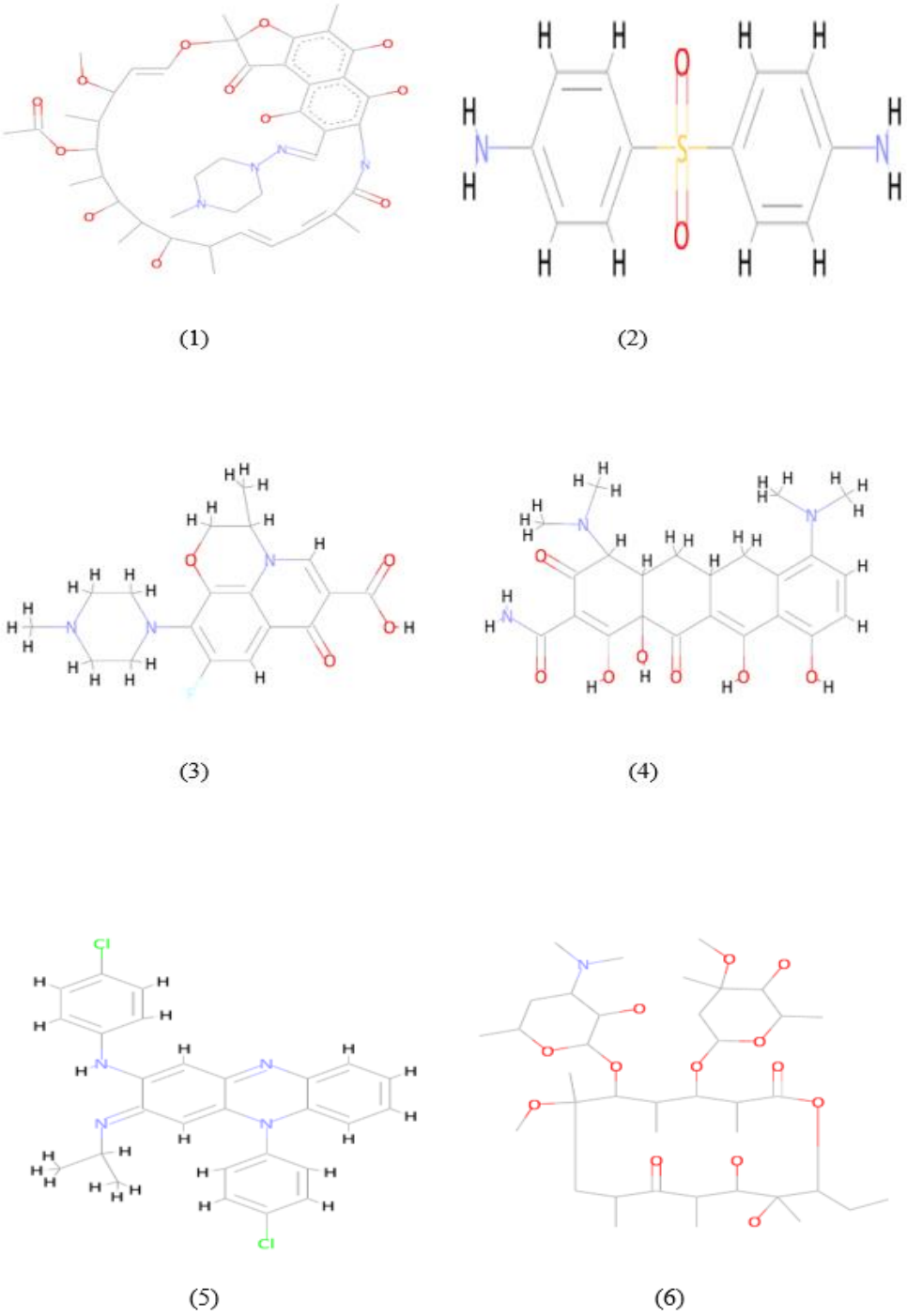

Fig.4 2D structures of all the Drugs currently in use for the treatment of Leprosy. (1) Rifampin, (2) Dapsone, (3) Ofloxacin, (4) Minocycline, (5) Clofazimine, (6) Clarithromycin.

Ofloxacin, minocycline, and clarithromycin are further medications used to treat leprosy.[20] **Ofloxacin** is an antibiotic that is prescribed to treat leprosy. Due to its negative effects, it is not suggested for children under the age of five, pregnant women, or those who are breastfeeding. **Minocycline** is the sole tetracycline medicine used to treat leprosy, and it can produce adverse effects such as tooth and skin discoloration, as well as anomalies of the central nervous system. **Clarithromycin** is a bactericidal antibiotic used to treat leprosy. Its adverse effects include nausea, vomiting, and diarrhoea.[21]

2.4 Rifampin

2.4.1 Introduction

Rifampicin belongs to the rifamycin class and is a semisynthetic antibiotic produced from Amycolatopsis rifamycinica, also known as Amycolatopsis mediterranei and Streptomyces mediterranei. It contains N-iminopiperazine, N-methylpiperazine, a hydrozone, and a cyclic keta. It is the tautomer of Rifampicin zwitterion. It performs a variety of functions, including RNA polymerase inhibition, DNA synthesis inhibition, antitubercular activity, leprosy, protein synthesis inhibition, neuroprotection, and antineoplastic properties [22]. Rifampin has a topological polar surface area (TPSA) of 220 angstrom and a molecular weight of 822.9 g/mol.
It has 6 hydrogen bond donors, 15 hydrogen bond acceptors, and 5 rotatable bonds, which are responsible for its binding to diverse enzymes with varying orientations. A variety of gram-positive cocci, such as Mycobacteria and Clostridium difficile, as well as several gram-negative species, such as Neisseria meningitidis, N gonorrhoeae, and Hemophilus influenza, are susceptible to the antibacterial action of Rifampin [23] .

