A combined study on the Molecular Docking, ADMET Profiling and Anti-tuberculosis Activity of Phytocompounds Obtained from the Barks of *Cassia auriculata* as Potential Inhibitors of *Mycobacterium tuberculosis* (H37Rv) Protein (5HKF)

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Abstract **-** As part of this study, we investigated the anti-tuberculosis properties of barks of *Cassia auriculata* from different solvent extracts and identified phytochemicals by gas chromatography-mass spectrometry (GC-MS).A high level of terpenoids, alkaloids, phenolic compounds, and steroids was found in petroleum benzene and ethanol extracts. We performed molecular docking studies with 5HKF, the selected inhibitor protein of Mycobacterium tuberculosis.It was determined by computer-aided drug design using virtual screening that previously known anti-tuberculosis drugs inhibit the *Mycobacterium tuberculosis* (H37Rv) Protein (5HKF). Using Molinspiration cheminformatics, repurposed drugs were evaluated for their drug-likeness in comparison to original drugs, while ADMET and pkCSM webservers were used to analyze their absorption, distribution, metabolism, excretion, and toxicity.According to this study, N-methyl-N-nitrosoadenosine (-7.2 kcal/mol),simiarenol (-6.9 Kcal/mol), 6,7,8,14-Tetradehydro-3-methoxy-17-methylmorphinan (– 6.5kcal/mol), and 4-Acetyl-5-(2-fluorophenyl)-3-hydroxy-1-methyl-1,5-dihydro-2H-pyrrol-2- one (–6.5 kcal/mol) were significantly inhibiting against *Mycobacterium tuberculosis* with superior pharmacokinetics, drug-likeness, oral bioavailability, bioactivity properties, and ADMET properties in comparison to isoniazid (-4.3 kcal/mol). These observations may lead to further research on the cure and management of tuberculosis.

Key words**:** *Cassia auriculata* bark, GCMS Analysis, Molecular docking study, ADMET, anti-tuberculosis activity

I. INTRODUCTION

About eight million people are infected with Mycobacterium tuberculosis every year, and about 2-3 million of them die from it [1]. It is estimated that 33% of the world's population is afflicted by tuberculosis (TB), with 40% only coming from India. [2]. As per the World Health Organization, tuberculosis is a severe illness caused by the Mycobacterium tuberculosis [3], which is predominantly associated with pulmonary tuberculosis (also called pulmonary

tuberculosis) [4].The current TB treatment strategies are only for active tuberculosis and do not address its associated side effects.There has been a worldwide search for new anti-TB drugs over the past few years due to the emergence of multidrug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB).In order to improve treatment strategy, enhance safety, and address infection and side effects, new drugs must be discovered and developed [5]. Due to their chemical diversity and important role as phyto-drugs, plant based natural products can serve as therapeutic drugs for TB in the absence of effective drugs [6]. It is an undying gift of nature that medicinal plants are used for the treatment of numerous diseases in humans since the beginning of time. In developing countries, 80% of the population relies on traditional medicines for primary health care, according to WHO estimates [7]. Many ailments, including TB, can be treated with medicinalplants extracts and phytochemicals.*In vitro* studies have shown that traditionally used medicinal plants, such as *Calpurnia aurea* roots, *Ocimumbasilicum* seeds, *Artemisia abyssinica* leaves, *Croton macrostachyus* leaves, *Artemisia afra* leaves and *Eucalyptus camaldulensis* leaves, possess antibacterial activity [8].

Anti-TB drugs have side effects such as GIT manifestations, hepatotoxicity, ototoxicity, nephrotoxicity, skin rashes, fever, peripheral neuritis, and rarely psychotic changes [9]. In the case of the first line anti-TB drugs isoniazid (INH), pyrazinamide (PZA) and rifampicin (RIF), toxicities are experienced inside tissues, primarily in the liver, leadingto hepatitis [10]. As well as developing Multi Drug Resistance (MDR) TB, liver toxicity caused by anti-TB drugs is one of the leading reasons for patients to discontinue treatment [11]. In the case of TB/MDR-TB patients, multi-chemotherapy is required, which further adversely affects the health of the individuals [12]. RIF, a drug used to treat tuberculosis, inhibits the export of bile salts, causing hyperbilirubinemia[13]. In addition, it affects the activity of several key enzymes, including alkaline phosphatase (ALP), serum glutamicoxalate transaminase (SGOT or AST), serum glutamic pyruvic transaminase (SGPT or ALT),and g-glutamyl transpeptidase (gGT) [14]. A plant extract can prevent cellular damage and significantly restore normal levels of hepatic enzymes when there are any hepatic anomalies. Plant compounds such as glycosides, flavonoids, triterpenes, and phenols have been shown tobe hepatoprotective in many studies [15]. Phytochemicals are potent antioxidants that also scavenge free radicals in the liver, preventing excessive lipid peroxidation, and decreasing glutathione, catalase, and superoxide dismutase levels [16-17]. There is no doubt that therapeutic plants play an important role in the treatment of human wellbeing. When extracted from the plant for human consumption, it produces a physiological activity that is essential for treating TB. Alkaloids, flavonoids, tannins, and phenolic mixtures are the main constituents [18]. One of the largest reserves of medicinal plants in the world and an abundance of traditional knowledge about home remedies for different ailments exist in India [19, 20]. Currently, there is no medication-safe strain of Mycobacterium tuberculosis, which has become increasingly entangled in the treatment of the disease.In light of the declining efficacy of the standard and modest TB prescriptions, new drugs are urgently needed for treating TB. New medications are derived from plants, so look for new prescriptions from plants [21, 22].In view of above maintaining facts, we were seeking molecular docking, ADMET profiling and antituberculosis studies on the extracts of *cassia auriculata* bark phytocompound as potential inhibitors of *Mycobacterium tuberculosis* (H37Rv) Protein (5HKF).

II. MATERIALS AND METHODS

A. Collection and Authentication of Plant Material:

Cassia auriculata bark was collected during the months of October and November (2019) from Kasilingapuram village, Thoothukudi district, Tamilnadu, India (8°46'38.7"N 77°52'50.6"E). Plant specimens have been identified and authenticated by Dr. C. Babu, Head and Associate Professor of Botany, Pioneer Kumaraswamy College, Nagercoil. To remove dust from bark, the leaves were thoroughly rinsed under running water, then shade-dried at room temperature for 7-8 days. Plant bark was then ground into a fine powder and stored inan air-tight container for future use.

B. Extract Preparation:

We prepared extracts by combining 50 grams of dry bark with 250 ml of petroleum benzene (40-60 ^{o}C), benzene, chloroform, ethanol, and water in a Soxhlet extractor. In a Soxhlet loop, the solvent is poured into the loop until it becomes colourless as it is extracted [23An airtight container was used to store the extracts at room temperature to allow the solvent to evaporate. A frozen solution of the solution was stored at 4℃ for further use [24].

C. Phytochemical Analysis:

An analysis of phytochemicals in *Cassia auriculata* bark was performed according to a previously described method [25]. Various qualitative chemical tests were conducted on the extracts to determine their chemical composition profiles. A standard procedure involves extracting crude powder with various solvents and testing it to determine what phytoconstituents are present. The majority of tests are performed to determine the presence of terpenoids, steroids, fatty acids, phenolic compounds, alkaloids, saponins, and flavonoids.

D. Gas Chromatography Mass Spectrum (GC-MS) Analysis:

In order to investigate *Cassia auriculata* phytochemistry, we used GCMS analysis from Heber Analytical Instrumentation Facility (HAIF), Bishop Heber College, Trichy-620 017.In this study, gas chromatography-mass spectrometry analyses were performed using GC-MS equipment (GC MS QP2020; SHIMADZU), which is composed of an autosampler, an injector, a gas chromatograph (GC-2010) and a mass spectrometer.A GC-MS system was composed of a capillary standard non-polar column SHRxi-5Sil-MS (dimensions: 30.0 m, diameter: 0.25mm, film thickness: 0.25µm) made from 100% Dimethyl polysiloxane. An electron ionization system was used with an electron ionization energy of 70 eV. It was carried out with helium gas (99.99%) at a rate of 1.20ml/min and an injection volume of 5µl (split ratio: 10). The oven temperature was programmed from 50℃ (isothermal for 2 min.), increasing to 280℃ for 10 min. Mass spectra were taken at 70eV at a scan interval of 0.3 seconds with scan range of 50 - 500 m/z. A total of 21 minutes were spent running the GC. We calculated the percentage of each component based on its average peak area divided by the total peak area. To analyze mass spectra and chromatograms, we used Shimadzu's GC- MS real-time software package.

E. Identification of components:

Based on data from the National Institute Standard and Technique (NIST14) [26] and WILEY8 [27] containing more patterns, GC-MS mass spectra were interpreted.A comparison was made between the spectrum of the unknown component and that of the known components in the NIST and WILEY libraries. Every component of the test material was identified by its molecular formula, name, molecular weight, and structure.

F. Molecular Docking Studies:

Preparation of Ligands:

We used 26 phytocompounds as ligands and clinical drugs (Isoniazid) as standards, both of which have been shown to be probable inhibitors of Mycobacterium tuberculosis (H37Rv) Protein (5HKF).The 3D structures of the ligands and standards were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov).

