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Targeting p53-MDM2 Interaction by Natural Plant Products: A Novel Approach for Future Cancer Therapy

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ABSTRACT- P53 is a tumor suppressor gene with a well established role in causation of different human cancers. The p53-MDM2 interactions have become the cornerstone of intensive cancer based research due to their effective anti-cancer properties. These potential compounds are found in many traditional natural plant products. In the present context, there is a tremendous level of enthusiasm to evaluate the pharmacological potential of various natural plants used in traditional systems of medicine. The experimental efforts to carry out such biological screening of plants are still considerably high, and therefore, computer-aided drug design approaches have become attractive alternatives. Virtual screening has established itself as a dynamic and cost-effective technology to isolate compounds with pharmacological potential. The main aim of the present study is to identify a novel or similar or better drug like compound in comparison with that of the FDA approved drug Nutlin (potent MDM2-p53 inhibitor) from the *Hamelia patens* plant, through the Structure Based Virtual Screening, Docking and Molecular Dynamic Simulation studies, for future anti-cancer therapy for future implications as a therapeutic model.

Key-words: Docking, MDM2-p53 interaction, Molecular dynamic simulation, Natural Plant products, Virtual screening

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INTRODUCTION

Cancer is an abnormal growth of cells, capable of invading surrounding tissue. This progression encompasses multiple stages, owing to the malignant potential. Heritable genetic mutation constitutes the nidus for uncontrolled proliferation. These mutations alter the quantity (or) function of the protein products that regulates cell growth and division with DNA repair.

There are two major categories of mutated genes; "oncogenes and tumor suppressor genes" [1].

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Oncogenes regulate the growth of cells whereas tumor suppressor genes suppress cell division through various processes. Tumor suppressor genes act as a check points for cell division and it is essential for cell division and their normal function [2]. If it does not function properly, cells can become awry leading to cancer. Inherited abnormalities of these tumor suppressor genes have been detected in some familial cancer syndromes. But most tumor suppressor gene mutations are acquired, not inherited. For example, acquired tumor protein gene (TP53 or p53) mutations have a wide implication in cancer causation and this defect has been detected in more than 50% of human cancers [3]. The p53, tumor suppressor gene, is in the center of normal cell division and growth. When silent these cells with spontaneous genetic mutation may progresses to tumors [1].

TP53

TP53 protein is a tumor suppressor protein and is related to control of cell proliferation [4]. TP53 has a short half life. It is localized in the nucleus of the cell, binding with DNA for regulation of cell. In time of stress, related to toxic agents

and chemicals, this protein will be the determine factor to decide whether the cell will undergo apoptosis or not. Depending on the reparability, p53 fixes the damaged DNA otherwise initiating apoptosis. This process halts the progression of the cell with mutated and damaged DNA, preventing future development of tumors. Based on this function TP53 is referred as the "guardian of the genome". [5,6]

More than 50% of human cancer occurs as a result of somatic mutation in TP53 gene. Most of these are single point mutation, producing large amount of defective proteins, which can build up preventing apoptosis and leading to malignant proliferation. Thus the damaged cells will keep on dividing in an uncoordinated way causing cancers [7].

MDM2

Mouse double minute 2 homolog (MDM2) protein, also referred as E3 ubiquitin-protein ligase, is encoded by the MDM2 gene in humans, visible in tumors only [8,9]. Being an important regulator of p53 tumor suppressor protein, MDM2 binds to N-terminal of TP53 suppressing its transcription [6]. MDM2 central zinc finger binds to ribosomal proteins degrading TP53, which was often disrupted by cancer associated mutation [10]. The high levels of MDM2 in many different human cancer types support the role of MDM2 in cancer causation.

MDM2 and p53 Interactions

TP53 can be turned "on and off" by direct gene altercation or interaction with MDM2. Development of small molecules capable of blocking MDM2-p53 interaction is a suitable technique to treat p53 related tumors. It is absolutely necessary to have an in-depth understanding of MDM2-p53 interaction at molecular level to apply modern technique for development of such compounds [11]. There has been continuous search for the natural plant products with chemotherapeutic properties [12]. The use of alternative natural plants products to target cancer cells is a promising field of research, because the conventional therapy alone is unable to curb the spread of the cancer [13].