2.4.2 Bactericidal action

Rifampin is a bactericidal drug which means this has the potency to kill the bacteria. RNA polymerase that is reliant on DNA is the target of Rifampin. The enzyme involved in transcription—the creation of RNA—is called RNA polymerase. This enzyme has several subunits. It is composed of five subunits: β′, β, αI, αII, and ω.[24] Our focus is on subunit Beta, which is produced by the gene rpoB which results in the production of 1178 amino acid long protein. When the transcript reaches a length of two to three nucleotides, Rifampin binds in the Deep groove of the RNAP beta subunit at the 5’ end and about 12 angstroms away from the active site, and obstructs the elongating RNA's course directly. Pathogens will eventually die if no RNA is created since this will result in the creation of either very little or none of the proteins and enzymes needed for *Mycobacterium* survival.[25]

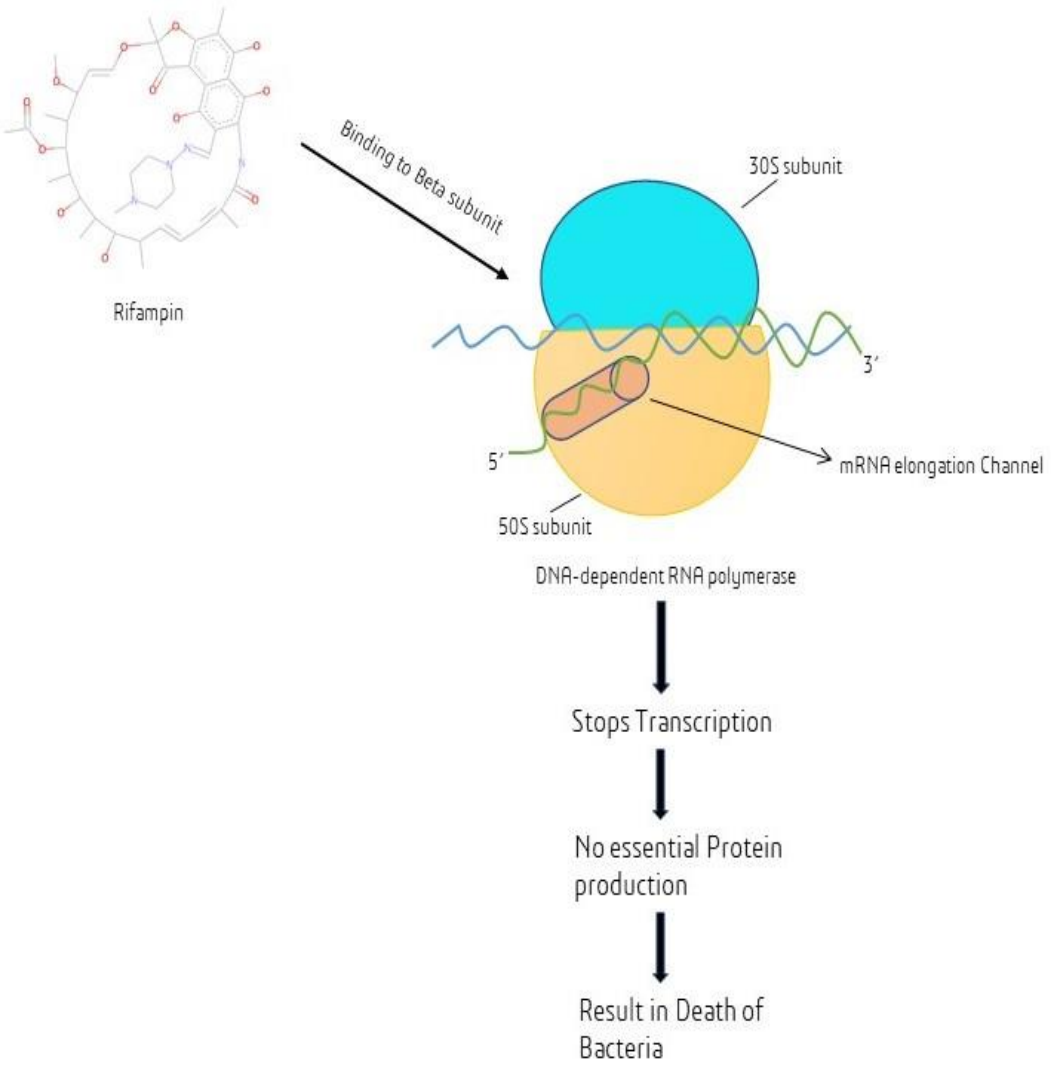

Fig.5 bactericidal action mechanism of Rifampin

2.4.3 Cyp3A4 induction mechanism

The most prevalent metabolizing enzyme in intestine and hepatic cells is human cytochrome p450 (Cyp) 3A4. It has the ability to bind to a wide variety of drugs. The enzyme known as cytochrome p450 is responsible for the metabolism of almost 50% of drugs that are marketed. Its high expression will cause drugs to be metabolized more quickly, reducing the bioavailability and half-life of the drug administered, whereas its low expression can cause drug-drug interaction and toxicity.[26]

Cyp3A4 is strongly induced by Rifampin. It attaches itself to the nuclear receptor known as the pregnane X receptor (PXR), activating it. After joining forces with the Retinoic receptor (RXR) to create a heterodimer, this activated PXR moves into the nucleus and functions as a transcriptional factor.[27] It is well established that rifampicin-activated PXR inhibits SHP gene expression while concurrently interacting with HNF4α, SRC-1, and PGC-1α to start CYP3A4 gene transcription, leading to a significant rise in Cyp3A4 levels. [28] This induction of Cyp3A4 not only impacts the medicines taking along with Rifampin but also decreases the half-life of itself also.