Preparation of Target Receptor:

In this study, *Mycobacterium tuberculosis* (H37Rv)Protein (PDB ID:5HKF) was used as the target receptor. The crystal structure was retrievedfrom the protein data bank (RCSB) (http://www.rcsb.org/pdb). In the host, *Mycobacterium tuberculosis* (H37Rv) [28] mediates replication and transcription of the bacteria. Based on various reports, it is one of the main targets of potential inhibitors in the development of anti-Mycobacterium tuberculosis (H37Rv) therapeutic agents, as shown in [29]. As such, it is a crucial enzyme for thedevelopment of antimicrobial agents against Mycobacterium tuberculosis (H37Rv).

Molecular Docking Analysis:

In this case, we docked the molecules using the AutoDock Vina program, which provides grid coordinates (grid centre) as well as grid boxes of a certain size for each receptor. The ligand has a flexible state when interfacing with macromolecules under rigid conditions. AutoDock Vina was run by opening notepad and executing the configuration file. In order to perform ADT on Protein (5HKF), a PDBQT file was prepared and the grid boxsize and center were set. Kollman charges and polar hydrogen atoms were added to the structure of 5HKF. The grid size was set at 14 × 14 × 14 (*x*, *y*, and *z*) points, and the grid centre was designated at *x*, *y*, and *z* dimensions of 48.652091, 8.446159 and 57.541273 (5HKF), with a grid spacing of 1000 Å. This file was prepared and saved in PDBQT format. Ligand-binding affinities were predicted as negative Gibbs Free Energy (ΔG) scores (kcal/mol), calculated according to the AutoDock Vina scoring function [30]. The post- docking analyses were visualized with PyMOL and Discovery Studio, which provided the sizes and locations of binding sites, hydrogen-bond interactions, hydrophobic interactions,and bonding distances as interaction radii of <5 A^{o} from the docked position. Following docking of compounds to the active site of 5HKF proteins, binding poses of each ligand were observed, their interactions with the protein were characterized, and the most energetically favourable conformation of each ligand was selected [31].

G. ADMET Studies:

Swiss ADME software of Swiss Institute of Bioinformatics (http://www.sib.swiss) and pkCSM software of University of Melbourne (http://biosig.unimelb.edu.au/pkcsm/prediction) were accessed in a web server that displays the Submission page of Swiss ADMET in Google was used to estimate individual ADME behaviours of the selected antibiotics. The list is made to contain one input per molecule, defined by a simplified molecular-input line-entry system (SMILES) and the results are presented for each molecule in tables and excel spreadsheet. Calculation were done onWindow 10 Pro, Version 2021[32, 33].

III. RESULTS AND DISCUSSION

The present study was carried out on the plant *Cassia auriculata* barks to identify the presence of bioactive components. Phytochemical tests, being economical and fast, are recommended for the quality control of antituberculosis secondary metabolism. Phytochemicals were confirmed to be present in different solvent extracts of *Cassia auriculata* bark.

A. Qualitative Phytochemical Analysis of *Cassia auriculata* Bark Extracts:

Barks of *Cassia auriculata* have a bioactive value due to some chemical substances that possess a strong physiological effect on *Mycobacterium tuberculosis*. The most important of these compounds are alkaloids, terpenoids, steroids, fatty acids, and phenols.The qualitative phytochemical analysis of various solvent extracts of *Cassia auriculata* bark is showed in **Table 1**. The phytochemical analysis results revealed the presence of alkaloids, terpenoids, steroids, fatty acid and phenolic compounds. There was a high intensity ofterpenoids in petroleum benzene extract and low intensity in water extracts. Fatty acids were detected in moderate intensity in petroleum benzene, chloroform, benzene, and ethanol extracts. The presence of steroids was found in high intensity in ethanol extracts. Alkaloids were found to be in moderate intensity in ethanol and petroleum benzene extracts. Phenolic compounds were present in high intensity in ethanolic extracts. Saponin was found in moderate amounts in ethanol extracts, while flavonoids were found in moderate amounts in petroleum benzene and ethanol extracts.

B. GC-MS Analysis of *Cassia auriculata* Barks extract:

The most effective way to determine the functional groups that make up bioactive constituents of terpenoids, steroids, fatty acids, phenolic compounds, alkaloids, saponins, and flavonoids is through GC-MS. In this study, we analyse the results of Gas Chromatography - Mass Spectroscopy on the various solvent extracts of *Cassia auriculata*, as shown in **Table.2 and Fig. 1**. Among twenty compounds identified in the peteroleum benzine extract five compounds are active for antituberculosis which are diethylhexyl phthalate(8.44), 2,5,6-Trichloro-4-(diethylamino)-nicotinic acid(1.57), Callitrin(1.04), ethyl7-amino[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate(3.58), and lucidulin(7.28).The benzene extract contains twenty five compounds, five of which are active against tuberculosis, thymol(0.9), alpha-Curcumene(1.13), xanthorrhizol(3.22), diethylhexyl phthalate(1.83), and beta-curcumene(0.91).An extract of chloroform contains thirty five compounds, of which six are active against tuberculosis, that include Thymol(0.56), alpha-curcumene(0.68), xanthorrhizol(2.24), uleine(0.52), diethylhexyl phthalate(14.99), and ethyl5-[(methylamino)acetyl]-10,11-dihydro-5H-dibenzo[b,f]azepin-3-ylcarbamate(2.13).The ethanol extract contains twenty five compounds, of which eight are active against tuberculosis, namely 1-ethoxy-4-(4-propylcyclohexyl)cyclohexane(1.73), 3-(4-ethoxyphenyl)-3-[(phenoxyacetyl)amino]propanoic acid(1.58),1-(4-butoxy-2-methylphenyl)-3-(1-piperidinyl)-1-propanone(1.96), diethylhexyl Phthalate(9.82), simiarenol(9.73), N-methyl-N-nitrosoadenosine(2.41), Bis(4-butylphenyl)terephthalate(2.23), and 6,7,8,14-Tetradehydro-3-methoxy-17-methylmorphinan(9.37). The water extract identified thirty compounds out of which two were Antituberculosis compound which are hexa(methoxymethyl)melamine(0.95) and 4-acetyl-5-(2-fluorophenyl)-3-hydroxy-1-methyl-1,5-dihydro-2H-pyrrol-2-one(1.51). Few compounds like thymol, alpha-curcumene and xanthorrhizol commonly appeared both in benzene and chloroform extracts.Aqueous extract

has very few phytochemical compounds. More number of active phytocompounds were found in ethanolic extract as when compared to peteroleum benzine, benzene, chloroform and water extracts.

Table 1 Preliminary phytochemical screening of extract of powdered barks of *Cassia auriculata*

S. No	Phytochemicals	Solvents				
		Petroleum benzene	Benzene	Chloroform	Ethanol	Water
1	Terpenoids	+++	+	+	++	+
2	Steroids	++	-	-	+++	++
3	Fatty acids	++	++	++	++	+
4	Phenolic compounds	++	++	-	+++	++
5	Alkaloids	++	-	-	++	-
6	Saponin	-	+	+	++	+
7	Flavonoids	++	+	+	++	-

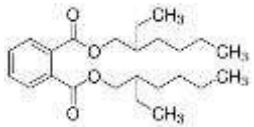
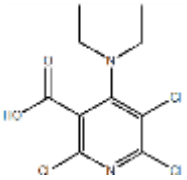
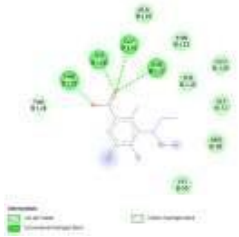
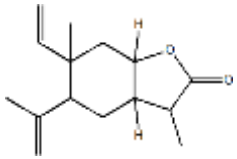
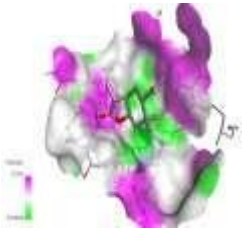
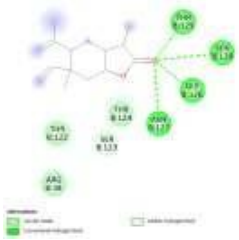
Note: + → present in small concentration; ++ → present in moderately high concentration; +++ → present in very high concentration; - → absent

