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Natural Compound in Cancer Therapy: Evaluating the Efficacy of Novel Triterpenoid from *Cassia fistula* as a Potential Therapeutic Agent for Prostate Cancer (PCa) Targets

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Abstract: Natural compound research has recently gained attention in the drug discovery process due to their range of pharmacological activities and reduced toxicity, when compared to synthetic compounds. Among the natural compound, Triterpenoids are diverse molecules exhibiting numerous pharmacological activities. Prostate cancer (PCa) is still a major health issue around the world, being the second most commonly diagnosed cancer and one of the leading causes of cancer death in men. Traditional treatment methods such as chemotherapy, hormone therapy, and targeted therapy are limited in their effectiveness. This study examines the effectiveness of a natural compound, novel triterpenoid that was sourced from the medicinal plant *Cassia fistula* on the PCa targets using a computational approach. The compound was screened for Lipinski's Rule of Five and was further investigated for ADMET properties. The results suggested that the novel triterpenoid followed Lipinski's rules more closely than the reference drug docetaxel and has favourable pharmacokinetic properties such as high intestinal absorption (98%) and low blood-brain barrier permeability. Further molecular docking studies showed that novel triterpenoids had strong binding affinities toward key-PCa targets such as steroid 5′-reductase, cytochrome P450, TGF-β1, and androgen receptors for potential therapeutic benefits. These findings underscore the potential of *Cassia fistula*-derived novel triterpenoid compound in PCa treatment and warrant further experimental validation.

Keywords: Prostate cancer, *Cassia fistula*, Triterpenoids, Phytotherapy, Drug-likeliness, Docking

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Introduction

Natural compound from medicinal plants have shown promising therapeutic potential over the years, and a number of them may have therapeutic efficacy as they possess various pharmacological activities and are less toxic than synthetic drugs. A class of natural compound is triterpenoid, which have long been recognized as a diverse class of phytochemicals characterized by vast structural diversity and extensive biological activities. Triterpenoids contain thirty carbon atoms made up of six isoprene building blocks that can be found as free acids, esters or glycoside derivatives (saponins) in various parts of the plant. (Rhourri-Frih *et al.*, 2012; Alqahtani *et al.*, 2013). The medicinal plant, *Cassia fistula*, which is well recognized for its ethnomedicinal importance, has been used in Ayurveda to manage a number of health problems (Siddiqua *et al.*, 2018). This plant is native to India and Sri Lanka but has been widely distributed around the world and has a number of bioactive phyto-constituents including triterpenoids present in stems, leaves, flowers, fruit and pulp (Rahmani, 2015). Triterpenoids are abundant in many fruits, vegetables, spices, cereals, and they are important in traditional medicinal systems such as Ayurveda and Traditional Chinese Medicine (Colorado *et al.*, 2013). They possess major pharmacological effects such as anti diabetic, anti-inflammatory, antioxidant, hepatoprotective, immunomodulatory, neuroprotective, anti-parasitic and anti-tumor activities (Yadav *et al.*, 2010; Wang *et al.*, 2010; Cruz *et al.*, 2016; Jiang *et al.*, 2017; Hill and Connolly, 2017; Kumar *et al.*, 2019; Indu *et al.*, 2021). Triterpenoids may serve as signalling molecules, membrane stabilization agents and active protectant of environmental stresses, and importantly as modulators of various pathways responsible for the development of cancer (Wang *et al.*, 2010; Sun *et al.*, 2019).