2.5 Phytochemicals

Since ancient times, plants have remained to be extremely vital. When there were no medications accessible in the past, people relied on plants for a variety of treatments, including the mending of wounds and the treatment of infections. This demonstrates that plants contain a wide variety of materials with therapeutic qualities that can aid in our defence against various infections and illnesses. These days we extract some of the medicinal substances from plants called phytochemicals, rather than using the portions of the plant directly [29]. According to their chemical structures, phytochemicals can be categorized into a number of classes. The main categories of phytochemicals include terpenoids, polyphenols, sulfur-containing phytochemicals, and alkaloids .

2.5.1 Terpenoids

The family of chemicals with antibacterial action is called terpenoids. One carbon isoprene unit serves as the basis for these molecules. The majority of terpenoids contain multicyclic structures with distinct functional groups and fundamental skeletons that set them apart from one another.[30] This is further classified in several classes:

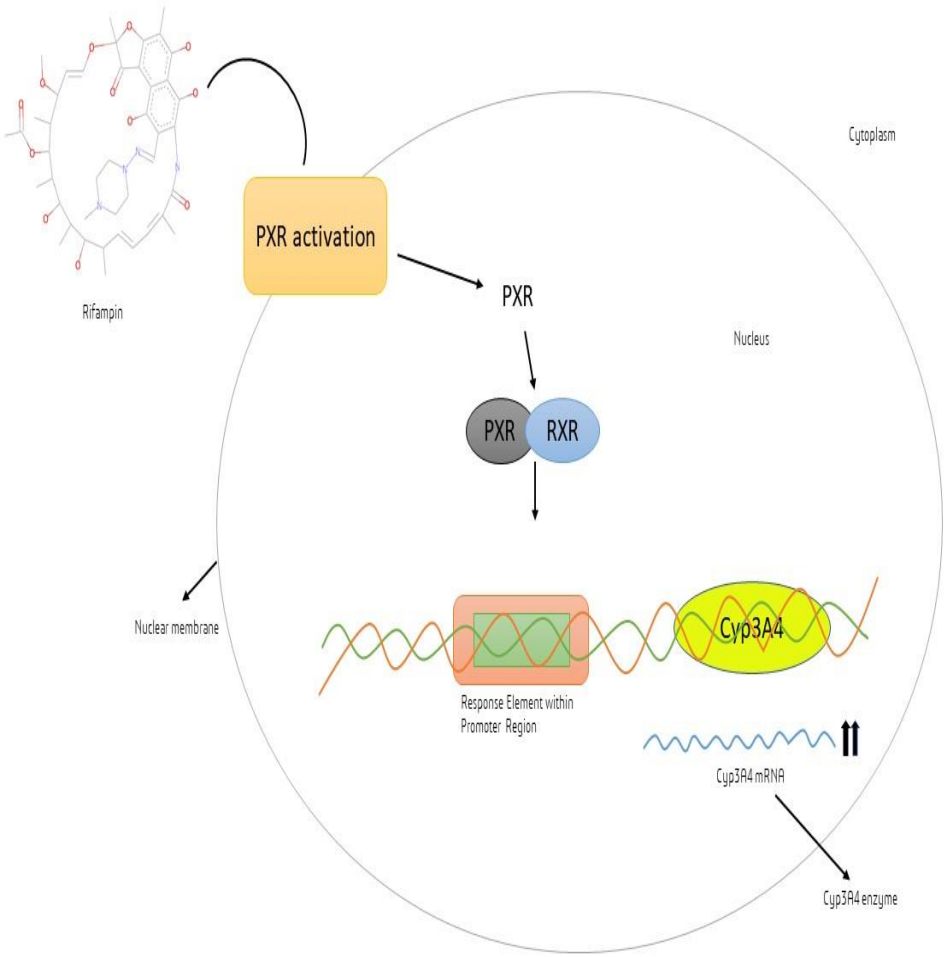

Fig.6 Induction of Cyp3A4 production due to Rifampin

Monoterpenes - Fruits, vegetables, spices, herbs, and other plants are the source of these chemicals that are present in essential oils. When extracted from plants, these chemicals add to the flavour and perfume of the plant., bicyclic, or acyclic C30 compounds are called monoterpenes. E.g., Thymol, Vanillin [31].

Diterpene - They exist in both terrestrial and marine habitats and are found in plants, bacteria, fungi, and mammals. They have four isoprene units. They exist in both terrestrial and marine habitats and are found in plants, bacteria, fungi, and mammals. They have four isoprene units. Commercial production of some isolated diterpenes is done for application in agriculture, food additives, medicine, and fragrance synthesis. E.g., Salvipisone, aethiopinone[32]

Sesquiterpenoids – These compounds come in a variety of frameworks and are composed of three isoprene rings. These may be isolated from bacteria, fungus, and plants. e.g. Farnesol[33]

2.5.2 Polyphenols

A significant amount of the phytochemicals that are present in fruits, vegetables, nuts, seeds, stems, flowers, and drinks like red wine, coffee, and tea are polyphenols. They possess both antibiotic modulation and antibacterial properties [34]. They are further categorized as flavonoids and non-flavonoids based on their chemical structure. E.g., Quercetin, Chrysin, Kaempferol etc.

2.5.3 Sulfur-containing phytochemicals

These compounds have antibacterial activity and targets both Gram positive and Gram-negative bacteria. E.g., Isothiocyanates, Allicin, Ajoene etc.

