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In Silico Assessment of Jatropha curcas Bioactive Compounds as CYP1A2 and CYP3A4 Inhibitors for Cancer Therapy

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Abstract:

Cytochrome P450 (CYP) enzymes break down both exogeneous and endogenous substrates in cancer therapy, potentially causing significant interactions due to their metabolic activity. They have been shown to be overexpressed in various types of tumor cells as a result of CYP polymorphism and presence of multi-drug resistance. This finding has sparked interest in using CYP inhibitors to reduce metabolic activity, thereby optimizing anti-cancer drugs for good therapeutic outcomes. Natural CYP inhibitors have been identified as an alternative to inhibit metabolic activity due to their low toxicity and higher bioavailability. Nonetheless, their effect of lessening the obstacle caused in chemotherapy due to CYPs remains untapped to the fullest. The aim of this study is to identify phytochemicals found in Jatropha curcas and assess their CYP1A2 and CYP3A4 inhibitory potential using in silico methods. 217 compounds were obtained from online database, and twenty eight compounds were docked against CYP1A2 and CYP3A4. Two compounds, 4-(P-Methoxyphenyl)-2-(4-phenyl-2-pyridyl)-6-(2-pyridyl)-pyridine (PMPPP) exhibited the maximum binding affinity (-11 Kcal/mol) against CYP3A4 and 3-Methoxy-D-homoestra-1,3,5(10),15-tetren-17a (MDHT) had -10 Kcal/mol against CYP1A2. ADMET and drug-likeness were studied to assess the pharmacokinetic properties of these compounds. Both MDHT and PMPPP have favorable pharmacokinetic profile, which reinforces their relevance. This study presents an approach for quickly screening of compounds as CYP inhibitors and demonstrate potential interactions between phytochemicals and CYPs using in silico techniques. Furthermore, in vitro studies are essential to assess the effects of these compounds on CYPs activity and their promising roles in cancer therapy.

Keywords: Cytochrome P450; cancer therapy; Jatropha curcas; Molecular docking

INTRODUCTION

Cytochrome P450 (CYP) is a large family of heme-containing monooxygenases that play a key role in phase I drug metabolism. They are primarily found in the lipid bilayer of the endoplasmic reticulum within hepatocytes and have a significant impact on drug, hormone, and carcinogen metabolism (Iacopetta et al., 2023). Diverse CYP isoenzymes are expressed across mammalian species, including humans, with many confined to the liver, playing specialized roles in drug and xenobiotic metabolism; however, mutant expression of specific isoforms of CYP has been found in various solid tumors, including breast cancer (Al-saraireh et al., 2023; Zhao et al., 2021). Several studies have reported overexpression of CYPs in breast cancer and other forms of cancer, where they play a major role in imparting resistance (Kawahara et al., 2021). Of these CYPs, CYP1A2 and CYP3A4 are a few of the promising therapeutic targets. Both have broad substrate recognition, metabolizing 90% of the prescribed drugs, including antineoplastic agents. CYPs can increase or reduce the pharmacological effect of anticancer drugs. CYP3A4-mediated metabolic inhibition of docetaxel, an anticancer drug, explains the mechanism of pharmacokinetic resistance in this drug (Hofman et al., 2021).

The combination of ketoconazole with docetaxel has been found to improve docetaxel sensitivity in cancer cells. Lopinavir, a potent CYP3A4 inhibitor, has therapeutic potential in the treatment of colorectal cancer through inhibition of CYP3A4 (Alaei et al., 2023a). Medicinal plants, including Jatropha curcas, contain flavonoids, alkaloids, phytosterols, and other phytochemicals that function as both CYP substrates and inhibitors. As a result, they are being explored as possible anticancer agents, cancer chemoprevention agents, and anticancer drug resistance inhibitors during cancer treatment.

J. curcas is a member of the Euphorbiaceae family that is native to Central America, and its ethnomedicinal usage has been documented in many countries (Zhou et al., 2023). The plant's medicinal properties stem from its diverse chemical composition, which includes bioactive compounds such as alkaloids, flavonoids, and saponins. Preparations of all parts of the plant—seeds, leaves, and bark—fresh or as a decoction, are high in phytochemicals, which explains their application in traditional medicine and veterinary purposes (Raju et al., 2021; Souza et al., 2024). The plant possesses antibacterial and anti-HIV activities, and its latex contains an alkaloid known as “jatrophine” that may

have anti-cancer properties (Souza et al., 2024). Despite its numerous applications, J. curcas remains unexplored as a potential natural product in the prevention of pharmacokinetic anticancer drug resistance due to its inhibitory activity against human CYP1A2 and CYP3A4. In this study, bioactive compounds present in J. curcas were assessed for their drug-likeness and CYP inhibition potential.