Cancer, a multifactorial genetic disease, is characterized by both genomic and epigenomic alterations that cause uncontrolled cell division and proliferation. It is the second major cause of morbidity and mortality, accounting for approximately 9.6 million deaths annually. The global burden of new cancer cases is rising (expected 420 million new cases world wide by 2025)(Copur, 2019; Saini *et al.*, 2020). Prostate cancer (PCa) is the second most common malignancy in men and fifth leading cancers that contribute to death globally (Shtivelman *et al.*, 2014). In 2020 alone, there were 1.41 million new cases and 3,75,304 deaths caused by PCa (Wang *et al.*, 2022). By 2040, PCa is estimated to account for around 2.43 million new cases and 7,40,000 deaths (Culp *et al.*, 2020; Varaprasad *et al.*, 2023). PCa typically presents in middle-aged men, with the major risk factors being: family history, age, race, obesity and environmental factors (Chen and Zhao, 2013). Diagnostic work-up generally includes prostate-specific antigen (PSA) testing, MRI, and/or Trans-Rectal Ultrasound (TRUS) guided biopsy. The management of localized PCa can be done with surgery, radiation, or androgen-directed therapies, advanced stages of PCa often require multimodality treatment including chemotherapy, immune therapy, bisphosphonates, radiopharmaceuticals, and/or androgen receptor inhibitors (Leslie *et al*., 2024). These treatment modalities are rarely curative and have serious adverse effects. This has driven the exploration of alternative therapies, particularly plant-based compounds with poly-pharmacological effects (Turanli *et al*., 2018).

Conventional drug discovery approaches are expensive, laborious, and time-consuming, resulting typically in more than a decade and over a billion dollars to have a new drug available on the market. In contrast, Computational prediction tools and *in silico* drug discovery allow for rapid and inexpensive methods of screening collections of natural compounds against molecular targets, enabling the prioritization of promising candidates for *in vitro* and *in vivo* validation and supporting their advancement towards pharmacological development (Li*et al*., 2020). In this study, we examined the anti-cancer activity of novel triterpenoid derived from *Cassia fistula* against molecular targets associated with prostate

cancer, using computational approach.

Materials and Methods

Target preparation

The PDB database were used to retrieve the target proteins in 3D X-ray crystallographic structures. The targets used are Transforming Growth Factor β1(TGF β1) (PDB ID: 1PY5), Tubulin (PDB ID: 1TUB), Steroid 5'reductase (PDB ID: 7BW1), Androgen Receptor (PDB ID 5T8E), Cyclooxygenase-2 (COX-2) (PDB ID: 4PH9), Cytochrome P450 (PDBID:3RUK), Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)(PDB ID: 8TQD), Tumor protein p53 (PDB ID: 8E7A), signal transducer and activator of transcription 3(STAT 3) (PDB ID 6TLC), Jun proto-oncogene (PDB ID:1JNM). By eliminating the hetero-atoms, water molecules, or other bound ligand groups, the receptor was prepared. Polarhydrogen atoms were added using the Discovery Studio Visualizer 2017 R2 Client programme.

Ligand preparation

The ligands were downloaded from PubChem database in SDF format and were converted into PDBQT format using Open Babel for use in molecular docking software. Energy minimization was performed, via force field optimization, to stabilize binding during docking.

Prediction of Lipinski rule of five and ADMET

The chosen ligands were subjected to know their drug likeness properties by calculating the Lipinski rule of five and ADMET parameters via PKCSM web server.

Docking

Molecular docking was conducted using the AutoDock utility in PyRx (Version 0.8), in order to evaluate the binding interactions of the target protein and ligand. The protein and ligand were prepared in the PDBQT file format, for adequate torsional flexibility and structural optimization that occurs before docking. A defined grid box was created around the target protein's active site, which allowed the docking simulation to be coupled specifically to the binding region. AutoDock Vinaalgorithm were used to generate multiple binding poses, which were ranked based on their binding affinity scores (in kcal/mol). The Discovery Studio 2017 R2 Client software was used to visualize the 2-dimensional (2D) interaction profile of the ligand and the target protein after docking. This visualization allowed for key molecular interactions (e.g. hydrogen bonds, hydrophobic interactions, and π–π stacking) to be observed to determine the binding mechanism.