2.5.4 Alkaloids

Organic nitrogenous bases are called alkaloids. Their chemical structure is quite varied. These substances include a variety of properties, including antitumor, vasodilator, anti-hypertensive, anti-malarial, and anti-asthma properties. Certain alkaloid chemicals prevent bacteria from growing bacterial biofilms. For example, Tomatidine, Reserpine, Sanguinarine, etc [35].

CHAPTER 3
METHADOLOGY

3.1 Database and search studies

This investigation is conducted in a very methodical way. A search was conducted using PubMed/MEDLINE, Google Scholar, and Scopus for literature. Additionally, the references cited in the chosen publications were examined for pertinent research. “Rifampin”, “Cyp3A4”, “Antibacterial Phytochemicals”, “Leprosy”, “Hansen's Disease”, and related terms are the primary keywords utilized to obtain the best-fitting publications. Approximately 300 papers were screened to get the required information, Data and phytochemicals to complete this study in best possible manner.

3.2 Selection of Papers

The Mendeley reference manager software was utilized to get the document intended for research at one place then all duplicates were eliminated. Papers were chosen based on both inclusion and exclusion criteria. The inclusion criteria include (1) research that accurately identify side effects related to Rifampin. (2) Full text research and review papers were chosen, along with (3) papers outlining the current and historical state of leprosy. Exclusion Criteria includes (1) Languages other than English, (2) full-text publications that aren't available, (3) case studies, and social commentary.

3.3 Selection of Phytochemicals

A total of 33 phytochemicals were chosen as antibacterial agents after reading various publications found in the literature on PubMed, Google Scholar, Scopus, and other databases. Following are the selected antibacterial phytochemical. screening process is employed to isolate the phytochemicals that are now being utilized in the fight against leprosy. Currently, the freshly created forms list is put through another screening process to exclude phytochemicals whose 3D structures are not accessible through PubChem.

3.4 Docking studies

3.4.1 Ligand preparation

For virtual screening and molecular docking against the DNA-dependent RNA polymerase beta subunit, 33 phytocompounds were chosen based on prior research on Rifampin and leprosy. Every ligand's three-dimensional structure was obtained from the DrugBank (https:// go. drugbank. com/k) and PubChem databases (https:// pubchem. ncbi. nlm. nih. gov/). Additionally, Open Babel was used to convert each ligand's file into PDB format. AutoDock vina 1.5.6 tools were then used to prepare and minimize each ligand.

3.4.2 Retrieval of protein models to be docked

Cyp3A4 enzyme and nuclear receptor PXR were retrieved from the Protein Data Bank (https://www. rcsb. org/), and the crystal structure of the RNA polymerase beta subunit of M. leprae was obtained from Uniprot (the structure is an estimated one built using Alpha Fold software). Three proteins are docked in the following order: -

- First, the RNAP beta subunit of M. leprae was used as the docking site for all phytocompounds.

- Following selection, Cyp3A4 was docked with all of the phytocompounds.

- Compounds having binding affinities greater than -8.4 (for Rifampin) against RNAP beta subunit and more than -8 (for Rifampin) were chosen after determining the binding energies of all ligands against Cyp3A4 and RNAP beta subunit.

- Next, specific ligands were docked against the Pregnane X receptor.

Table 1: List of 33 phytochemicals selected for study

Source	Phytochemicals
Piper betle leaves	Phytol
	Acyclic Diterpene alcohol
	4-Chromanol
	Hydroxychavicol
	Allylpyrocatechols
Sargassum siliquastrum	Critinol
Clitoria ternatea	Anthocyanins
	Quercetin
	Kaempferol
Berberis vulgaris	Berberine
Solanum lycopersicum	Tomatidine
Vinca minor	Reserpine
Piper Nigrum	Piperine
Pimenta dioica, Syzygium aromaticum	Eugenol
Withania somniferum	Withaferin A
Capsicum Annuum	Capsaicin
Lawsonia	Lawsone
Glycyrrhiza	Glabrol
Curcuma longa	Curcumin
Schinus terebinthifolius	Terebinthone
Ranunculus	Protoanemonin
Erythroxylum coca	Cocaine
Gloriosa superba	Colchicine
Camellia sinensis	Catechin
Arnica montana	Helenine
Olea europaea	Hexanal
Carica papaya	Latex
Anemone pulsatilla	Anemonins
Glycyrrhiza glabra	Liquiritin
	Isoliquiritin
	Liquiritigenin
	Isoliquiritigenin
	Liquiritin Apioside
	Glycyrrhetic Acid
	Licochalcone A
	Glabridin

3.4.3 Target Protein Preparation

The AutoDock Vina 1.5.6 tool and the discovery studio were used to prepare the protein. To improve the binding capability, polar hydrogen was added after the removal of water molecules. The binding site for the target's protein-ligand interaction was appropriately encircled inside the grid box with the adjustment of the grid parameter values for x, y, and z coordinates.

3.4.4 Virtual Screening and Molecular Docking

AutoDock Vina 1.5.6 is used to do a molecular docking analysis of the chosen ligands utilizing the three enzymes in the previously described order. Discovery Studio (https:// discover.3ds. com/d) is used to analyse binding once the docking search is finished, with the best confirmation having an RMSD value of zero or below selected.

3.4.5 ADMET analysis

To ascertain the ligands' drug-likeness (Dong et.al. 2018), toxicity, and absorption characteristics, ADMET screening was carried out. The three-dimensional structures of ligands were uploaded to ProTox-II and SWISSADME for ADMET screening. The ProTox-II (http:// tox. chari te. de/ protox_ II) (Singh et. al. 2021a, b, c, d; Banerjee et. al. 2018). web server was utilized to forecast the chemical's toxicity profile. Measured toxicity endpoints for ligands include carcinogenicity, mutagenicity, and so on. Additionally, it can be quantified using methods like LD50 (lethal dose) values, where Class I (LD50 & 5) and II (5 < LD50 & 50) are deemed fatal if ingested and Class VI (LD50 > 5000) is non-toxic, as well as qualitatively using methods like binary (active or inactive) for specific cell types and assays or indication areas like cytotoxicity, immunotoxicity, and hepatotoxicity.