Phytochemicals such as flavonoids, phenolic alcohol, phenolic acids, terpenes, and saponins present in J. curcas and other medicinal plants are known as inhibitors of CYP1A2 and CYP3A4 (Oyetayo and O.E., 2023). Over the past two decades, information about plant-derived CYP inhibitors has been gradually accumulating. Thus, this study aims to investigate the CYP1A2 and CYP3A4 inhibitory potential of phytochemicals from J. curcas using detailed computational analyses involving molecular docking and ADMET prediction tools to uncover potential interactions and therapeutic implications of these compounds.

2. MATERIALS AND METHOD

2.1.Compound acquisition and preparation

Two hundred and seventeen bioactive compounds from all parts of J. curcas were sourced from the IMPPAT 2.0 database, a manually curated extensive phytochemical atlas of Indian medicinal plants that is available at https://cb.imsc.res.in/imppat/ (Bhatt et al., 2022; Guleria et al., 2025). The top compounds with minimum binding energy (BE, -8.1 kcal/cal) cutoff were selected for further screening. This cutoff was based on comparison between the standard CYP1A2 and CYP3A4 inhibitors and the compounds present in J. curcas. Figure 1 showed some of the compounds present in J. curcas acquired from the IMPPAT 2.0 database. The 3D structures of these compounds, as well as those of the standard ligands, ciprofloxacin and ketoconazole (Alaei et al., 2023b; Hofman et al., 2021), were downloaded in simple data format (SDF) from the PubChem server using Open Babel in PyRx (version 0.8), which energetically transformed the ligands into the most stable structures using the Merck molecular force field 94 (MMFF94) (Mohanraj et al., 2018; Vivek-Ananth et al., 2023).

2.2. Protein preparation

The 3D structures of Cytochrome P450 1A2 (CYP1A2) (PDB ID: 2HI4) and Cytochrome P450 3A4 (CYP3A4) (PDB ID: 4D78) enzymes were obtained from the Protein Data Bank (PDB) website (http://www.pdb.org/pdb/home/home.do). Receptor preparation was performed using the "Prepare Protein" method in BIOVIA Discovery Studio 2020 (Bello et al., 2024). At physiological pH 7.4, native ligands and water molecules were removed from the crystal structure. Subsequently, the coordinates for both structures, 2HI4 (X=5.067395 Y=25.124837 Z=22.789581 RADIUS=8.702407) and 4D78 (X=-17.804055 Y=28.767932 Z=-8.952877 RADIUS=11.516019), corresponding to the active sites, were determined. Polar hydrogen was added to these structures, and the resulting 2HI4 and 4D78 structures in PDB format were extracted and saved for utilization as targets in the virtual screening process (Bello et al., 2024).

2.3 Molecular docking

The AutoDock Vina engine was utilized on the PyRx (version 0.8) platform. The docking was carried out utilizing a flexible docking strategy based on a previously described method with slight modifications (Trott, O., & Olson, 2010). The combined ligand file (.sdf) was imported as a chemical table SDF file. After importing the protein, Vina Wizard was used to dock each of the ligands, and the active sites of the protein were identified through a literature review and PDB structure visualization. The key region of interest in CYP1A2 is made up of Phe226, Gly316, and Thr385 (Dutkiewicz & Mikstacka, 2018). The active sites of interest for CYP3A4 include Val101, Asn104, Arg105, and several other amino acid residues (Kaur et al., 2016). The grid was placed such that amino acids implicated in catalytic activity, together with the other active site residues, were selected (Table 1).

To rigidify the receptor, all bonds of the ligand were allowed to rotate freely, and the grid box was adjusted based on the active sites of the protein molecule (Furth et al., 2024). Upon completion of the molecular docking studies, text files containing scoring results were created for manual comparative analysis, and ten configurations for each protein-ligand complex were generated for all phytocompounds, focusing on minimum binding energy (BE, kcal/mol). Binding energy validation and cross-comparison were not carried out in this work due to the lack of available experimental or computational data for the specific interactions between J. curcas bioactive compounds and the CYP1A2 and CYP3A4 isoforms. Consequently, the reported binding energies should be interpreted as preliminary theoretical assessment for further investigation and experimental validation.