Results and Discussion

ADMET profiling and Lipinski's Rule of Five are important during early drug discovery to identify compounds with favourable bioavailability and safety. Lipinski's rules examine drug-likeness through a set of physical chemical properties, whereas ADMET profiling examines that selected compounds are not only biologically active but also safe and effective within the human body. Together, these two methods reduce the risk of late-stage failures. According to the drug-likeness prediction rule, the molecular weight of a compound should be between 150 and 500 g/mol. The novel compound and reference drug showed a molecular weight of 424 g/mol and 807 g/mol. Total polar surface area (TPSA) should be between 20 and 130 Å, and it was found to be 187 Å and 336 Å, respectively, The average of all predicted Log po/w should not be higher than six, which was found for compound as 5 and for reference drug as 3. The hydrogen bond donors (HBDs) should be < 5, and hydrogen bond acceptors (HBAs) should be <10. The novel compound shows H-bond donor as 2 and acceptor as 3, reference drug shows 5 donors and 14 acceptors. The molecular weight, H-bond donor, H-bond acceptor, log p-value, rotatable bond, and surface area of novel triterpenoid (compound) and docetaxel, the reference drug, are all shown in Table 1, where the novel compound adheres to all the Lipinski's rules than the reference drug.

Table 1: Drug-Likeliness parameter (Lipinski rule of five) for the compound novel triterpenoid and reference drug Docetaxel

Ligand	Mol. weight	Log P	#Rotatable bonds	#Acceptors	#Donors	Surface area
Novel triterpenoid	424	5	5	3	2	187
Docetaxcel	807	3	8	14	5	336

Table 2: Drug-Likeliness parameter Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) for the compound novel triterpenoid and Reference drug Docetaxel

Properties	Novel triterpenoid	Docetaxel
Water solubility	-5.932 log mol/L	-3.258 log mol/L
$CaCO_2$ permeability	1.401 log Pappin10^{-6}cm/s	0.521 log Pappin10^{-6}cm/s
Intestinal absorption (human)	98.237%	100
P-glycoprotein substrate	Yes	Yes
VDss (human)	-0.027 log L/kg	1.163 log L/kg
Fraction unbound (human)	0Fu	0Fu
BBB permeability	-0.716 log BB	-1.531 log BB
CNS permeability	-1.803 log PS	-3.699 log PS
CYP2D6 substrate	No	No
CYP3A4 substrate	Yes	Yes
CYP2D6 inhibitior	No	No
CYP3A4 inhibitior	No	No
Total Clearance	0.552 log ml/min/kg	-0.411 log ml/min/kg
Renal OCT2 substrate	No	No
hERGI inhibitor	No	No
hERGII inhibitor	No	No
Oral Rat AcuteToxicity (LD_{50})	2.579 mol/kg	3 mol/kg
Hepatotoxicity	Yes	No

A favourable candidate drug should have the appropriate ADMET properties at a therapeutic dose plus appropriate activity against the therapeutic target. Table 2 depicts terms of absorption, distribution, metabolism, excretion, and toxicity properties for the novel compound and reference drug. As per PkCSM theory, the value of $CaCo_2$ must be greater than 0.90. The compound demonstrates high $CaCo_2$ permeability. The intestine is the most common site for absorption; thus the absorption value should not be less than 30% which is considered poor absorption. The compound has a good absorption value of 98%. The compound is a P-glycoprotein

substrate, which can extrude xenobiotics and toxins from the cell. Considering the distribution parameter VDss - Volume of distribution illustrates that the total dose of drug need to be uniformly distributed to give the same concentration as in blood plasma, the value is considered high if it is >2.81 but this compound has low value when compared to the reference drug. The fraction unbound value of the compound has greater efficiency to traverse through cellular membrane. If compound or drug exhibited the value >0.3 log BB it readily cross the Blood Brain Barrier (BBB) and if the value is >-2 logPS it is considered to penetrate the Central Nervous System (CNS). The novel compound has a low level of penetration through the blood-brain barrier, compound has low value and it cannot penetrate the CNS and has poor absorption through the brain. Under metabolism, there are two main isoforms CYP2D6 and CYP3A4, if the drug/compound is a substrate for both isoforms the drug metabolism and clearance are affected by things that affect the activity of the enzyme, where the compound is only the substrate of CYP3A4. The compound does not act as an inhibitor for the CYP3A4 and CYP2D6 enzymes. In terms of excretion, the compound is not a substrate for renal OCT2, and it does not inhibit hERG I/II. Thus, it is shown that there is a reduced risk of cardiotoxicity, and the compound does exhibit hepatotoxicity.