CHAPTER 4
RESULTS

33 phytochemicals in total were chosen for molecular docking against the metabolizing enzyme Cyp3A4 and the DNA-dependent RNA polymerase beta subunit. A selection of six compounds was made based on their binding affinity. Here are the names of these compounds: Glabrol, Glycyrrhetic acid, withaferin A, tomatidine, reserpine, and Glabridin. The binding affinities of these six compounds to the nuclear receptor Pregnane X receptor protein and ADMET analysis were then used for screening. This results in the identification of just five compounds these are Glabrol, Glycyrrhetic, Withaferin A, Tomatidine and Glabridin, the best of which is determined to be tomatidine. Reserpine's carcinogenic properties, extremely low bioavailability score of 0.17, and noncompliance with the Lipinski rule for drug likeliness result in its removal from the list.

All 5 compounds have binding affinity for the DNA-dependent RNA polymerase beta subunit of M. leprae greater than -8.4; binding affinity for Cyp3A4 greater than -8.0; and binding affinity against the nuclear receptor Pregnane X receptor protein, which is significantly less than that of Cyp3A4.

To test for Lipinski's rule, toxicity, and ADME, respectively, all of the medications were analysed using SwissADME and ProTox 3.0. This investigation determined that all five chemicals had good water solubility, high GI absorption, and fall into either class IV (least or non-toxic) or class III (less harmful). According to In Silico drug likeliness prediction all the drugs showed zero violation except Tomatidine and Glycyrrhetic acid both with 1 violation to the rule.

Table: 2 Binding affinity of different Selected phytochemicals against the given targets (pink coloured shows finally selected ones)

S.No.	Phytochemicals	Binding affinity against RNAP beta subunit	Binding affinity against Cyp3A4	Binding affinity against PXR
1	Phytol	-4.6	-6.1	-5.8
2	4- Chromanol	-5.5	-5.8	-6.9
3	Hydxychavicol	-5.8	-6.3	-7.1
4	Allylpytcatechols	-5.3	-6	-7.1
5	Critinol	-5.4	-6.3	-7.7
6	Quercetin	-8.2	-8.2	-8.2
7	Kaempferol	-7.9	-8.3	-7.5
8	Berberine	-8.8	-8.3	-7.2
9	Tomatidine	-10.2	-10.6	-8.5
10	Reserpine	-8.9	-9.6	-7
11	Piperine	-7.4	-7.9	-7.7
12	Eugenol	-5.5	-6.2	-7.1
13	Withaferin A	-8.6	-9.8	-8.5
14	Capsaicin	-5.6	-6.7	-6.3
15	Lawsone	-6.1	-7.2	-8.4
16	Glabrol	-8.6	-9.5	-8.7
17	Curcumin	-7.6	-8.1	-8.1
18	Terebinthone	-6.5	-9.9	-7.4
19	Protoanemonin	-4.7	-4.8	-5.1
20	Cocaine	-6.2	-7.6	-6.4
21	Colchicine	-6.8	-7.7	-6.5
22	Catechin	-8.2	-8.2	-7.2
23	Helenine	-6.5	-7.3	-9.4
24	Hexanal	-3.6	-4.2	-4.6
25	Latex	-3.7	-4	-4.7
26	Anemonins	-6.3	-6.5	-7.1
27	Liquiritin	-8.4	-9.8	-9.9
28	Isoliquiritin	-7.8	-8.5	-8.1
29	Liquiritigenin	-6.9	-8.5	-8.3
30	Isoliquiritigenin	-7	-7.6	-8.4
31	Liquiritin Apioside	-8.5	-9.6	-8.2
32	Glycyrrhetic Acid	-9.8	-10.1	-8.4
33	Glabridin	-9.2	-9.8	-7.6