H. patens and secondary plant products

There is a continuous trend of the use of plant products for variety of disease process although little is known about them. *H. patens,* one of the natural plants commonly used in many forms. It is a perennial shrub to tree and commonly known as scarlet, fire or humming bird bush (Fig. 1). It has elliptical to oval whorled leaves and gray-pubescent underneath with reddish veins and petioles.

H. patens have been studied chemically. It is known to contain pentacyclicoxiindole alkaloids. A number of active compounds have been found in firebush, such as apigenin, ephedrine, flavanones, isomaruquine, isopteropodine, maruquine, narirutins, oxiindole alkaloids, palmirine, pteropodine, rosmarinic acid, rumberine, rutin, seneciophylline, speciophylline, isopteropodine, stigmast-4-ene-3,6-dioneand tannin [14]. It has been used traditionally to cure multiple common ailments like skin lesion and inflammatory conditions. The objective of this study is to identify the potent MDM2-p53 interaction inhibitors for cancer therapy from the active compounds extracted from the *H. patens* leaf extracts by methanol extraction by the structure based virtual screening, docking and molecular simulation studies.

Fig. 1: *Hamelia patens* plant

MATERIALS AND METHODS

Collection and Preparation of Sample

The leaves of *H. patens* were randomly collected from Andhra Loyola College, Vijayawada, and the experiment was performed in the pharmaceutical lab of the Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, India.

Extraction of active compounds

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. Polar solvents, such as methanol, ethanol or ethyl-acetate were used for obtaining hydrophilic compounds. Different sequences of events were followed, starting with washing, drying, freezing and grinding of the plants to achieve a homogenous product. Continuous extraction was applied to extract the alkaloid through Soxhlet apparatus. Isolation and partial purification of the alkaloid was done with thin-layer chromatography (TLC).

Identification test for the presence of Alkaloids

Dragendorff's and Mayer's reagents, on crude ethanol extract of *H. patens* were used to indicate the presence of alkaloids. This extract was converted into thick syrup through evaporation. It was followed by the addition of 2N HCL (5 ml) heated for 5 minutes with stirring in water bath and cooled. 0.5 g of NaCl was added to the mixture for avoiding false positive outcome. The overall residue was then stirred, filtered and washed with 2 N HCL again and thereafter 5 ml of the final solution was obtained. The final filtrate was divided into three test tubes, each

with 1 mL of solution. The first 1 mL of the filtrate was mixed with 2–3 drops of Mayer's and second with Dragendorff's reagent respectively. The third test tube with 1 mL of the solution was used as a control after comparing with that of the known alkaloid compound (Morphine).

The isolation and partial purification of alkaloid-rich fraction of Grounded leaves of *H. patens* were done using Thin-Layer Chromatography by comparing with a known alkaloid Morphine. The mobile phase used was toluene: acetone: ethanol: ammonia (40: 40: 6: 2).

Structure Based Virtual Screening, Docking and Molecular Simulation Studies

Software and Program

Schrodinger's maestro visualization program (Maestro, v9.6, 2013) and Accelry's discovery studio 4.0 is utilized for mapping the receptors, structure of ligand, hydrogen bonding to determine the length of the bonds and to visualized the images. Autodock Vina is the primary docking program used in this work for the structure based virtual screening [15]. The Auto-Dock Tools version 1.5.6 (The Scripps Research Institute, La Jolla, USA) was used to obtain the ligands and proteins in pdbqt file and finding of the grid box size [16,17]. Schrodinger's Desmond module (Desmond v3.6, 2013) was used for molecular dynamic simulation studies.

Preparation of protein structure

The three-dimensional structures of the MDM2-p53 complex were retrieved from the Protein Data Bank [18]. These structures were prepared by removing all bound crystal water molecules and hetero atoms including ligands. Missing hydrogen bonds were added. Obtained structures were energy minimized using charms force field for optimal docking results.