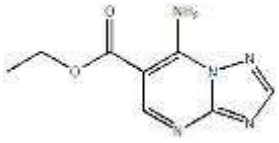
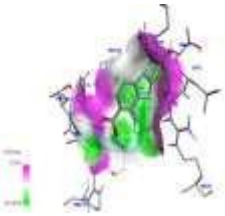
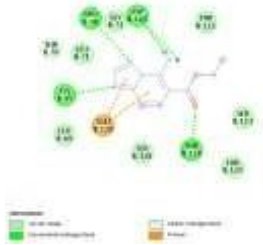
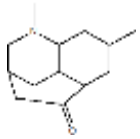
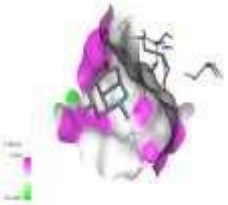
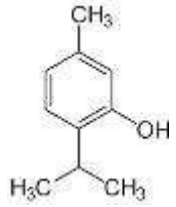
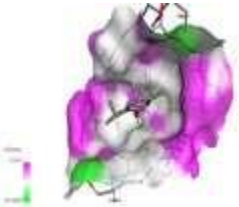
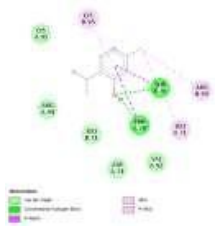
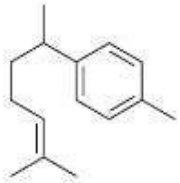
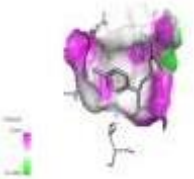
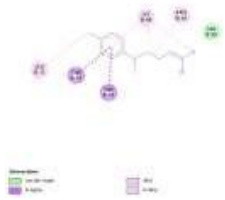
Table 2 Major phytocompounds in different solvent extracts of *Cassia auriculata* bark by GC-MS chromatogram

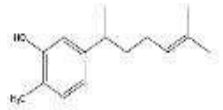
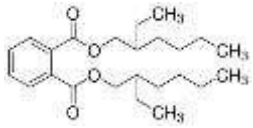
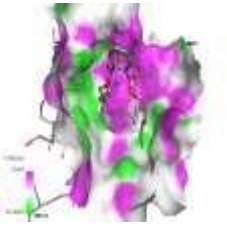
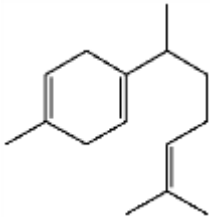
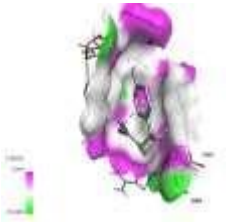
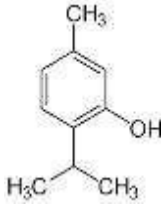
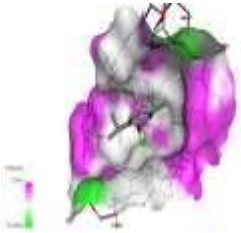
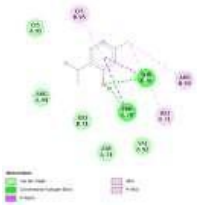
Solvent	Retention Time	Peak Area %	Name of the Compound	Molecular formula	Molecular weight	Name of the phytocompounds
Peteroleumbenzine	34.887	8.44	Diethylhexyl phthalate	$C_{24}H_{38}O_4$	390	Phthalate ester
	38.26	1.57	2,5,6-Trichloro-4-(diethylamino)-nicotinic acid	$C_{10}H_{11}Cl_3N_2O_2$	296	Alkaloid
	38.824	1.04	Callitrin	$C_{15}H_{22}O_2$	234	Elemane sesquiterpenoids
	38.855	3.58	Ethyl7-amino[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate	$C_8H_9N_5O_2$	207	Alkaloid
	38.35	7.28	Lucidulin	$C_{13}H_{21}NO$	207	Quinoline
Benzene	13.703	0.9	Thymol	$C_{10}H_{14}O$	150	Monoterpenoid
	17.885	1.13	alpha-Curcumene	$C_{15}H_{22}$	202	sesquiterpene
	23.013	3.22	Xanthorrhizol	$C_{15}H_{22}O$	218	Sesquiterpenoid
	34.884	1.83	Diethylhexylphthalate	$C_{24}H_{38}O_4$	390	Phthalate ester
	18.478	0.91	beta-Curcumene	$C_{15}H_{24}$	204	sesquiterpene
Chloroform	13.702	0.56	Thymol	$C_{10}H_{14}O$	150	Monoterpenoid
	17.884	0.68	alpha-Curcumene	$C_{15}H_{22}$	202	Sesquiterpene
	23.012	2.24	Xanthorrhizol	$C_{15}H_{22}O$	218	Sesquiterpenoid
	37.92	0.52	Uleine	$C_{19}H_{24}N_2$	280	Monoterpinoid indole alkaloid
	34.888	14.99	Diethylhexyl phthalate	$C_{24}H_{38}O_4$	390	Phthalate ester
	38.700	2.13	Ethyl5-[(methylamino)acetyl]-10,11-dihydro-5H-dibenzo[b,f]azepin-3-ylcarbamate	$C_{20}H_{23}N_3O_3$	353	Carbamate
	36.565	1.73	1-ethoxy-4-(4-propylcyclohexyl)cyclohexane	$C_{17}H_{32}O$	252	Ester

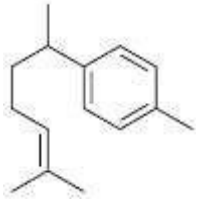
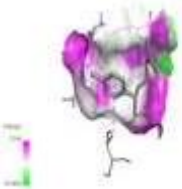
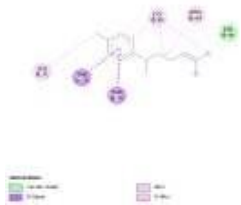
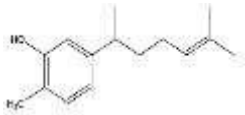
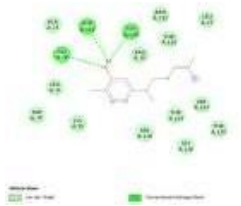
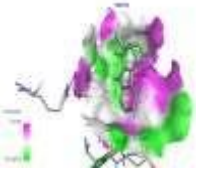
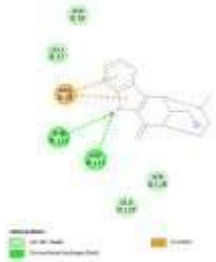
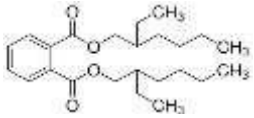
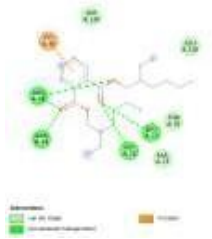
Ethanol	39.07	1.58	3-(4-ethoxyphenyl)-3-[(phenoxyacetyl)amino]propanoic acid	$C_{19}H_{21}NO_5$	343	Amino acid
	39.34	1.96	1-(4-Butoxy-2-methylphenyl)-3-(1-piperidinyl)-1-propanone	$C_{19}H_{29}NO_2$	303	Alkaloid
	34.887	9.82	Diethylhexyl Phthalate	$C_{24}H_{38}O_4$	390	Phthalate ester
	36.654	9.73	Simiarenol	$C_{30}H_{50}O$	426	Pentacyclic triterpenoid
	38.33	2.41	N-Methyl-N-nitrosoadenosine	$C_{11}H_{14}N_6O_5$	310	Alkaloid
	38.140	2.23	Bis(4-butylphenyl)terephthalate	$C_{28}H_{30}O_4$	430	Schiff base
	39.245	9.37	6,7,8,14-Tetradehydro-3-methoxy-17-methylmorphinan	$C_{18}H_{21}NO$	267	Alkaloid
Water	39.258	0.95	Hexa(methoxymethyl)melamine	$C_{15}H_{30}N_6O_6$	390	Triazine
	38.344	1.51	4-Acetyl-5-(2-fluorophenyl)-3-hydroxy-1-methyl-1,5-dihydro-2H-pyrrol-2-one	$C_{13}H_{12}FNO_3$	249	Pyrrole derivative

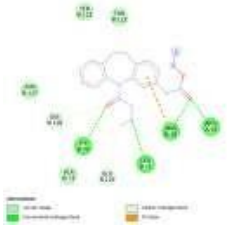
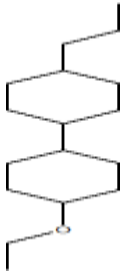
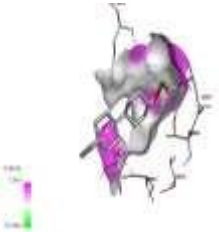
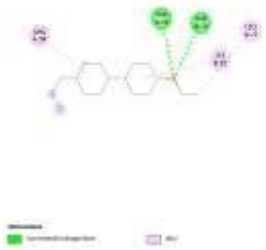
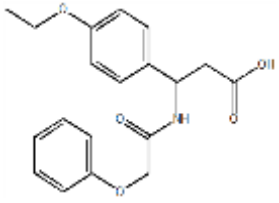
Table 3 Molecular docking analysis of *Cassia auriculata* bark phytocompounds with tuberculosis 5HKF protein

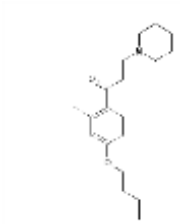
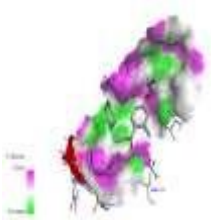
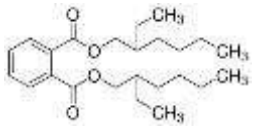
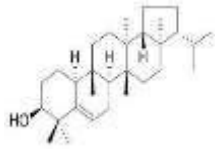
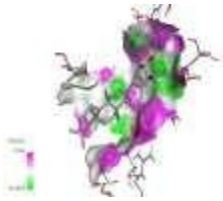
Solvent	Name of the compound	Structure	Binding Site	Binding Interaction	Interacting amino acid	Docking Score
Peteroleum benzine	Diethylhexyl Phthalate				ARG38,LEU71,GLY72,ARG94- Hydrogen Bond LYS95 –Electrostatic Bond	-4.5
	2,5,6-Trichloro-4-(diethylamino)-nicotinic acid				THR125,GLY126,ASN127,SER128- Hydrogen Bond	-4.8
	Callitrin				THR125,GLY126,ASN127,SER128- Hydrogen Bond	-5.1