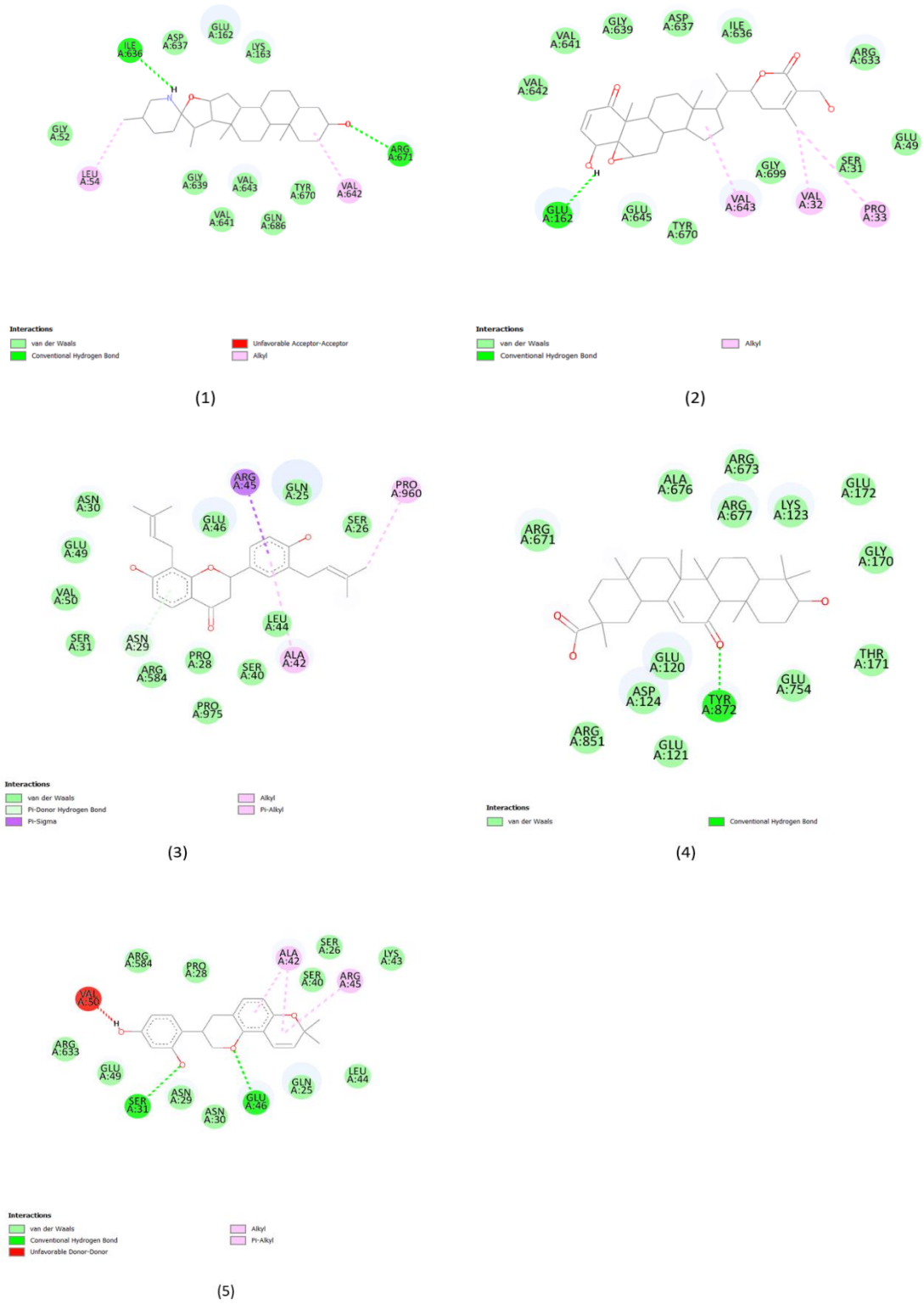

Fig. 7 2D diagrams showing interactions of phytochemicals with DNA-dependent RNA polymerase beta subunit. Where (1) Tomatidine, (2) Withaferin A, (3) Glabrol, (4) Glycyrrhetic acid, (5) Glabridin

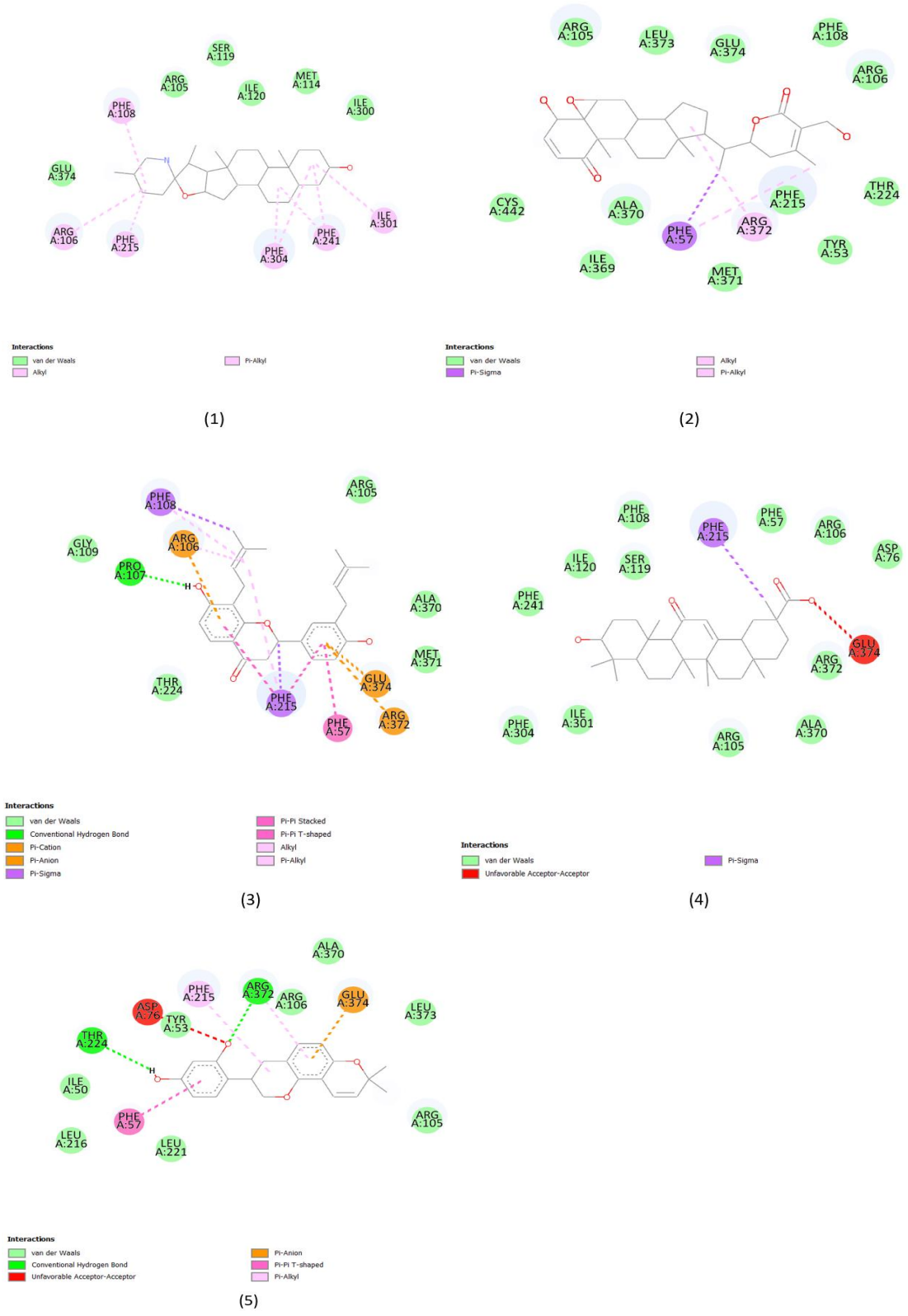

Fig. 8 2D diagrams showing interactions of Pytochemicals with Cyp3A4 where (1) Tomatidine, (2) Withaferin A, (3) Glabrol, (4) Glycyrrhetic acid, (5) Glabridin