Virtual screening

Virtual screening based on the structure of MDM2 inhibitor was carried out. Autodock Vina was utilized to search for potential inhibitors amongst the compounds found through ligand based virtual screening, targeting MDM2 active site (p53 binding site). AutoDock is very effective in locating docking modes in relation to the X-ray crystal structures. A spacing of 0.4 Å between the grid points was used. Lamarckian Genetic Algorithm (LGA) was selected as docking engine with the default parameters. Flexibility of the ligand helps in exploring the spatial degrees of freedom for rotation and translation, for given the number of torsional degrees of freedom. Interaction energy for every new location and conformation of the ligand was evaluated by applying a random perturbation for each time step.

The grid box was set to 28.81; -18.40 and -3.87 Å (x, y, and z) cube at MDM2 active site. After each LGA run, Autodock reports the best docking solution (lowest docked free energy), and results were reported based on cluster analysis and Gibbs free energy. The summation of hydrogen bonding, dispersion/repulsion, electrostatic interactions and deviation from covalent geometry, internal ligand torsional constraints, and desolvation effects accurately gives the Gibbs free energy (ΔG). The lowest energy docking mode was selected from each docking simulation after obtaining a total of 10 docking modes represented by the LGA cluster analysis.

Molecular dynamic simulations

''Desmond v3.6 Package'' was used to study the thermo dynamical stability of the receptor-ligand system. OPLS2005 force field was used to simulate water molecules using the pre-defined TIP3P water model [19,20]. The exact shape and size of the repeating unit buffered distanced at 10 Å distances were specified by orthorhombic periodic boundary. The calculation and minimization of the boundary conditions box volume were performed simultaneously.

In order to neutralize the system electrically, appropriate counter Cl-/Na+ ions were added to balance the system charge and were placed randomly in the solvated system. After building the solvated system containing protein in complex with the ligand, the system was minimized and relaxed using the default protocol integrated within Desmond module using OPLS 2005 force field parameters.

Molecular dynamic simulations were carried out with the periodic boundary conditions in the NPT ensemble [21]. Nose-Hoover temperature coupling and isotropic scaling were used to maintain the temperature and pressure at 300K and 1 atmospheric respectively; it was followed by 10ns NPT production simulation, saving the configurations thus obtained at 5ps intervals.

RESULTS

Identification of the presence of alkaloids in the *H. patens* leaf extract

Thin-layer Chromatography (TLC) on pre-coated silica gel 60F254 plate with toluene: acetone: ethanol: ammonia (40:40:6:2) solvent system afforded five alkaloids (Table 1). Alkaloid 1, 2, 3 and 4 had a smaller Rf value, which is 0.16, 0.26, 0.35 and 0.46 respectively. All of them exhibited lesser affinity towards the mobile phase. On the other hand, alkaloid 5 had Rf value of 0.52, higher than the previous alkaloids and showed greater affinity toward the eluent. All alkaloids produced orange-brown spots when sprayed with Dragendorff's reagent. These spots, fluorescent were blue at U.V. light at 366 nm, indicating the presence of alkaloids.

Table 1: Retention factor (Rf) of the extract compound of the HpLEt

S. No	Compound's Name	Rf Value	Observation
1	Alkaloid -1	0.16	Produced orange-brown spot with Dragendorff's reagent; fluoresced blue light at U.V. 366 nm
2	Alkaloid -2	0.26	Produced orange-brown spot with Dragendorff's reagent; fluoresced blue light at U.V. 366 nm
3	Alkaloid -3	0.35	Produced orange-brown spot with Dragendorff's reagent; fluoresced blue light at U.V. 366 nm
4	Alkaloid -4	0.46	Produced orange-brown spot with Dragendorff's reagent; fluoresced blue light at U.V. 366 nm
5	Alkaloid -5	0.52	Produced orange-brown spot with Dragendorff's reagent; fluoresced blue light at U.V. 366 nm
6	Morphine	0.34	Produced orange-brown spot with Dragendorff's reagent; fluoresced blue light at U.V. 366 nm

This observation established the presence of the five alkaloids of the extracted sample of HpLEt compared to that of the known alkaloid called morphine. These five alkaloids are: Palmirine, Rumberine, Alakaloid A, Isopteropodine, and Maruquine (Fig. 2). There have been multiple reported extractions of different chemical compounds from HpLEt in the past. Based on the chemical analysis, the leaf extracts of *H. patens* has also revealed the presence of other chemical constituents like essential oils, alkaloids, tannins, saponins, carotenoids, flavonoids and triterpenes [22,23].