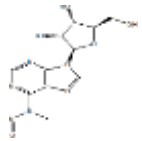
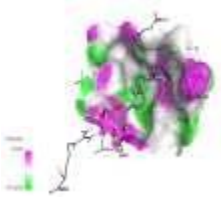
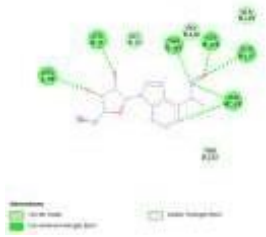
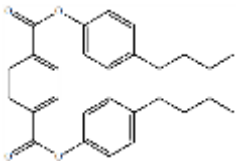
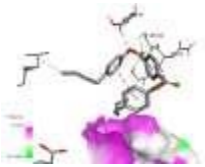
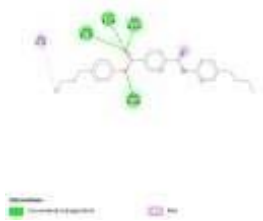
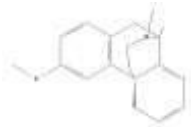
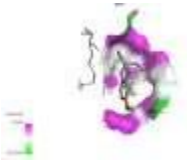
	Ethyl7-amino[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate				ARG38,LYS95,THR124,ASP121-Hydrogen Bond GLU120- Electrostatic Bond	-5.2
	Lucidulin				LYS95- Hydrogen Bond	-6.0
Benzene	Thymol				THR70 – Hydrogen Bond THR70,LEU71,ARG94,LYS95-Hydrophobic Bond	-4.9
	alpha-Curcumene				THR70,ARG94,LYS95,LEU71 – Hydrophobic Bond	-5.5

	Xanthorrhizol				GLY72,GLU120,ASP 121- Hydrogen Bond	-4.7
	Diethylhexyl Phthalate				ARG38,LEU71,GLY72,ARG94- Hydrogen Bond LYS95 –Electrostatic Bond	-4.5
	beta-Curcumene				LYS95,ARG94,LEU71 – Hydrophobic Bond	-4.7
Chloroform	Thymol				THR70 – Hydrogen Bond THR70,LEU71,ARG94,LYS95- Hydrophobic Bond	-4.9

	alpha-Curcumene				THR70,ARG94,LYS95,LEU71 – Hydrophobic Bond	-5.5
	Xanthorrhizol				GLY72,GLU120,ASP121- Hydrogen Bond	-4.7
	Uleine				ASP121,THR122 – Hydrogen Bond ARG38 –Electrostatic Bond	-5.7
	Diethylhexyl Phthalate				ARG38,LEU71,GLY72,ARG94- Hydrogen Bond LYS95 –Electrostatic Bond	-4.5

	Ethyl5-[(methylamino)acetyl]-10,11-dihydro-5H-dibenzo[b,f]azepin-3-ylcarbamate				ARG94,ARG38,LEU71,LYS95-Hydrogen Bond ARG38-Electrostatic Bond	-6.0
Ethanol	1-ethoxy-4-(4-propylcyclohexyl) cyclohexane				THR70-Hydrogen Bond ARG94,LYS95,LEU71-Hydrophobic Bond	-4.7
	3-(4-ethoxyphenyl)-3-[(phenoxyacetyl)amino]propanoic acid				ARG38,LEU71,GLY72,THR125,LEU69-Hydrogen Bond	-6.1

	1-(4-Butoxy-2-methylphenyl)-3-(1-piperidinyl)-1-propanone				LEU37,ARG38,THR124,ARG152-Hydrogen Bond	-5.0
	Diethylhexyl Phthalate				ARG38,LEU71,GLY72,ARG94- Hydrogen Bond LYS95 –Electrostatic Bond	-4.5
	Simiarenol				THR124 – Hydrogen Bond	-6.9

	N-Methyl-N-nitrosoadenosine				ARG94,LEU71,THR124,THR125,ASN127, SER128- Hydrogen Bond	-7.2
	Bis(4-butylphenyl)terephthalate				THR124,GLY126,ASN127,SER128-Hydrogen Bond LEU37-Hydrophobic Bond	-5.4
	6,7,8,14-Tetradehydro-3-methoxy-17-methylmorphinan				ARG94-Hydrogen Bond LYS95,ARG94-Hydrophobic Bond	-6.5

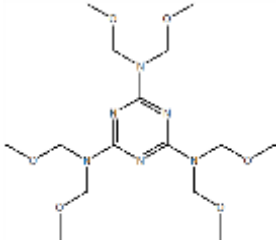
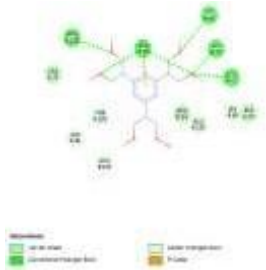
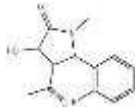
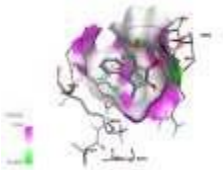
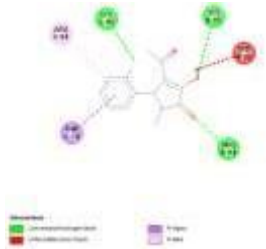
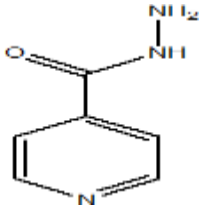
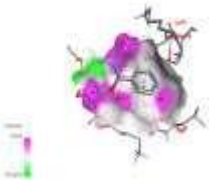
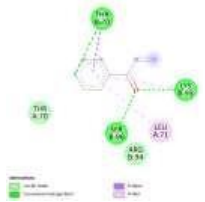
Water	Hexa(methoxymethyl)melamine				ARG38,LEU71,GLY72,LYS95,ARG152 – Hydrogen Bond ARG38-Electrostatic Bond	-5.0
	4-Acetyl-5-(2-fluorophenyl)-3-hydroxy-1-methyl-1,5-dihydro-2H-pyrrol-2-one				LYS95,ARG94-Hydrogen Bond THR70,ARG94-Hydrophobic Bond	-6.5
Standard	Isoniazid				THR70,LYS95,SER96 - Hydrogen Bond THR70,LEU71,LYS95- Hydrophobic Bond	-4.3

Table 4 Pharmacokinetic profile and toxicity prediction of cassia auriculata bark extracts

Property	Peteroleum benzine					Benzene					Water		Standard drug
	Pb1	Pb2	Pb3	Pb4	Pb5	B1	B2	B3	B4	B5	W1	W2	Isoniazid
Absorption Water solubility (log mol/L)(**ADME**)	Poorly soluble (-6.06)	Moderately soluble (-4.07)	Soluble (-3.79)	Very soluble (-1.70)	Soluble (-2.23)	Soluble(-3.19)	Moderately soluble (-4.52)	Moderately soluble (-4.65)	Poorly Soluble (-6.06)	Moderately soluble (-4.92)	Soluble (-2.09)	Soluble (-2.40)	Soluble (-0.56)
Caco2 permeability (log Papp,cm/s)(**pkCSM**)	1.413	1.335	1.019	0.222	1.493	1.466	1.555	1.633	1.413	1.402	0.477	1.307	1.208
Intestinal absorption(human)(**pkCSM**)	92.68	93.702	97.257	73.578	96.001	92.154	93.896	90.443	92.68	93.874	52.621	92.921	99.568
Skin Permeability (log Kp)(**pkCSM**)	-2.675	-2.729	-2.389	-2.75	-2.148	-2.055	-1.271	-1.617	-2.675	-1.21	-2.721	-3.523	-2.735
P-gp substrate(**ADME**)	Yes	No	No	No	No	No	No	No	Yes	No	Yes	No	No
TPSA(Å^2)(**ADME**)	52.60	53.43	26.30	95.40	20.31	20.23	0.00	20.23	52.60	30.00	103.77	57.61	68.01
Bioavailability Score(**ADME**)	0.55	0.85	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.85	0.55
Lipinski Rule(**ADME**)	1 violation	No violation	No violation	No violation	No violation	No violation	1 violation	No violation	1 violation	1 violation	1 violation	No violation	1 violation
Distribution(pkCSM) VDss	0.372	-1.166	0.269	-0.387	0.773	0.441	0.847	0.885	0.372	0.635	-0.352	-0.461	-1.134
BBB pereability(logBB)	-0.144	-0.047	0.583	-0.647	0.718	0.403	0.599	0.423	-0.144	0.777	-2.046	0.172	0.5
CNS permeability(logPS)	-2.189	-2.921	-2.475	-3.182	-2.619	-1.487	-1.456	-1.821	-2.189	-2.091	-3.254	-2.458	-2.82
Metabolism (Swiss ADME) CYP1A2	No	Yes	No	No	No	Yes	No	No	No	No	No	No	No