Cancer is an extremely complex, multifactorial disease that includes numerous molecular pathways, which sets it apart from most other human disease. The human genome contains approximately 30,000 genes, between 6,000 to 8,000 genes are believed to be promising pharmacological targets. Out of these, only approximately 400 of the protein targets have been successfully utilized in drug discovery (Cui *et al*., 2020). The discovery and characterization of a different proteins that regulate cell proliferation, differentiation, motility, and apoptosis have triggered a revolution in the development of targeted cancer therapies. Recent research has highlighted several promising molecular targets involved in cancer pathogenesis, particularly in prostate cancer (PCa). Among these, Transforming Growth Factor-β1 (TGF-β1), tubulin, steroid 5α-reductase, androgen receptor (AR), cytochrome P450 enzymes, NF-κB, TP53, STAT3, JUN, and cyclooxygenase-2 (COX-2). These proteins have critical functions in PCa signalling cascades and have become interesting drug targets.

Tubulin, is essential for maintaining the integrity of the cytoskeleton and carrying out cellular functions like mitosis, intracellular transport, and angiogenesis. Microtubule disturbing drugs which block these cellular functions are commonly used in chemotherapy (Shaikh *et al*., 2024). Similarly, TGF-β1 is a multifunctional cytokine that has received significant attention within the literature which is based on its role in PCa as both a tumor suppressor and promoter depending on the state of disease (Ishabiyi *et al*., 2024). Steroid 5α-reductase is another important enzyme, especially in benign and malignant tissues of the male reproductive system. 5α-reductase over-expression leads to an excessive production and accumulation of dihydrotestosterone (DHT), a potent androgen contributing to androgen-dependent diseases including benign prostatic hyperplasia and prostate cancer (Apeh *et al*., 2023). Androgen signaling, which includes testosterone and DHT, plays a critical role in the development of PCa, with DHT binding the androgen receptor (AR) with high-affinity and inducing the transcription of genes that propagate cancer progression. Therefore, targeting the AR directly or inhibiting 5α-reductase are proven strategies for PCa therapy (Brito *et al*., 2019; Ikuw *et al*., 2020). In relation to this, cyclooxygenase-2(COX-2), an inducible isozyme of cyclooxygenase, is largely over-expressed within PCa cells. The COX-2 catalyzes the conversion of arachidonic acid into prostaglandins which inducing pro-inflammatory mediators undergo tumorigenesis, promote angiogenesis, and become resistance to apoptosis (Kirschenbaum *et al*., 2001).

Table 3: Molecular Docking analysis between the Prostate cancer targets with the novel compound novel triterpenoid and reference drug docetaxel

Ligand	Target	Binding affinity	H-bondinteraction
Noveltriterpenoid	TGF- β	-8.2	HIS: 283
	Tubulin	-5.5	-
	Steroid5'Reductase	-10.5	ARG:114
	AndrogenReceptor	--8.0	GLN:711;TYR:763
	CytochromeP450	-8.4	LYS:312
	NFκB	-6.8	CYS:118;HIS:117;GLY:115
	TP53	-6.8	ASP:208;ASN:210
	STAT3	-7.8	GLY:254
	JUN	-5.4	ARG:279
	COX-2	-8.8	ASN:382;THR:212
Docetaxel	TGF- β	-7.5	ARG:472;ARG:451,TRP:475;ALA:474
	Tubulin	-5.9	GLU:282;LYS:285
	Steroid5'Reductase	-7.0	SER:74
	AndrogenReceptor	-7.5	ASN:756
	CytochromeP450	-8.2	LYS:231;ARG:45;LYS:211
	NFκB	-7.8	ALA:155,ARG:156,THR:145
	TP53	-7.5	LEU:111;PHE:113,GLN:144
	STAT3	-7.0	CYS:259;ARG:350;LEU:260;LYS:348
	JUN	-4.7	-
	COX-2	-10.0	LYS:468,LEU:472,SER:471