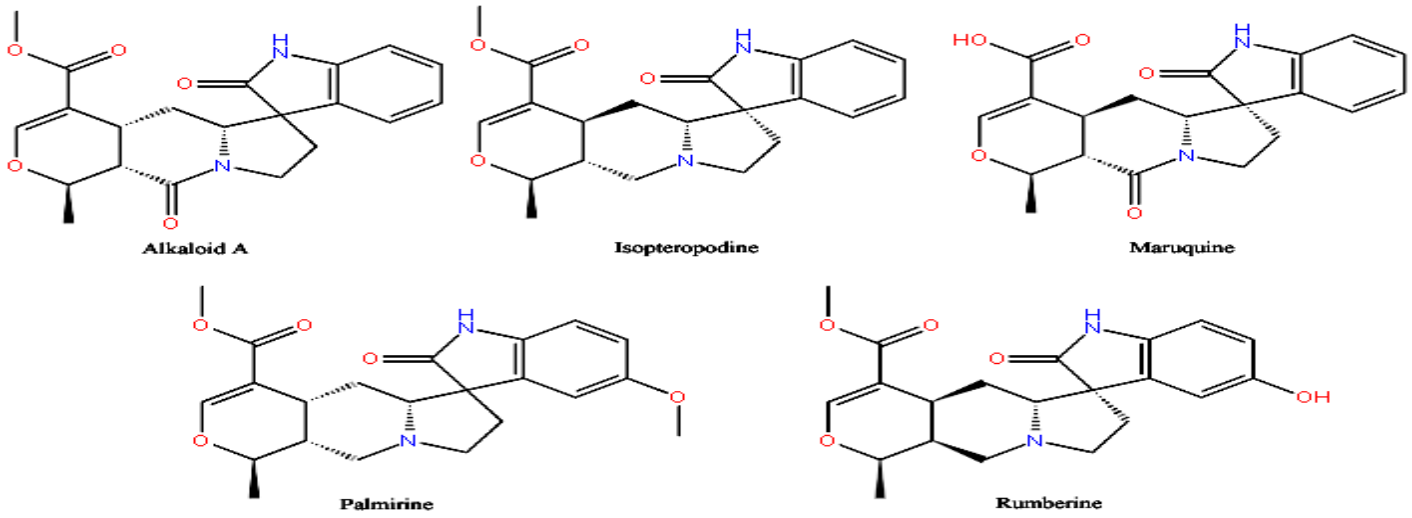


Fig. 2: Structures of *H. patens* compounds

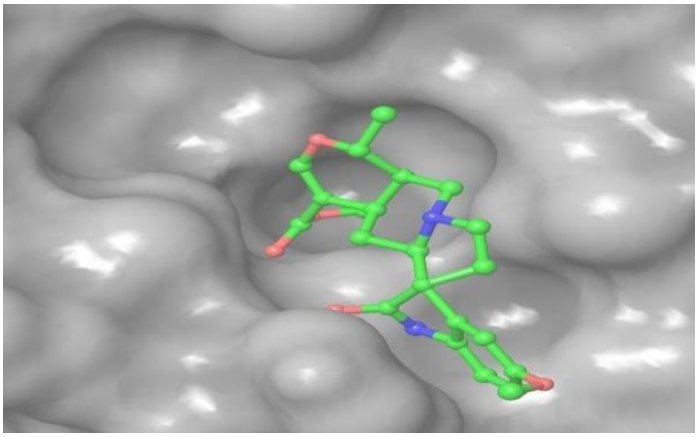
Active constituents of *H. patens* as promising anti-cancer drug candidates targeting MDM2