CYP2C19	No	No	Yes	No	No	No	No	No	No	No	No	No	No
CYP2C9	Yes	Yes	Yes	No	No	No	No	Yes	Yes	Yes	No	No	No
CYP2D6	No	No	No	No	No	No	Yes	Yes	No	No	No	No	No
CYP3A4	Yes	No	No	No	No	No	No	No	Yes	No	Yes	No	No
Excertion(pkCSM) Total Clearance (log ml/min/kg)	1.898	0.407	0.236	0.625	0.504	0.211	1.511	1.224	1.898	1.44	0.763	0.162	0.366
Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No
Toxicity(pkCSM) AMES toxicity	No	No	No	No	No	No	Yes	No	No	No	No	Yes	Yes
hERG I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No
hERG II inhibitor	Yes	No	No	No	No	No	No	No	Yes	No	No	No	No
Oral Rat Acute Toxicity (LD50)	1.37	2.682	1.817	2.095	2.178	2.347	1.788	2.179	1.37	1.581	2.884	2.259	2.482
Hepatotoxicity	No	No	No	Yes	No	Yes	No	No	No	No	Yes	No	No

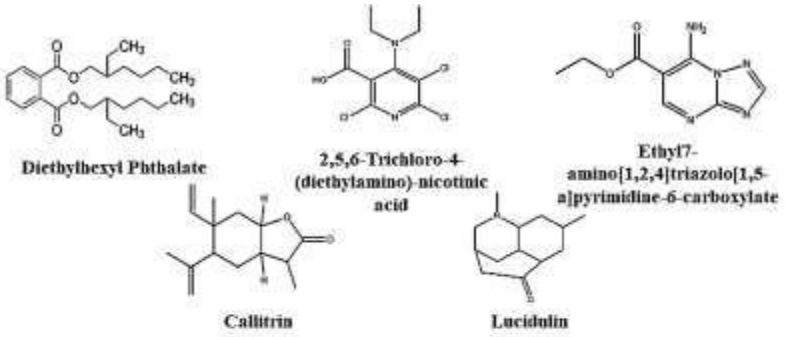
Active components: Pb1-Diethylhexyl phthalate,Pb2 - 2,5,6-Trichloro-4-(diethylamino)-nicotinic acid, Pb3- Callitrin, Pb4- Ethyl7-amino[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate, Pb5–Lucidulin; B1-Thymol, B2-alpha-Curcumene, B3-Xanthorrhizol,B4-Diethylhexyl Phthalate,B5- beta-curcumene; W1-Hexa(methoxymethyl)melamine,W2-4-Acetyl-5-(2-fluorophenyl)-3-hydroxy-1-methyl-1,5-dihydro-2H-pyrrol-2-one.

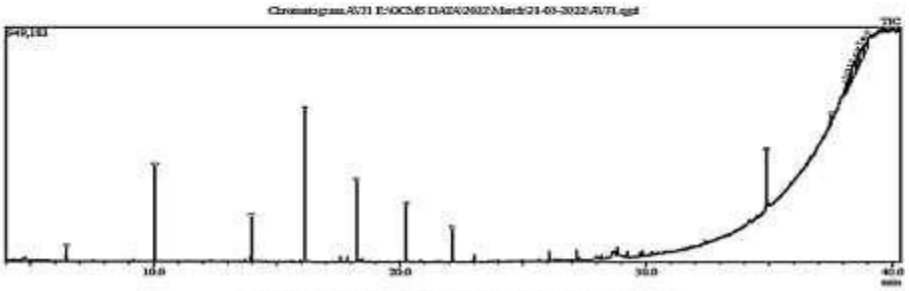
Table 5 Pharmacokinetic profile and toxicity prediction of cassia auriculata bark extracts

Property	Ethanol								Chloroform					
	E1	E2	E3	E4	E5	E6	E7	E8	Cf1	Cf2	Cf3	Cf4	Cf5	Cf6
Absorption Water solubility (log mol/L)(**ADME**)	Moderately soluble (-4.89)	Soluble (-3.30)	Moderately soluble (-4.39)	Poorly Soluble (-6.06)	Poorly soluble (-8.10)	Very soluble (-1.46)	Poorly soluble (-7.49)	Soluble (-3.66)	Soluble(-3.19)	Moderately soluble (-4.52)	Moderately soluble (-4.65)	Moderately soluble (-4.18)	Poorly Soluble (-6.06)	Soluble (-3.60)
Caco2 permeability (log Papp,cm/s)(**pkCSM**)	1.5	1.221	1.513	1.413	1.232	0.426	1.058	1.39	1.466	1.555	1.633	1.394	1.413	0.892
Intestinal human absorption(%)(**pkCSM**)	93.435	94.86	91.749	92.68	95.366	98.063	94.982	91.644	92.154	93.896	90.443	90.86	92.68	91.29
Skin Permeability(log Kp)(**pkCSM**)	-1.829	-2.729	-2.508	-2.675	-2.767	-2.735	-2.733	-2.545	-2.055	-1.271	-1.617	-2.807	-2.675	-2.938
P-gp substrate(**ADME**)	No	No	Yes	Yes	No	No	Yes	No	No	No	No	Yes	Yes	Yes
TPSA(Å^2)(**ADME**)	9.23	84.86	29.54	52.60	20.23	146.19	52.60	12.47	20.23	0.00	20.23	19.03	52.60	70.67
Bioavailability Score(**ADME**)	0.55	0.56	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Lipinski Rule(**ADME**)	1 violation	No violation	No violation	1 violation	1 violation	1 violation	1 violation	No violation	No violation	1 violation	No violation	No violation	1 violation	No violation
Distribution(pkCSM) VDss	0.63	-1.584	1.07	0.372	0.109	-0.591	-0.396	1.313	0.441	0.847	0.885	1.668	0.372	0.55
BBB pereability(logBB)	0.755	-0.39	0.627	-0.144	0.723	-1.562	-0.508	0.754	0.403	0.599	0.423	0.448	-0.144	0.143
CNS permeability(logPS)	-2.103	-2.926	-2.729	-2.189	-1.648	-3.939	-1.835	-2.599	-1.487	-1.456	-1.821	-1.094	-2.189	-2.221
Metabolism(Swiss ADME) CYP1A2	No	No	No	No	No	No	No	Yes	Yes	No	No	No	No	No
CYP2C19	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	Yes

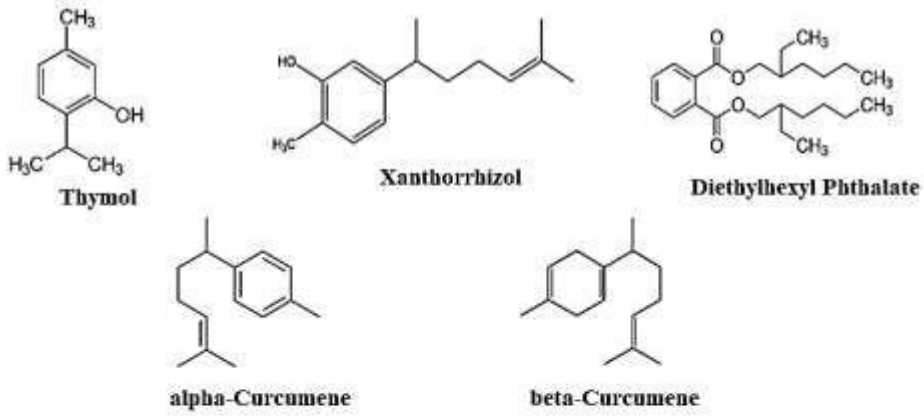
CYP2C9	Yes	No	No	Yes	No	No	No	Yes	No	No	Yes	No	Yes	No
CYP2D6	Yes	Yes	Yes	No	No	No	No	Yes	No	Yes	Yes	Yes	No	Yes
CYP3A4	No	No	No	Yes	No	No	No	Yes	No	No	No	No	Yes	Yes
Excertion(pkCSM) Total Clearance (log ml/min/kg)	1.382	0.331	1.198	1.898	0.141	0.314	1.656	0.929	0.211	1.511	1.224	0.812	1.898	0.853
Renal OCT2 substrate	No	No	Yes	No	No	No	No	Yes	No	No	No	No	No	Yes
Toxicity(pkCSM) AMES toxicity	No	No	No	No	No	Yes	No	No	No	Yes	No	Yes	No	No
hERG I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No
hERG II inhibitor	No	No	No	Yes	Yes	No	Yes	Yes	No	No	No	Yes	Yes	Yes
Oral Rat Acute Toxicity (LD50)	1.651	2.419	2.712	1.37	2.428	2.364	2.524	3.215	2.347	1.788	2.179	2.849	1.37	2.106
Hepatotoxicity	No	No	Yes	No	No	Yes	No	Yes	Yes	No	No	Yes	No	Yes