Table 1: Active site residue of CYP1A2 and CYP3A4.

Protein	Active sites residue	References
CYP1A2 PDB ID: 2HI4	Phe226, Gly316, and Thr385	(Sansen et al., 2007), (Dutkiewicz & Mikstacka, 2018).
CYP3A4 PDB ID: 4D78	Val101, Asn104, Arg105, Met114, Ser119, Leu211, Arg212, Asp214, Asp217, Pro218, Ile301, Ala305, Thr309, Ile369, Ala370, Leu373, Glu374, Ser478, Leu479, and the "Phe-cluster" (Phe108, Phe215, Phe219, Phe220, Phe241, Phe304, Phe215).	(Kaur *et al*., 2016), (Jayakanthan et al., 2010)

2.4 Data visualization and Validation of the results

After obtaining notable docking scores, outcomes of the compounds were analyzed and validated, and a comparison of their positioning within the active site was done using the Discovery Studio visualizer (Sonawani et al., 2024). Furthermore, the specific interactions formed by each molecule within the active site were scrutinized in comparison with the standard drug.

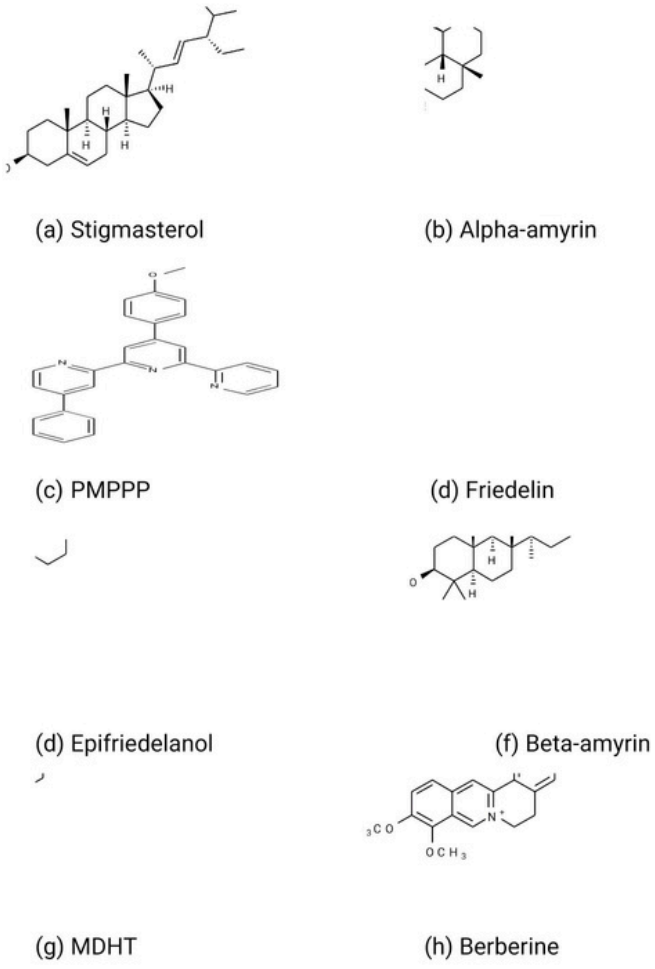


Figure 1: 2-D Structure of compounds present in J. curcas.
PMPPP - 4-(P-Methoxyphenyl) -2-(4-phenyl-2-pyridyl)-6-(2-pyridyl)-pyridine, MDHT - 23-Methoxy-D-homoestra-1,3,5(10),15-tetren-17a-one.

2.5 ADMET Profiling

To assess the ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of the selected test compounds, in silico predictive models were used. The SwissADME server was critical in determining various ADME properties, including lipophilicity (Log P), water solubility (ESOL Log S), drug-likeness based on the Lipinski rule, bioavailability score, and pharmacokinetics-related factors such as gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, permeability glycoprotein (Pgp) binding, and Cytochrome P450 (CYP) inhibition (Allouche, 2012; Fakih & Dewi, 2021). Furthermore, the ProTox-3.0 web server was utilized to anticipate the acute toxicity class, LD_{50}, and potential for hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, and immunotoxicity associated with the compounds (Allouche, 2012).