Docking of the compounds with MDM2 active site

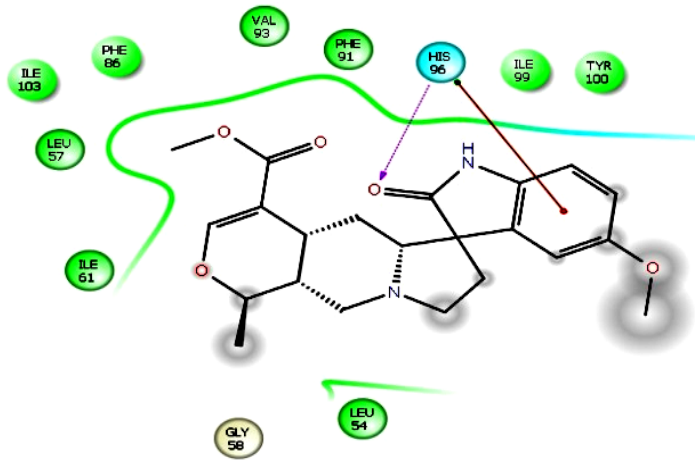
The experiment was followed by performing the docking studies for the extracted compounds from *H. patens* with the MDM2 protein, targeting its active p53 binding site, in order to know whether these compounds are capable of binding at the same binding site of MDM2 protein where active p53 peptide binds. It will also help us to find the binding energy involved in this complex formation along with molecular interactions responsible for this target specific inhibition.

All the five compounds studied in this present work have shown to successfully dock inside the same active binding site of MDM2 protein, where p53 peptide binds with a binding energy in a range of –7.42 to –6.79 Kcal/mol. Among the five tested compounds Palmirine has shown to be the best MDM2 inhibitor with a binding energy -7.42 Kcal/mol, whereas Maruquine compound showed the least binding affinity towards MDM2 with a binding energy -6.79 kcal /mol. When the docked conformation of MDM2 protein in complex with Palmirine compound was investigated, it was revealed that this compound is a single hydrogen bond with HIS96 residue. Apart from hydrogen bonds, this compound was found to be forming

hydrophobic interactions with ILE99, TYR100, LEU54, ILE61, LEU57; ILE103, PHE86, VAL93 and PHE91 residues (Fig. 3). Along with above interactions, HIS96 residue was also found to be forming pi-pi interaction with Palmirine compound contributing towards stabilizing the docked compound inside the active site of MDM2 protein.



(A)



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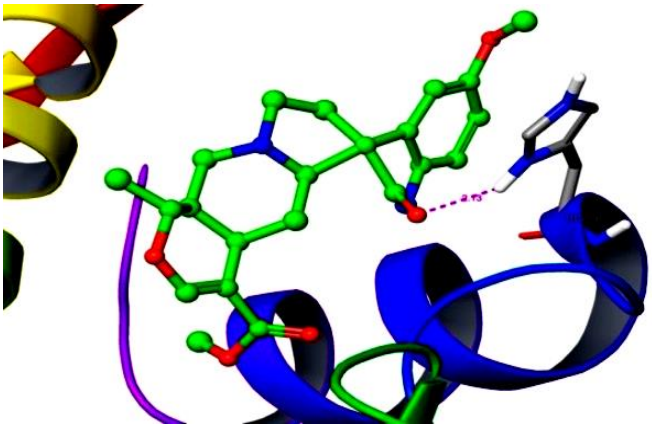


(C)

Fig. 3: Docking snapshots of Palmirine compound with MDM2 drug target showing A) surface binding B) 2d interactions, and C) 3d interactions

IC50 prediction

In order to understand the plausible experimental anti-cancer activity of the present studied compounds from *H. patens* plant, we have carried out the half maximal inhibitory concentration (IC50) value predictions. IC50 value is a reliable tool to quantitatively measure the usefulness of the compound to inhibit a given biological process by half and is widely applied to symbolize the inhibitory effect of given compounds. The predicted IC50 values for the compounds were within a range of 3.66 to 10.59 micro molar. Among which Palmirine compound has shown the best possible inhibitory potential with 3.66 micro molar. IC50 values obtained clearly demonstrated plausible high inhibitory potential of Palmirine compound among the five studied compounds from *H. patens* plant with MDM2 protein.

Molecular Dynamics simulations of MDM2 protein in complex with palmirine compound

Based on the promising inhibitory potential shown by Palmirine compound towards MDM2 protein as evident with docking and IC50 value predictions, we have taken this compound for further analysis to reveal underlying molecular interactions which might not have been revealed during docking studies and to better understand the effect of Palmirine compound binding with MDM2's p53 binding active site. The MDM2- palmirine protein-ligand binding complex with the binding energy of -7.42 kcal/mol obtained using AutoDock calculations was used for carrying out MD simulations (Table 2). The predicted IC50 values for the compounds were within a range of 3.66 to 10.59 micro molar. Among which Palmirine compound has shown the best possible inhibitory potential with 3.66 micro molar. IC50 values obtained clearly demonstrated plausible high inhibitory potential of Palmirine compound among the five studied compounds from *H. patens* plant with MDM2 protein.