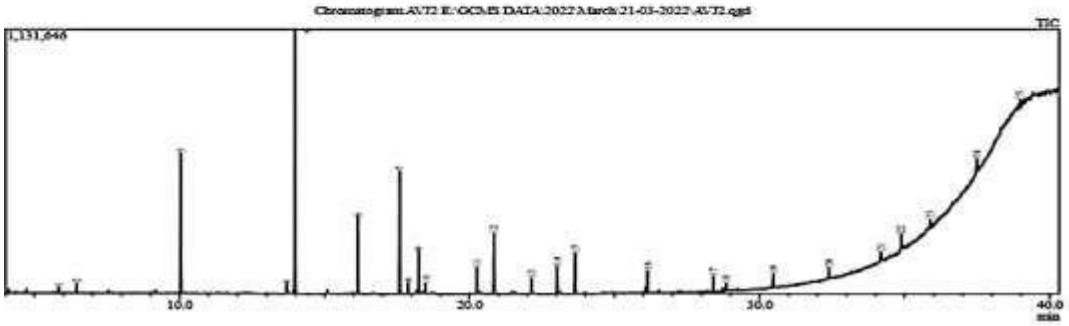
Active components: E1- 1-ethoxy-4-(4-propylcyclohexyl)cyclohexane, E2 – 3-(4-ethoxyphenyl)-3-[(phenoxyacetyl)amino]propanoicacid, E3- 1-(4-Butoxy-2-methylphenyl)-3-(1-piperidinyl)-1-propanone, E4- Diethylhexyl Phthalate , E5- Simiarenol, E6- N-Methyl-N-nitrosoadenosine, E7- Bis(4-butylphenyl)terephthalate, E8- 6,7,8,14-Tetradehydro-3-methoxy-17-methylmorphinan; Cf1- Thymol, Cf2- alpha-curcumene, Cf3- Xanthorrhizol, Cf4- Uleine, Cf5-Diethylhexyl Phthalate, Cf6-Ethyl5-[(methylamino)acetyl]-10,11-dihydro-5H-dibenzo[b,f]azepin-3-ylcarbamate.



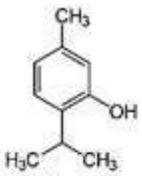


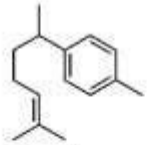
GC-MS Chromatogram of petroleum benzene extract

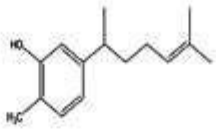


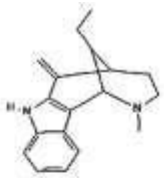


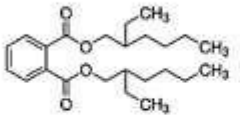
GC-MS Chromatogram of benzene extract

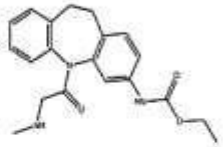

Thymol

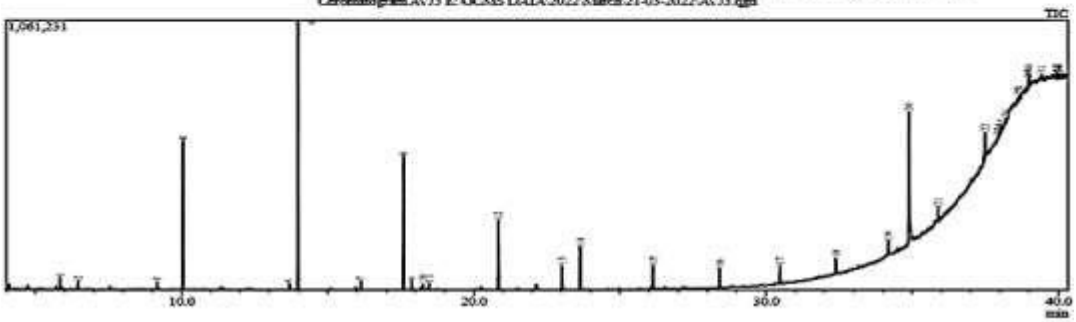

alpha-Curcumene


Xanthorrhizol

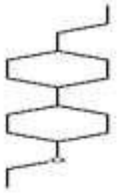

Uleine

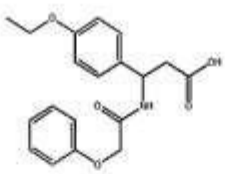

Diethylhexyl Phthalate

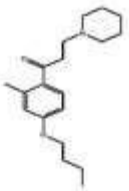

Ethyl5-[(methylamino)acetyl]-10,11-dihydro-5H-dibenzo[b,f]azepin-3-ylcarbamate

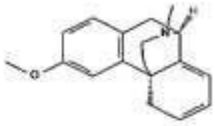


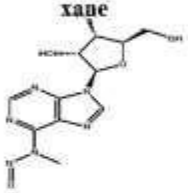
GC-MS Chromatogram of chloroform extract