3. RESULTS AND DISCUSSION

3.1 Molecular docking and interaction studies

The top twenty-eight (28) compounds of J. curcas obtained from the Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0 (IMPPAT 2.0) online database were docked into the active sites of CYP1A2 and CYP3A4, respectively. After visual inspection of the top-ranked compounds, the potential binding sites were found for the two models, which were the active site cavities corresponding to the known inhibitors, ciprofloxacin and ketoconazole, respectively (Figures1, 2, and 3). The binding energies and type of interactions with test compounds are shown in Tables 2 and 3. MDHT exhibited the lowest binding energy (-10.7 kcal/mol) for CYP1A2, whereas PMPPP had the lowest binding energy (-11.5 kcal/mol) for CYP3A4 compared to the others (Tables 2 and 3). According to the docking data (Figure 1), the interactions between MDHT and the active site of human CYP1A2 are quite similar to those of ciprofloxacin. Similarly, PMPPP interactions with the active site of CYP3A4 are remarkably considerable with those of ketoconazole. Figures 1A and 3I show MDHT forming three alkyl bonds (Ala317, Leu497, and Phe260) and two alkyl bonds (Phe108 and Leu211) with CYP1A2 and CYP3A4, respectively. In contrast, PMPPP formed a single hydrogen bond (Gly481) with CYP3A4 (Figure 2G). PMPPP, like the standard ketoconazole, interacted with the amino acid residues in the CYP3A4 active site, including Phe57, Leu210, Leu211, Phe213, Phe215, Phe304, Ile369, Gly481, and Leu482 (Figure 2).

The accuracy of the docking procedure was assessed by comparing the lowest energy pose (binding conformation) predicted by the scoring function to the object scoring function. The metrics studied were G-score, H-bond energy, and residual interaction, which served as the foundation for discussions about the ligand's binding affinity to the protein. A negative standard free energy change suggests a higher binding affinity. Residual interactions show where the ligand binds to a specific amino acids in the protein (Wong et al., 2021).

3.2 ADMET and Drug likeness studies

The inhibition of CYP1A2 and 3A4 exhibited by the phytochemicals present in different parts of J. curcas could be explored to develop a CYP inhibitor for cancer therapy. Thus, it is crucial to consider drug likeness and ADMET properties of these compounds. In silico chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction is a fast and low-cost approach to determine compounds' therapeutic effectiveness (Lipinski et al., 2012). The Lipinski filter, based on molecular weights, hydrogen bond acceptors and donors, and lipophilicity, was applied (Idowu et al., 2020). Compounds like MDHT, berberine, isocryptotanshinone, 2-methoxyanthraquinone, heudelotine, PMPPP, phorbol, and 4-pregnene-3,6,20-trione-tri-methyl oxime, with zero Lipinski violations, are likely to be orally active. This is supported by their oral bioavailability scores (Tables 4 and 5). Out of the twenty-eight (28) J.curcas compounds that exhibited strong binding affinity with the CYP1A2 and 3A4, only eight (8) compounds with a 0.55% bioavailability score have a 55% probability of oral absorption (Sing et al., 2012). Only isocryptotanshinone has a higher bioavailability score of 0.85%, indicating an 85% probability of minimum oral absorption. This is also reflected in the GI absorption potential of the compounds, which is high for all the compounds that passed Lipinski's rule. The top compounds from both compound-CYP complexes show blood-brain barrier (BBB) permeability except PMPPP.

3.3 Toxicity analysis

MDHT toxicity projections tend to be favorable (Table 5). It belongs to the oral toxicity class 4, with LD50 values of 2000 mg/kg, whereas PMPPP belongs to oral toxicity class 3, with LD50 values of 200 mg/kg, indicating that it is safe to use within these dosage limits (Table 6). Both compounds are unlikely to be hepatotoxic, carcinogenic, mutagenic, or cytotoxic, but they may cause immunotoxicity. Further modifications may improve their safety as possible CYP1A2 and CYP3A4 inhibitors.