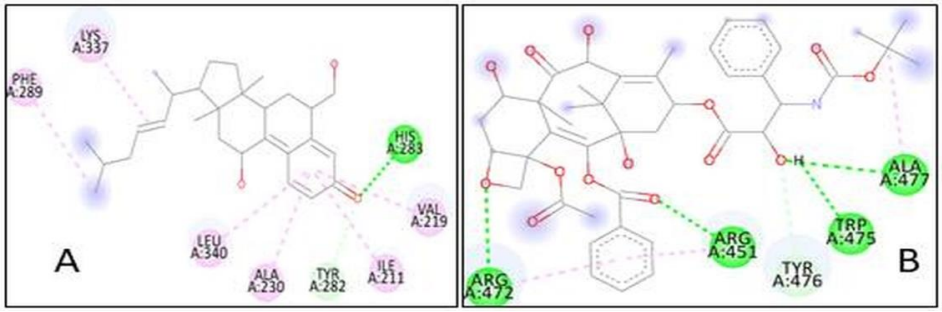

Fig. 1: TGF-β with (A) novel triterpenoid, and (B) Docetaxel.

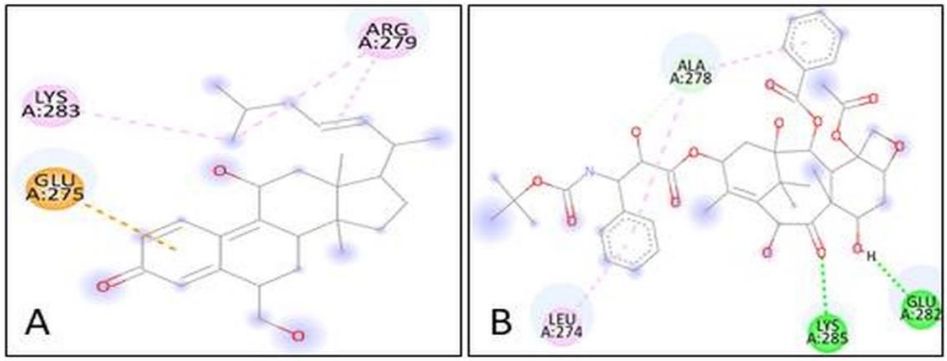

Fig. 2: Tubulin with (A) novel triterpenoid, and (B) Docetaxel.

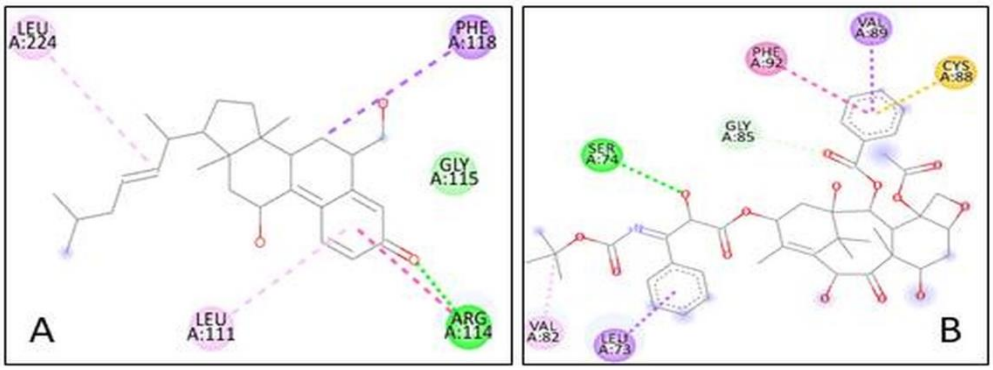

Fig. 3: Steroid 5' reductase with (A) *Cassia*, and (B) Docetaxel.