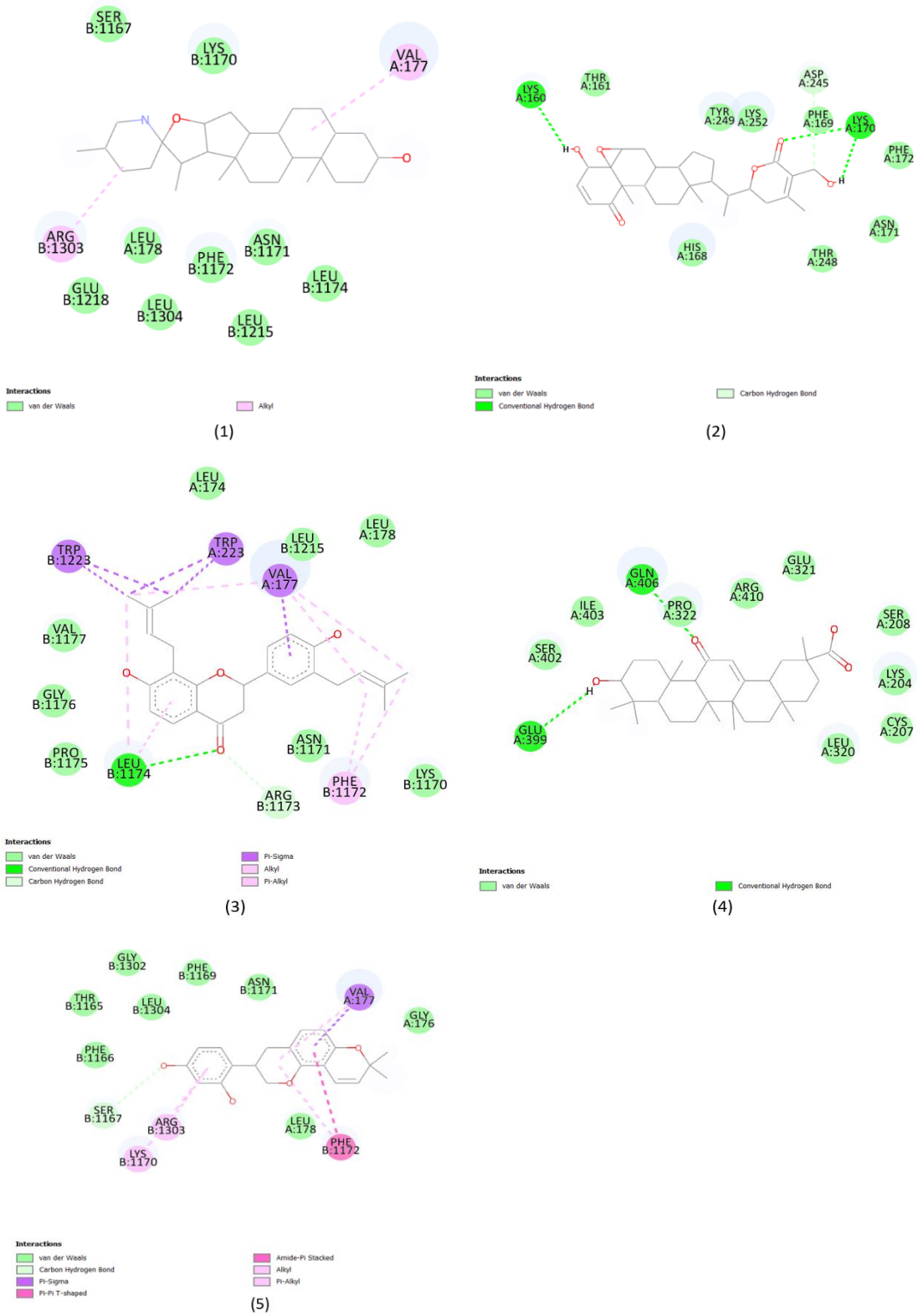

Fig. 9 2D diagram showing interactions of Phytochemicals with Nuclear Receptor PXR Receptor where (1) Tomatidine, (2) Withaferin A, (3) Glabrol, (4) Glycyrrhetic acid, (5) Glabridin

Table: 3 ADME Prediction of Phytochemicals selected on the basis of binding affinity

Phytochemicals	**Tomatidine**	**Withaferin A**	**Glabrol**	**Glycyrrhetic acid**	**Glabridin**
TPSA	41.49	96.36	66.76	74.6	58.92
Solubility	Moderate	Moderate	Poorly	Moderate	Moderate
LogP	4.9	3.45	5	5.17	3.45
GI absorption	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	Yes	Yes
Drug likeliness	Yes	Yes	Yes	Yes	Yes
Bioavailability	0.55	0.55	0.55	0.85	0.55
Lipinski rule	Yes,1	Yes,0	Yes,0	Yes,1	Yes,0