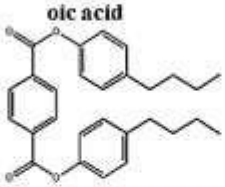

1-ethoxy-4-(4-propylcyclohexyl)cyclohexane

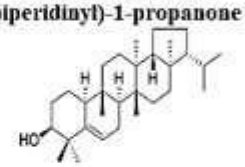

3-(4-ethoxyphenyl)-3-[(phenoxyacetyl)amino]propanoic acid

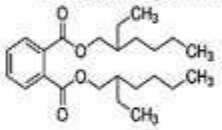

1-(4-Butoxy-2-methylphenyl)-3-(1-piperidinyl)-1-propanone

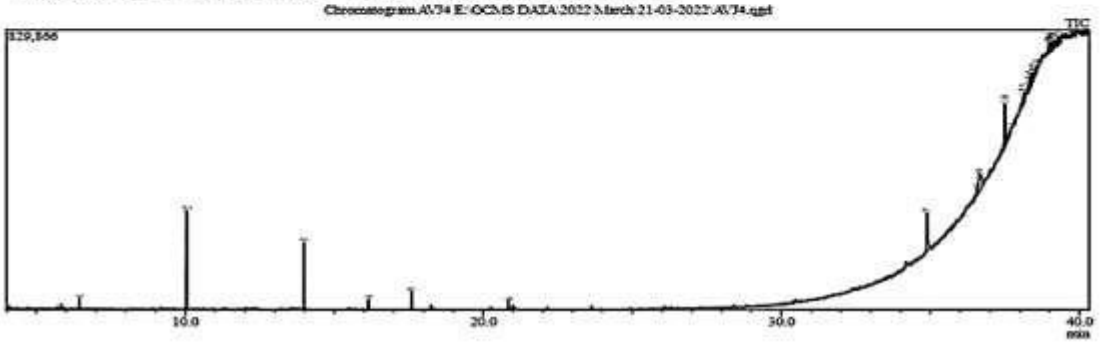

6,7,8,14-Tetradehydro-3-methoxy-17-methylmorphinan


N-Methyl-N-nitrosoadenosine


Bis(4-butylphenyl)terephthalate


Simiarenol


Diethylhexyl Phthalate



GC-MS Chromatogram of ethanol extract

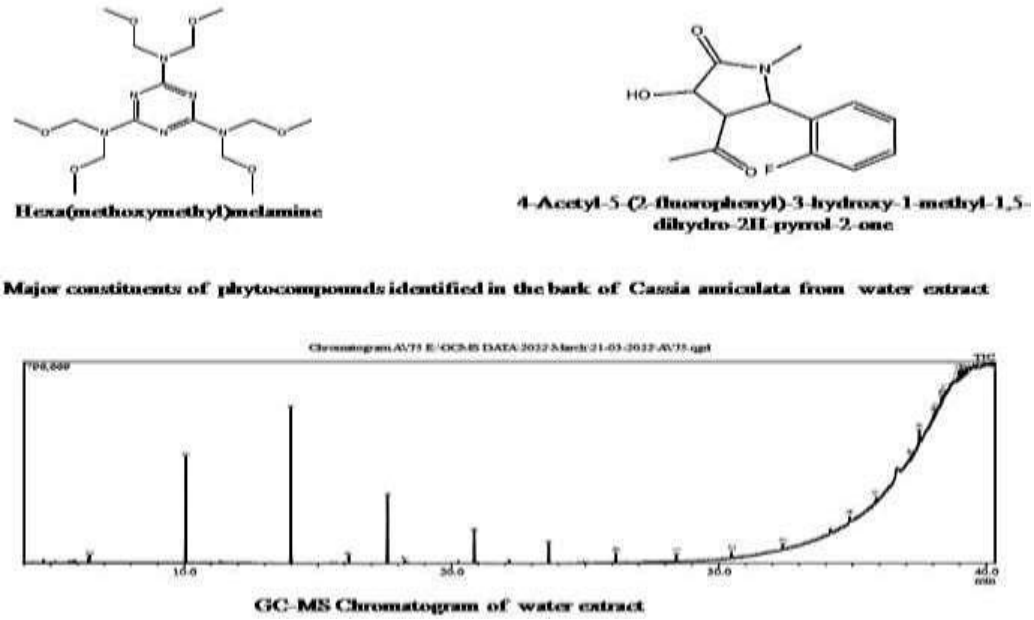


Fig. 1. GC-MS chromatogram different solvent extracts of cassia auriculata bark

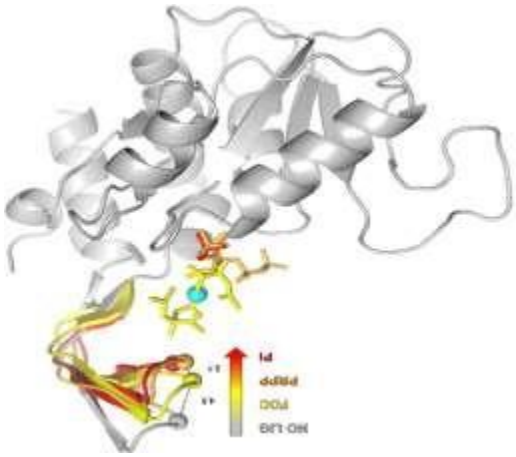


Fig. 2. ***Mycobacterium tuberculosis*** **(H37Rv) Protein (5HKF) Structure**

C. *Mycobacterium tuberculosis* (H37Rv) Protein (5HKF) Structure and Active Gauge Analysis:

The structure of *Mycobacterium tuberculosis* (H37Rv) main protease (PDB ID: 5HKF) is shown in **Fig 2**. 5HKF is a 189 amino acid protease complexed with 5-phospho- alpha-D-ribosyl 1-diphosphate (PRPP). The study of its X-ray diffraction, a = 56.2 Å, b = 58.6 Å, c = 57.2 Å, α = 90° β = 116.9° γ = 90.0°, was to a resolution of 2.25 Å. 5HKF has TASA (total accessible surface area) of 14,043(A°)^2 and R-values (fee = 0.235, work = 0.202,and observed = 0.204). The *Mycobacterium tuberculosis* main protease has two domains with

residues (4–129), and (130–189) in domain 1, and 2 [34] repectively. Its active site/binding region is located in the cleft between Domain 1 and 2.

D. Molecular Docking Analysis:

Recent developments in drug discovery/design have led to a renewed interest in this computational strategy (CADD), as it has been proved easier, faster and cheaper with an outstanding success rate in the screening of molecules for biological and chemical interactions compared to the traditional methods [35]. It speeds up the process of designing small-molecule ligands, identification of lead and optimization and sorting out of drug candidates of best fit toward the development of novel therapeutic agents. Molecular docking is an important tool in computational drug discovery that provides predictive information on the binding of small molecules to target receptor [36].The molecular docking approach has found wide application because it offers predictions with a higher degree of accuracy of binding affinities, intermolecular interactions, and conformations of ligand's molecule at receptor's binding sites [37]. The docking scores of the ligands and standards against *Mycobacterium tuberculosis* (H37Rv) Protein (5HKF) were as presented in **Table 3**. From the results, a good number of ligands displayed activity comparable to those of standards. Binding affinities for the standard isoniazid was – 4.3 kcal/mol while those of the ligands were between – 4.5 kcal/mol and – 7.2 kcal/mol. Overall, N-methyl-N-nitrosoadenosine with binding affinity – 7.2 kcal/mol had the most outstanding inhibitory activity.Amino acids involved in its hydrogen bond interaction with receptor molecules are ARG94,LEU71,THR124,THR125,ASN127,SER128. Simiarenol exhibited one hydrogen bond interactions (THR124), which resulted in a binding energy of -6.9 Kcal/mol. In 6,7,8,14- Tetradehydro-3-methoxy-17-methylmorphinan, there was one hydrogen bond interaction (ARG94) and two hydrophobic interaction (LYS95,ARG94), with amino acid residues, with the binding energy being -6.5 Kcal/mol. 4-Acetyl-5-(2-fluorophenyl)-3-hydroxy-1-methyl- 1,5-dihydro-2H-pyrrol-2-one exhibited two hydrogen bond interaction (LYS95,ARG94) and two hydrophobic interactions (THR70,ARG94), with amino acid residues, with a binding energy of -6.5 kcal/mol. The high binding affinity recorded, could be attributed to themultiple hydrogens, electrostatic and hydrophobic bonds involved in binding to amino acidsat the active site of *Mycobacterium tuberculosis* (H37Rv) Protein (5HKF).

To validate the significance of using in-silico approach to elucidate the bindingaffinity and interactions of ligands to the receptor as reported above, it is worthy of mentioning that this method has been widely used in notable research in the studies and design of therapeutic agents for tuberculosis diseases as observed in [38]. The inhibition potential of the drugs as ranked by the binding affinity (**Fig. 2**) and inhibition constant are as shown below:

N-Methyl-N-nitrosoadenosine(-7.2) >Simiarenol(-6.9) > 6,7,8,14-Tetradehydro-3-methoxy-17-methylmorphinan (-6.5) > 4-Acetyl-5-(2-fluorophenyl)-3-hydroxy-1-methyl-1,5-dihydro-2H-pyrrol-2-one(-6.5) >3-(4-Ethoxyphenyl)-3 [(phenoxyacetyl)amino]propanoic acid(-6.1) >Ethyl-5-[(methylamino)acetyl]-10,11-dihydro-5H-dibenzo[b,f]azepin-3-ylcarbamate(-6.0) >Lucidulin(-6.0) >Uleine(-5.7) > alpha-Curcumene(-5.5) >Bis(4-butylphenyl)terephthalate(-5.4) > Ethyl-7-amino[1,2,4]triazolo[1,5-a]pyrimidine-6-

carboxylate (-5.2) >Callitrin (-5.1)>1-(4-Butoxy-2-methylphenyl)-3-(1-piperidinyl)-1-propanone(-5.0) >Hexa(methoxymethyl)melamine(-5.0) > Thymol(-4.9) > 2,5,6-Trichloro-4-(diethylamino)-nicotinic acid (-4.8) > 1-Ethoxy-4-(4-propylcyclohexyl)cyclohexane(-4.7) >Xanthorrhizol(-4.7) > beta-Curcumene(-4.7) >Diethylhexylphthalate (-4.5) > Isoniazid (4.3)

This study furnishes N-methyl-N-nitrosoadenosine, simiarenol, 6,7,8,14-tetradehydro-3-methoxy-17-methylmorphinan, and 4-Acetyl-5-(2-fluorophenyl)-3-hydroxy-1-methyl-1,5-dihydro-2H-pyrrol-2-one (**Table 3**) as significant potential inhibitors of *Mycobacterium tuberculosis* (H37Rv) Protein (5HKF) as compared to isoniazid. Similarly, these phytocompounds with good oral and pharmacokinetic properties demonstrated broad-spectrum activity against bacterial infections including lower and upper respiratory tract infections (RTI) [39].

E. Drug-likeness and Oral Bioavailability Analysis:

Analysis of the pharmacokinetic properties of potential drug candidates is very essential in the early stage of drug discovery. According to Lipinski and his team, drug-like compounds must obey the rule of five (RO5) i.e. molecular weight (MW) $\leq$ 500 Da, number of hydrogen bond donor (HBD's) $\leq$ 5, number of hydrogen bond acceptor HBAs) $\leq$ 10 and octanol–water partition coefficient (LogP) $\leq$ 5. No more than one violation is allowed [38]. As shown in **Tables 4 and 5**, the HA, MW, HBD, HBA, and Log P values of all the selected compounds are within the acceptable range as stated in the RO5 and no compound violated more than one rule, whereas, the standard drugs used (isoniazid) have one violation respectively.

The oral bioavailability and other physicochemical properties of the selected compounds and standards obtained using the SwissADME web tool are shown in **Table 4**. The bioavailability radar gives a swift catch sight of the important physicochemical properties and drug-likeness of the selected compounds and standards [40]. As shown in **Fig. 5**, the coloured portion (pink) shows the most desirable area for each of the bioavailability properties (LIPO, SIZE, INSOLU, POLAR, INSATU, and FLEX). The octanol–water partition coefficient (XLOGP3) (**Table 4**) was used to determine the LIPO (Lipophilicity) of the selected compounds and standards.

Surprisingly, all the selected compounds and standards were in the coloured region and fall within the LIPO recommended range of - 0.7 to + 5.0. According to Lipinski rule of five (RO5), the SIZE (Molecular Weight) of a good drug candidate is expected not to be more than 500gmol^{-1}, of which of all selected compounds obey the rule.The INSOLU (insolubility) requirement of the selected compounds and standards as depicted in their ESOL(Log S) and ESOL Class revealed that N-methyl-N-nitrosoadenosine are very soluble, while simiarenol, 6, 7, 8, 14-tetradehydro-3-methoxy-17-methylmorphinan, 4-acetyl-5-(2- fluorophenyl)-3-hydroxy-1-methyl-1,5-dihydro-2H-pyrrol-2-one and standard isoniazid are moderately soluble respectively. The Total Polarity Surface Area (TPSA) whose recommended value is between the range of 20 and 130 A° was used to examine the POLAR (polarity) of the selected compounds and standards. As shown in **Table 4 and Fig. 5**, only C- 1 and C-2 fall within the optimal range while others fell apart. The fraction of carbon Sp3 (CSP3) which is expected to be the range of 0.25 and 1 and the number of the rotatable bond

which should not exceed nine are used to determine the INSATU (unsaturation) and FLEX (flexibility) of the selected compounds are standards. Interestingly, all the selected compounds fall within the INSATU recommended range of values while only isoniazid disobey the FLEX requirement. Putting together, 4-acetyl-5-(2-fluorophenyl)-3-hydroxy-1- methyl-1,5-dihydro-2H-pyrrol-2-one, simiarenol, N-methyl-N-nitrosoadenosine, and 6,7,8,14-tetradehydro-3-methoxy-17-methylmorphinan have the best oral bioavailability since all their physicochemical properties fall within the optimal coloured (pink) region.