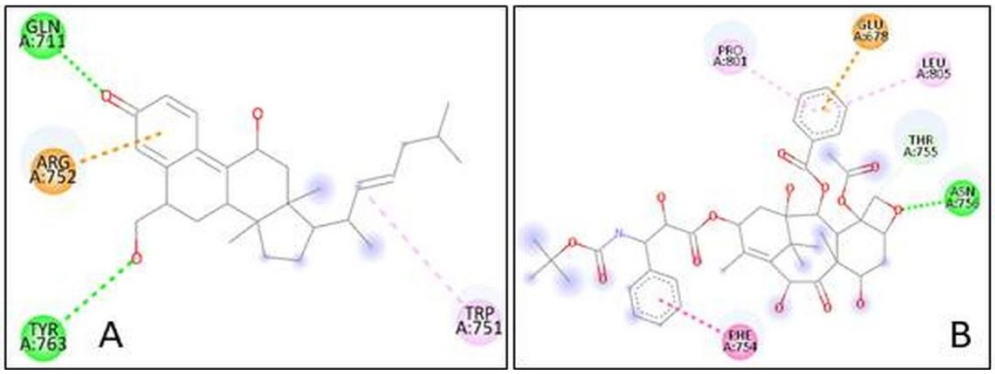

Fig. 4: Androgen receptor with (A) novel triterpenoid, and (B) Docetaxel.

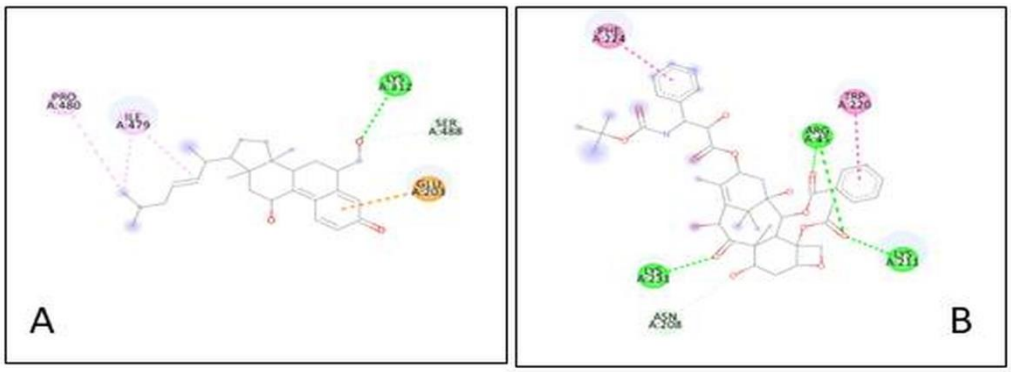

Fig. 5: Cytochrome p450 with (A) novel triterpenoid, and (B) Docetaxel.