3.4 Biological implications

This study examined the CYP1A2 and 3A4 inhibitory potential of MDHT and PMPPP, phytochemicals found in J. curcas. The implications for this inhibition in cancer therapy include cancer chemoprevention, prevention of anticancer drug resistance, and the development of new anticancer agents that inhibit CYP. The activation and detoxification of most carcinogens and anticancer drugs are mediated by CYP enzymes belonging to the families of CYP1 or 3 (Shimada, 2017). CYP3A4 is the most prominent CYP isoform expressed in the liver and gut, and it is also responsible for the metabolism of many anticancer drugs, including paclitaxel, docetaxel, and tamoxifen. The inhibition of CYPs by natural products, with consequent reduction in CYP expression, has been explored as a possible strategy to prevent cancer development. In a study, the distinctive expression of CYP1B1 in cervical cancer cells compared to healthy cervical cancer cells revealed the possibility that CYP1B1 could be exploited as a primary prevention target for cervical cancer (Alshammari et al., 2021). In another study, natural compounds such as quercetin and apigenin were reported to inhibit CYP1A2 and 3A4 in vitro and have the potential to prevent tumorigenesis in mice through the inhibition of enzymes in the activation of 7,12-dimethylbenz[a]anthracene (7,12-DMBA), a carcinogen implicated in skin cancer (Shimada, 2017; Silvan et al., 2011). Quercetin, a natural flavonoid commonly found in medicinal plants and useful in preventing breast cancer, downregulates CYP3A4 expression and activities to reduce the metabolism of arachidonic acid to epoxyeicosatrienoic acids, which are known to promote breast cancer (Tang et al., 2023). Thus, MDHT and PMPPP, which showed strong inhibition of CYP1A2 and CYP3A4, may have the potential to be utilized as cancer chemoprevention.

Pharmacokinetic-mediated herb-drug interactions have been recognized as key drivers of drug resistance in cancer therapy. Although there is stronger evidence linking drug transporter activity to anticancer drug resistance, newer research have linked drug metabolizing enzymes to the development of anticancer drug resistance (Hofman et al., 2021). Because CYPs metabolize the majority of anticancer drugs, CYP-mediated interactions between anticancer drugs and natural products account for a significant category of metabolic herb-drug interactions (Yim et al., 2020). This interaction may have beneficial or harmful effects, for instance inhibition of CYP1A2 metabolism of vincristine by thymoquinone commonly found in Nigella sativa will increase vincristine plasma concentration and increases its therapeutic effect and adverse effects (Manthalkar et al., 2022). Similarly, overexpression of CYPs in breast, cervical, prostate, and liver cancers (Ren et al., 2016) may cause the pharmacologically active drug to be rapidly metabolized and changed to a less active form, altering the drug's pharmacokinetic profile and, as result, reducing therapeutic outcomes. Therefore, CYP1A2 and CYP3A4 play crucial role in assessing herb-drug interactions and avoiding treatment failure in cancer therapy. The current study indicated that CYP1A2 and CYP3A4 were inhibited by MDHT and PMPPP, phytochemicals present in J. curcas, a medicinal plant with anticancer activity. These compounds, may act by inhibiting the expression of CYPs in cancer cells or preventing the deactivation of anticancer drugs, thereby preventing cancer cells proliferation and deactivation of anticancer drugs.

Hoffman et al. (2020) reported the effect of ketoconazole, a potent inhibitor of CYP3A4, on the antiproliferative effect of various taxanes on HepG2_CYP3A4 and HepG2_EV cells. Attempts were made to overcome docetaxel resistance with coadministration of ketoconazole; however, little success was achieved in a clinical study (Engels et al., 2004). Therefore, the approach of preventing CYP-mediated resistance with the use of CYP inhibitors from plants should serve as an alternative. In this current study, whether compounds found in J. curcas can inhibit human CYP1A2 and CYP2A4 enzyme activities was done using molecular docking methods. Based on these results, two compounds (MDHT and PMPPP) were shown to inhibit CYP3A4, and only MDHT inhibits CYP1A2 in silico. Further in vitro and mechanistic studies could be conducted to confirm these findings. MDHT, like the standard ligand (ciprofloxacin), occupied the active site of CYP1A2, interacting with important amino acid residues. On the other hand, PMPPP, like the standard ligand (ketoconazole), interacted with the amino acid residues in the CYP3A4 active site.