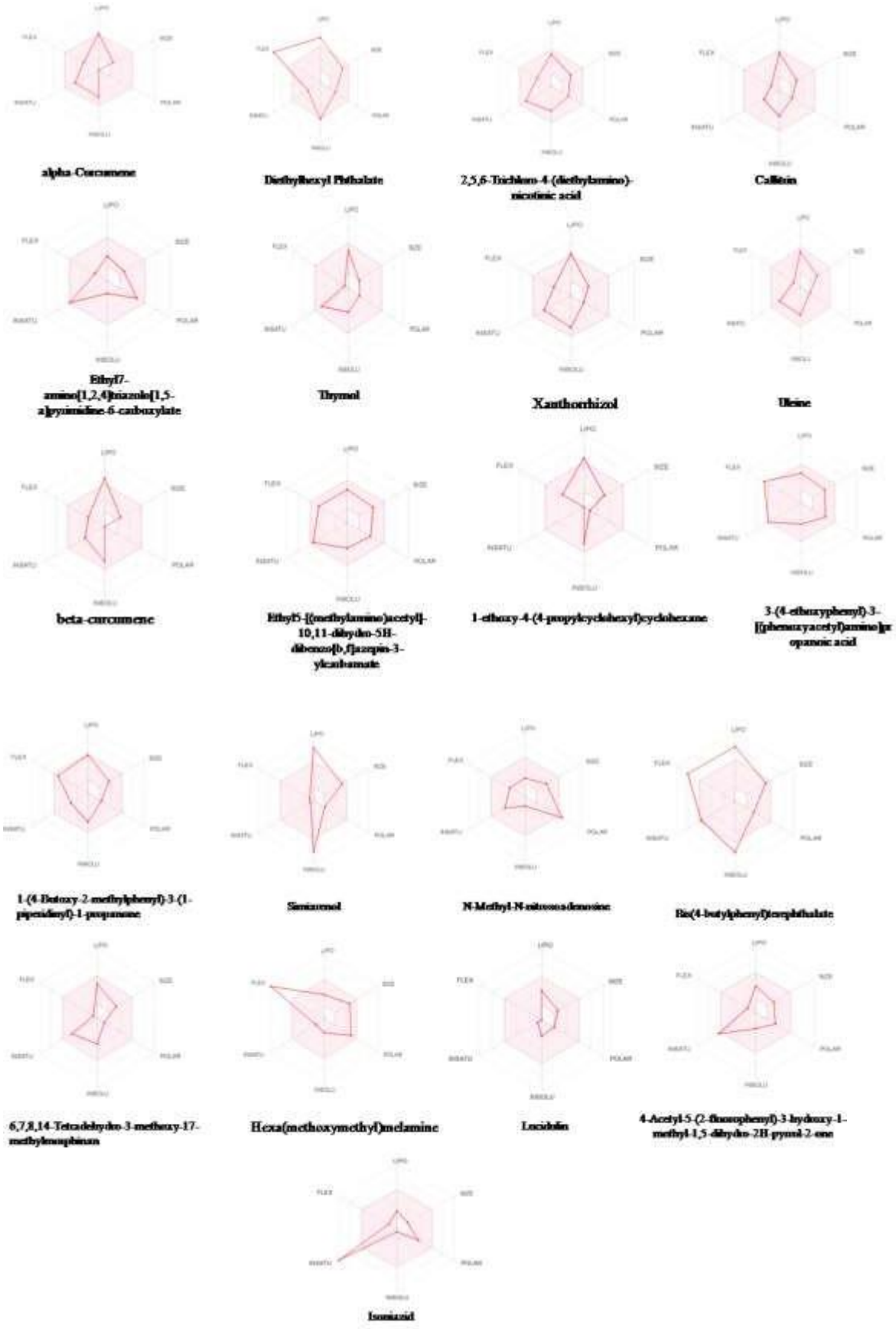


Fig. 3. Bioavailability radar picture of selected phytocompounds

F. Bioactivity of the Selected Compounds and Standards:

The bioactivity properties of the selected phytocompounds are summarized in **Table 4**. For a compound to be a Hit, its Ki value should be in a micro-molar range of 0.1–1.0 lM and not more than 10 nM for a drug [41]. For other bioactivity parameters like Ligand Efficiency (LE), Fit Quality (FQ), and Ligand-efficiency- dependent lipophilicity (LELP), theirrecommended values for a hit are C 0.3, C 0.8 and - 10 to 10 respectively [42]. Similarly, the (LE), (FQ) and (LELP) values were observed within the recommended range, although all the selected compounds obey (LELP) recommended value (see **Table 3 and 5**).

G. ADMET Properties of the Selected Phytocompounds and Standards:

The results of ADMET (absorption, distribution, metabolism, excretion, and toxicity shown in **Tables 5 and 6** are computed using the ADMET SAR2 web server. ADMET properties play significant roles in the early stage of drug discovery and development since high-quality drug candidates are to possess both sufficient efficacies against the therapeutic target as well as appropriate ADMET properties at a therapeutic dose [43]. Interestingly, all the selected antibiotics and standards have an excellent probability of being absorbed in the human intestine with HIA + values of 95.366%, 98.03%, 98.64%, 94.98% and 99.56% for 4- acetyl-5-(2-fluorophenyl)-3-hydroxy-1-methyl-1,5-dihydro-2H-pyrrol-2-one, simiarenol, N-methyl-N-nitrosoadenosine, 6,7,8,14-tetradehydro-3-methoxy-17-methylmorphinan and isoniazid. Also, these phytocompounds have an excellent probability of crossing the blood– brain barrier, an important pharmacokinetic property in drug discovery. Other selected drug candidates and the standard show negative BBB potential; although this may not be a threat since our focus in this study is not directed towards finding potential drug candidates that target receptors in the brain, like antipsychotics, antiepileptic, and antidepressant drugs do. Furthermore, a drug molecule is expected to be in an aqueous solubility range of - 1 to – 5 and the Log S values of all the selected phytocompounds and standards fall within the range, indicating that the selected phytocompounds have good absorption and distribution potential. Furthermore, microsomal enzymes (Cytochrome P450 inhibitors) were used to predict the metabolic activities of the selected drug candidates. All the selected drugs and standards are non-inhibitors of all the Cytochrome P450 which enhances their metabolism as potential therapeutic drugs. All the selected phytocompounds and standards are predicted to be non- biodegradable nevertheless, they are non-carcinogenic. Considering the AMES toxicity of theselected phytocompounds and standards *i.e*., their mutagenic abilities, all phytocompoundsare non-AMES-toxic. Also, all the selected compounds and standards possess type III oral acute toxicity indicating that they are slightly toxic although they show no eye irritation and corrosion. However, type III toxicity can easily be upgraded to type IV and become (non- toxic) during the lead optimization stage of drug discovery. The ability of a drug molecule to inhibit human hERG is very dangerous, as it can lead to blockage of the potassium ion channel of the myocardium which disrupts the electrical activity of the heart and may resultin untimely death [44]. Interestingly, all the selected phytocompounds and standards are non- inhibitor of hERG with compound C-1 and C-2 having better potential of being non-inhibitor of hERG. To summarize, all the selected compounds are safer and excellent drug candidates against the target receptor.

IV. CONCLUSION

As an indispensable CADD approach, molecular docking enables us to study drug-receptor interactions in target receptor active/binding sites. Biological and catalytic activity take place in the active gauge where potential drug candidates (ligands) inhibit replication and transcription of the target receptor. The effectiveness of some drugs for treating multiple diseases has been proved and approved. As a result, the current study used molecular docking in conjunction with other relevant analyses to screen some commercial antituberculosisagents against Mycobacterium tuberculosis (H37Rv) Protein (5HKF). Phytocompounds identified in this study (acetyl-5-(2-fluorophenyl)-3-hydroxy-1-methyl-1,5-dihydro-2H- pyrrol-2-one, simarenol, N-methyl-N-nitrosoadenosine, 6,7,8,14-tetradehydro-3-methoxy-17-methylmorphinan) may be inhibitors of Mycobacterium tuberculosis replication and transcription.As compared to standards whose randomized clinical trials have been completed, these four phytocompounds displayed outstanding bioactivity and oral bioavailability properties. Further, ADMET profiling of four phytocompounds confirmed that they were readily absorbed by humans, were non-inhibitors of Cytochrome P450, were non- carcinogenic, and were non-hERG inhibitors. However, their potency, efficacy, pharmacokinetics, and reduced toxicity can be improved during the Hit-Lead optimization stage of drug discovery, and molecular dynamics and free energy calculations can be used for further stability studies.Accordingly, we recommend further clinical trials and experimental studies of these four compound hits to find a lasting solution to the Mycobacterium tuberculosis (H37Rv) Protein (5HKF).

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