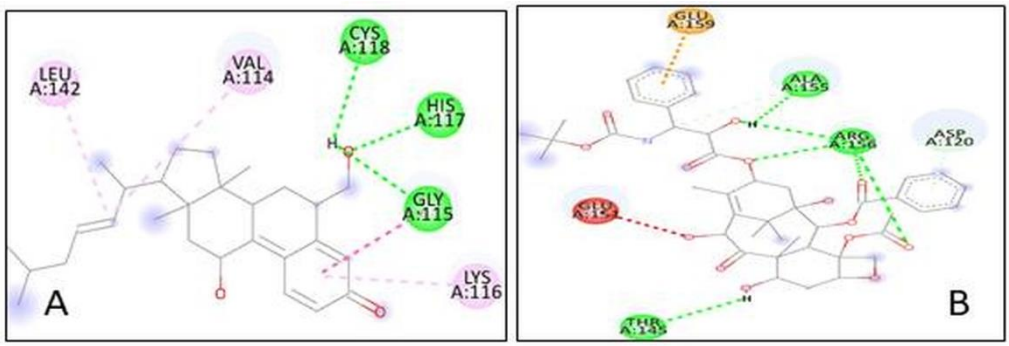

Fig. 6: NFκB with (A) novel triterpenoid, and (B) Docetaxel.

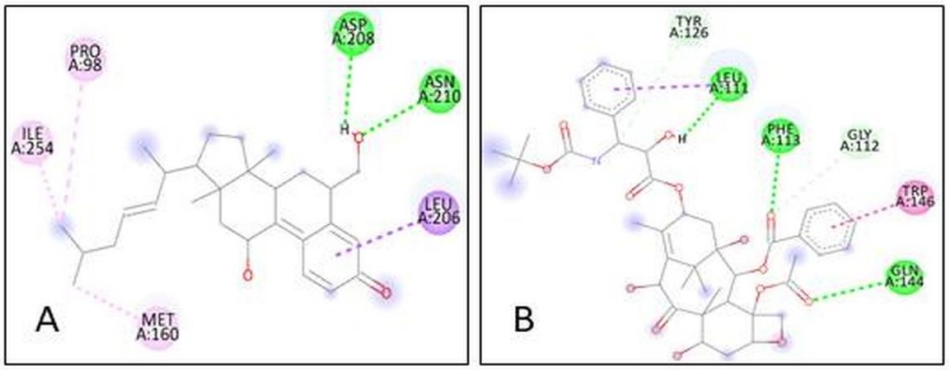

Fig. 7: TP53 with (A) novel triterpenoid, and (B) Docetaxel.

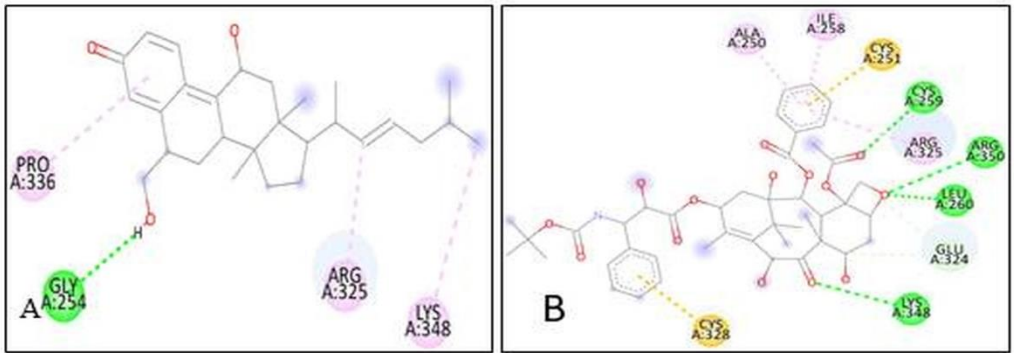

Fig. 8: STAT3 with (A) novel triterpenoid, and (B) Docetaxel.