Table: 4 Toxicity type and class of Phytochemicals

Phytochemicals	**Tomatidine**	**Withaferin A**	**Glabrol**	**Glycyrrhetic acid**	**Glabridin**
Respirotoxicity	Active	Inactive	Active	Active	Inactive
Nephrotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive
Cardiotoxicity	Inactive	Inactive		Active	Inactive
immunotoxicity	Active	Active	Active	Active	Active
Mutagenicity	Inactive	Inactive	Inactive	Inactive	Inactive
Carcinogenicity	Inactive	Inactive	Inactive	Inactive	Inactive
Toxicity class	4	3	4	4	4
LD50	500	300	2000	560	500

4.1 Conclusion

This study had shown that although leprosy doesn't cause death but it should not be neglected as it causes deformity to great extent. it has been discovered that Rifampin is the most potent against leprosy but has some limitation because of which it is given on monthly bases. In this study we found the 5 phytochemicals out of 33, which can prove to be more potent than that of Rifampin as these compounds have more binding affinity towards the target and will not cause early metabolism of the drugs used along with them by inducing the enzyme Cyp3A4 because they have high binding affinity towards it also and hence can cause its inhibition. These phytochemicals have High GI absorption and less toxicity and can give them on weekly basis or more frequently than that of Rifampin which can shorten the treatment period and patient can get relieve at the earliest.

4.2 Discussion

Leprosy is a neglected disease and is not considered a major problem in current situation as its prevalence has decreased in the world . But it is still affecting the life of majority of people in the countries like India, Indonesia etc. where thousands of leprosy cases registered each year. It is treatable if detected early, otherwise the damage to nerve caused by it will not be retreated with antibiotics. To treat the condition of leprosy there are many drugs but all have some limitations.

A good drug which has least or no major toxicity, so that a new critical condition not arise during the treatment of the disease. A good drug must have high GI absorption so that it will be effectively absorbed by the body and complete dose will be utilise to get the best result. A drug with good water solubility is considered best because a drug with more water solubility will have more GI absorption. In our study most of the drugs are not permeant to Blood brain barrier which is good but if they are permeant to BBB, it will be not harmful as these drugs do not have toxicity related to brain or nervous system. Out of 33 only 5 phytochemicals are selected because all the other phytochemicals have binding affinity against DNA- dependent RNA polymerase beta subunit is less than that of Rifampin against this target, while these selected phytochemicals have high good water solubility, High GI absorption, less toxicity, bioavailability score between 0.55 and 1, none of these have mutagenicity, carcinogenicity, cytotoxicity, having good LD50 value, none of these phytochemicals violets the Lipinski Rule.

4.3 Future Scope

The discovery of phytochemicals as a potential Rifampin Substitute for leprosy has several encouraging aspects. Firstly, Mycobacterium leprae is evolving resistance to these drugs; therefore, compounds like phytochemicals may be the most effective in preventing the development of resistance. (2) In addition to their antibacterial and immunomodulatory qualities, phytochemicals can help fight leprosy's inflammatory responses and strengthen our bodies' defences against infection. (3) While traditional medications such as Rifampin have a variety of adverse effects, phytochemicals, which have been used for a long time, are thought to be safer and have fewer minor side effects. (4) In regions where leprosy is an endemic illness, such as India, Brazil, etc., traditional herbs may be more beneficial since the plants are adapted to the local environment and culture and complement regional medical procedures, which will improve treatment acceptability and adherence. (5) Access to costly antibiotics may be restricted in underdeveloped nations with high leprosy rates. In such instances, treating patients using substances derived from readily available native plants would be a quick and affordable solution for everyone.

Although phytochemicals have many therapeutic benefits, there are certain drawbacks to these substances that need to be taken into account. (1) Because each plant has so many differences, it is necessary to assure the optimum extraction techniques for phytochemicals in order to preserve consistency and quality of phytochemicals. (2) Strict scientific proof of these substances' safety and effectiveness is needed. (3) To confirm their safety and efficacy, more investigation is needed, as well as preliminary clinical studies.

4.4 Social Impact

Discovering phytochemicals as a Rifampin substitute has a number of important societal ramifications. (1) For those living in places where antibiotics are expensive or hard to get by, this will provide an accessible and cheap choice. (2) By proving that phytochemicals are effective against leprosy, we can support the integration of traditional medicines into contemporary healthcare systems and empower traditional practitioners. (3) These studies promote better acceptability and treatment uptake among afflicted populations and are more culturally and trust-worthy than contemporary medications. Local communities are involved in the process of phytochemical-based therapy, which promotes a sense of ownership and participation. (5) The preservation of cultural legacy and natural resources, as well as the protection of plant biodiversity, will be aided by public awareness of the numerous medical uses of plants. (6) Leprosy is frequently linked to prejudice and stigma, which causes affected people to become socially isolated and marginalized. Treatments that are easily accessible and efficient, like those based on phytochemicals, can help lessen the stigma attached to the illness by providing hope for recovery and reintegration into society.

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Work history

- As volunteer at Nanhe Pakshi NGO (2022 - current)

- Worked well in a team setting, providing support and guidance.
- Strengthened communication skills through regular interactions with others.
- Self-motivated with a strong sense of personal responsibility.

- As a home tutor (2018 - current)

Certification

- Diploma in application software
(good at computer fundamentals, MS office, Photoshop, Corel draw, PageMaker, Illustrator, Tally ERP 9/Tally prime)

- Internship done in biochemistry, microbiology and pathology laboratories from ESIC hospital, Basaidarapur, New Delhi.

Interests

- Teaching
- Management
- Research in the field of health and environment.

Strengths

- Honesty
- Hard Work
- Presence of mind
- Concentrate
- Punctuality

Languages Known (Hindi and English)