The physicochemical properties affect the pharmacological activity and pharmacokinetics of compounds. The ADMET properties of compounds are a major factor in drug development (Bultum et al., 2022). The two compounds (MDHT and PMPPP) possess favorable ADMET profiles, and both showed no tendency for hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity; however, both showed a tendency for immunotoxicity. In this study, Lipinski's rule of 5 was used to assess the drug likeness of phytochemicals found in J. curcas (Lipinski et al., 2012). However, it is not all the successful drugs that meet these guidelines, and many oral drugs from natural products violate 2 or 3 of these rules (Cosconati et al., 2011). Both MDHT and PMPPP do not violate any of these rules, indicating the drug-likeness of these compounds. Therefore, these compounds could be considered for experimental studies and further development as CYP1A2 and CYP3A4 inhibitors.

The ADMET-related properties, such as molecular weight (MW), octanol-water coefficients (Log p), water solubility, gastrointestinal absorption (GIA), and blood-brain barrier permeability (BBB), are important factors in determining the drug-likeness of any compound. The log p and bioavailability score of greater than zero indicate that the compounds have a substantial bioavailability and penetrate cell membranes efficiently (Doak et al., 2014). GIA and BBB properties of a compound are also critical for drugs intended for wide use (Azman et al., 2022). All the compounds screened in this study exhibited acceptable GIA and penetrated the BBB.

Permeability glycoprotein (P-gp) influences ADMET profiles of many chemicals, and it is important to assess the interaction between the transporter protein and drug molecule (Bultum et al., 2022). It reduces cellular uptake and metabolism of chemicals by serving as an efflux pump to extrude its substrate from the interior to the exterior of cells (Amin, 2013). In the current study, MDHT is not a substrate of P-glycoprotein (Table 5), suggesting its desirable properties as a potential therapeutic agent. CYP enzymes can be induced or inhibited by phytochemicals, resulting in clinically significant herb-drug interactions that can cause increased efficacy or therapeutic failures. CYP inhibitors block the expression or metabolic activity of CYP enzymes. Hence, CYP inhibitors may be beneficial in reducing inactivation of anticancer drugs, resulting in an improved therapeutic outcome, preventing chemoresistance, and preventing cancer progression. However, these findings are preliminary theoretical assessment and there is a need for biological validation.

4. Conclusion

In conclusion, our study provides preliminary theoretical evidence that J. curcas phytoconstituents inhibit CYP1A2 and CYP3A4. Considering the fact that increased activities of these enzymes are capable of modulating anticancer drug metabolism, that could lead to treatment failure. The findings that 3-methoxy-D-homoestra-1,3,5(10),15-tetren-17a-one (MDHT) and 4-(P-Methoxyphenyl)-2-(4- phenyl-2-pyridyl)-6-(2-pyridyl)-pyridine (PMPPP) inhibit CYP1A2 and CYP3A4 may alter drug metabolism, causing improved therapeutic outcomes. However, these findings should be subjected to compound optimization and in vitro validations.

Table 2: Binding affinity of the top eight phytochemical constituents of *J. curcas* with CYP1A2.

S/N	Compounds	Binding affinity (Kcal/mol)
1.	3-Methoxy-D-homoestra-1,3,5(10),15-tetren-17a-one	-10.7
2.	2-Quinazolinamine, 3,4-dihydro-N,3-diphenyl-4- (phenylimino)-	-10.3
3.	Berberine (seed)	-9.7
4.	Vitexin	-9.4
5.	Isocryptotanshinone	-9.1
6.	1,7-Dicyclohexyl-4-(3-cyclohexylpropyl)heptane	-9.1
7.	2-Methoxyanthraquinone	-9
8.	Heudelotine	-9
STANDARD	Ciprofloxacin	-8.9

Table 3: Binding affinity of the top twenty phytochemical constituents of *J. curcas* with CYP3A4.