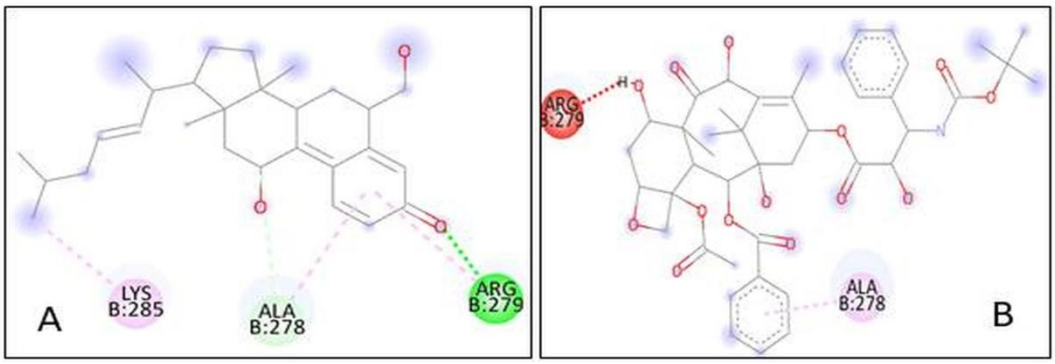

Fig. 9: JUN with (A) novel triterpenoid, and (B) Docetaxel.

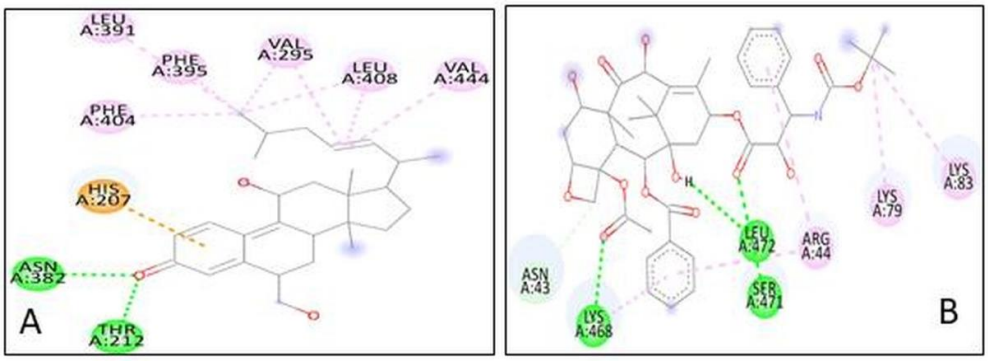

Fig. 10: COX-2 with (A) novel triterpenoid, and (B) Docetaxel.

Dysregulation of other transcription factors including TP53, STAT3, and JUN is frequently seen in PCa and are important nodes in cancer progression pathways (Prakash. 2023).

In the current study, we used molecular docking to study the interaction of a novel triterpenoid from *Cassia fistula* with multiple prostate cancer targets. Based on the docking results, the triterpenoid had significantly higher binding affinity for steroid 5α-reductase, cytochrome P450, TGF-β1, and androgen receptor compared to the standard treatment drug. The corresponding binding energies were -10.5 kcal/mol (steroid 5′-reductase), -8.4 kcal/mol (cytochrome P450), -8.2 kcal/mol (TGF-β1), and -8.0 kcal/mol (androgen receptor), indicating stable and strong interactions. The interactions stabilized by hydrogen bonding were ARG:114 (steroid 5′-reductase), LYS:312 (cytochrome P450), HIS:283 (TGF-β1), and GLN:711, TYR:763 (androgen receptor) (Table 3, Figs. 1-10). Overall, these interactions suggest that the triterpenoid compound has the potential to impact important oncogenic pathways in the management of prostate cancer by interactionwith multiple relevant targets.

Conclusion

This study demonstrates novel triterpenoids, a bioactive compound derived from *Cassia fistula,* which has promising therapeutic potential in the management of prostate cancer. The compound showed good pharmacokinetic characteristics and strong molecular interactions with important targets related to PCa, such as androgen receptors, cytochrome P450, TGF-β1, and steroid 5′-reductase. The novel triterpenoid demonstrated better adherence to drug-likeness standards and a lower risk of toxicity when compared to the reference medication. These results imply that natural substances could be effective supplements or substitutes for traditional treatments for prostate cancer. The clinical applicability of novel triterpenoid in the treatment of prostate cancer will be investigated, and these computational predictions will be further validated.

Ethical Statement

No animal or microbes have been used or sacrificed for this study, hence Ethical Approval not required.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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