S/N	Compounds	Binding affinity (Kcal/mol)
1.	4-(P-Methoxyphenyl)-2-(4-phenyl-2-pyridyl)-6-(2-pyridyl)-pyridine	-11.5
2.	Friedelin	-11.1
3.	Epifriedelanol	-10.9
4.	alpha-Amyrin	-10.8
5.	Stigmasterol	-10.7
6.	3-Hydroxystigmast-5-en-7-one	-10.6
7.	beta-Amyrin	-10.6
8.	Taraxerol	-10.5
9.	Campesterol	-10.5
10.	Oleanolic acid	-10.2
11.	Cholesterol	-10.1
12.	beta-Sitosterol-beta-D-glucoside	-10.1
13.	3-Methoxy-D-homoestra-1,3,5(10),15-tetren-17a-one	-9.8
14.	Phorbol	-9.7
15.	Daucosterol	-9.6
16.	beta-sitosterol-3-O-beta-d-glucoside	-9.6
17.	4-Pregnene-3,6,20-trione-tri-methyl oxime	-9.6
18.	1,2,3,3a,3b,4,5,6,7,7a,9,10,11,12-Tetradecahydrobenzo[b]fluoranthene	-9.5
19.	Vitexin	-9.4
20.	2-Quinazolinamine, 3,4-dihydro-N,3-diphenyl-4-(phenylimino)-	-9.4
STANDARD	Ketoconazole	-9.2

Table 4: Drug-likeness and pharmacokinetics prediction of topmost *J. curcas* compound for CYP1A2.

S/N	MDHT	Berberine	Isocryptotanshinone	2-Methoxyanthraquinone	Heudelotine
Molecular weight	296.40	336.36	296.36	238.24	268.35
ESOL log S	-4.45	-4.55	-4.56	-4.24	-4.13
Solubility class	Soluble	Soluble	Soluble	Soluble	Soluble
Mean log P	3.82	2.19	2.36	1.51	3.28
Lipinski violations	0	0	0	0	0
Bioavailability score	0.55	0.55	0.85	0.55	0.55
GI absorption	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	Yes	Yes
Pgp substrate	No	Yes	No	No	No
CYP1A2 inhibitor	Yes	Yes	Yes	Yes	No
CYP2C19 inhibitor	Yes	No	Yes	Yes	Yes
CYP2C9 inhibitor	No	No	Yes	No	Yes
CYP2D6 inhibitor	Yes	Yes	No	No	No
CYP3A4 inhibitor	Yes	Yes	Yes	No	Yes

MDHT: 3-Methoxy- D-homoestra- 1,3,5(10),15-tetren-17a- one

TABLE 5: Drug-likeness and pharmacokinetics prediction of topmost *J. curcas* compounds for CYP3A4.

S/N	PMPPP	MDHT	Phorbol	4-Pregnene-3,6,20-trione-tri-methyl oxime	1,2,3,3a,3b,4,5,6,7,7a,9,10,1,12-tetradehydrobenzo[b]fluoroethane
Molecular weight	415.5	296.4	364.43	415.57	266.42
ESOL log S	-6.14	-4.44	-1.5	-5.2	-5.8
Solubility class	Insoluble	Soluble	Soluble	Soluble	Soluble
Mean log P	3.03	3.82	0.35	3.57	6.64
Lipinski violations	0	0	0	0	1
Bioavailability score	0.55	0.55	0.55	0.55	0.55
GI absorption	High	High	High	High	Low
BBB permeant	No	Yes	No	Yes	No
Pgp substrate	Yes	No	Yes	No	Yes

PMPPP: 4-(P-Methoxyphenyl)-2-(4-phenyl-2-pyridyl)-6-(2-pyridyl)-pyridine; MDHT: 3-Methoxy-D-homoestra-1,3,5(10),15-tetren-17a-one.

Table 5. Toxicity profile of the top-scoring *J. curcas* with CYP1A2.

	MDHT	Berberine	Isocryptotanshinone	2-Methoxyanthra-quinone	Heudelotine
LD50 (mg/kg)	2000	200	2000	5000	680
Toxicity class	4	3	4	5	4
Hepatotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive
Carcinogenicity	Inactive	Active	Active	Inactive	Inactive
Immunotoxicity	Active	Active	Active	Active	Active
Mutagenicity	Inactive	Active	Inactive	Active	Inactive
Cytotoxicity	Inactive	Active	Inactive	Inactive	Inactive

Table 6. Toxicity profile of the top-scoring *J. curcas* with CYP3A4.

	PMPPP	MDHT	Phorbol	4-Pregnene-3,6,20-trione trimethyl oxime	1,2,3,3a,3b,4,5,6,7,7a,9,10,11,12-tetradecahydrobenzob] fluoroethane
LD50 (mg/kg)	200	2000	9000	3000	1200
Toxicity class	3	4	6	5	4
Hepatotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive
Carcinogenicity	Inactive	Inactive	Inactive	Active	Active
Immunotoxicity	Active	Active	Active	Active	Active
Mutagenicity	Inactive	Inactive	Active	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive

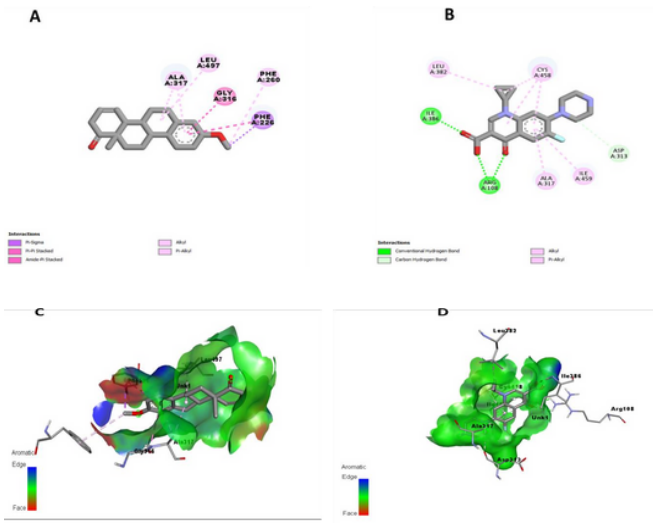


Figure 1: The 2D view of the molecular interaction of the CYP1A2 complex with (A) MDHT and (B) ciprofloxacin; the 3D view and the active site pose of the molecular interaction of the CYP1A2 complex with (C) MDHT and (D) ciprofloxacin.

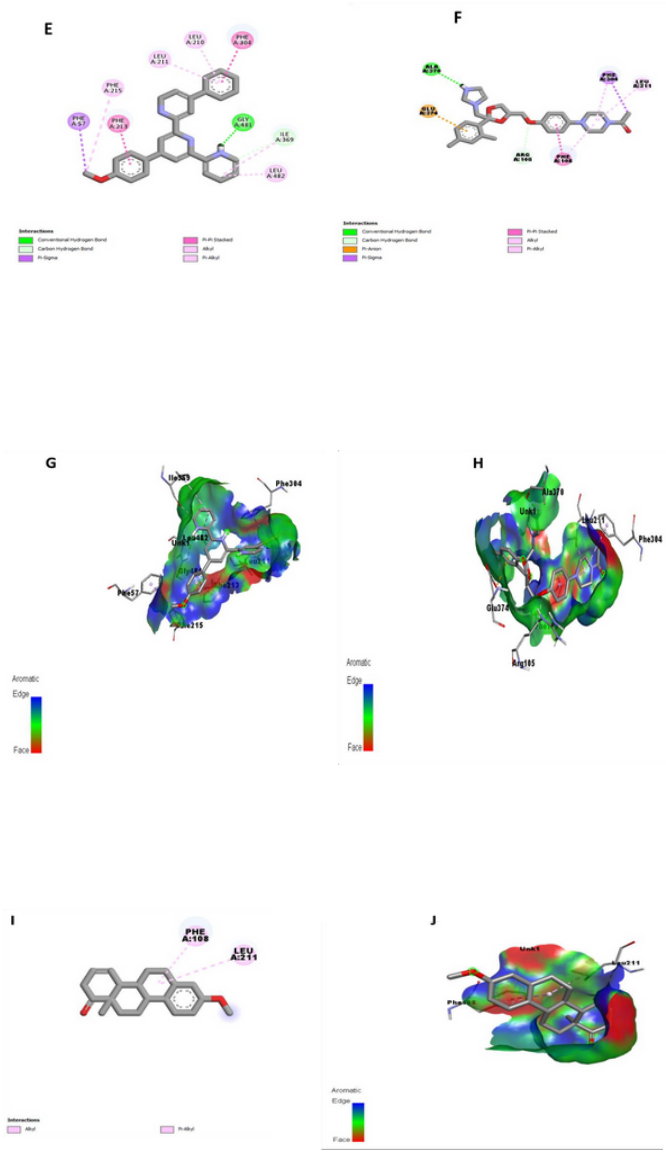


Figure 3: (I) 2D view of the molecular interaction of the CYP3A4 complex with MDHT, (J) 3D view and the active site pose of the molecular interaction of the CYP3A4 complex with MDHT.

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Conflicts of Interest
The authors did not declare any conflicts of interest.

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