Table 2: Docking energies of *H. patens* compounds with MDM2 protein

S. No	Drug target	Compound Name	Docking binding energy in Kcal/mol	Predicted IC50 value in micro molar
1.	MDM2	Palmirine	-7.42	3.66
2.		Rumberine	-7.3 0	4.48
3.		Alakaloid A	-7.09	6.34
4.		Isopteropodine	-7.04	6.92
5.		Maruquine	-6.79	10.59

After MD simulations, we calculated Root mean square Deviation (RMSD) for the trajectory MDM2 complexed with Palmirine using its initial model as a reference structure (Fig. 4). The results show that the protein backbone RMSD [green] for the complex were always less than 1.7 Å, which is comparably equal to the RMSD of the MDM2 in complex with Nutlin compound suggesting that this compound has similar confirmatory effect on the MDM2 similar to FDA approved drug Nutlin and also evidences the overall stability of our simulated system of protein (MDM2) in complex with this Palmirine compound. On the other hand, ligand superimposed on itself throughout the simulated time was quite stable in its binding conformation showing well below 0.2 Å RMSD hinting towards its adaptability with the MDM2 active site conformational changes.

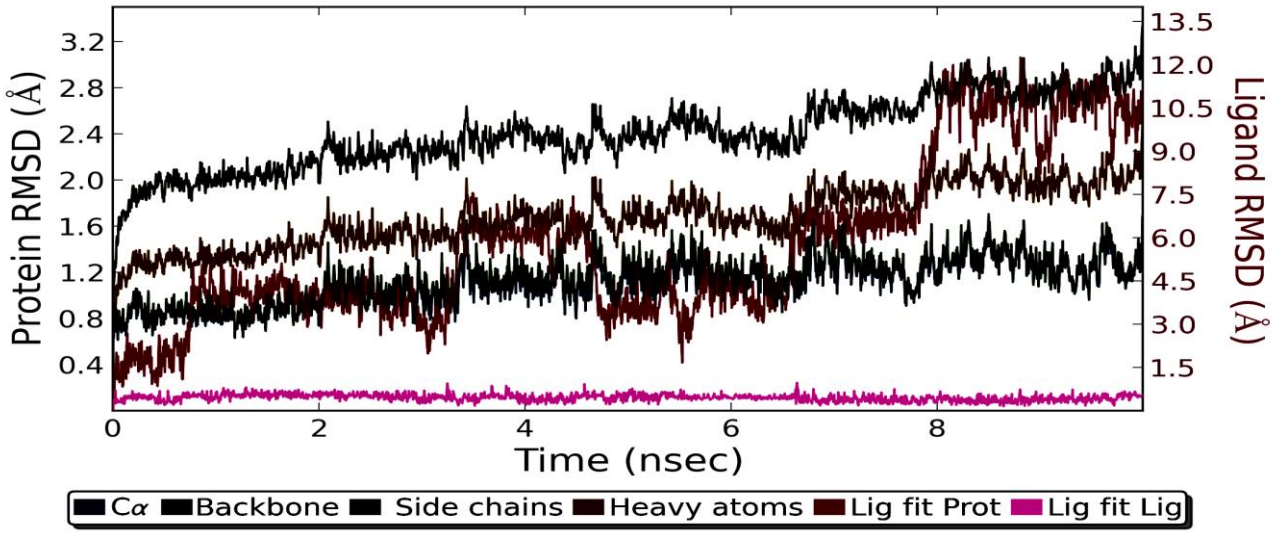


Fig. 4: Root mean square Deviation [RMSD] graphs of MDM2 protein in complex with Palmirine compound

When MDM2 protein's residue fluctuations were calculated in the presence of ligand Palmirine compound, it was observed that the backbone of the protein was quite stable throughout the simulation with well below 1.2 Å of fluctuating distance (Fig. 5).

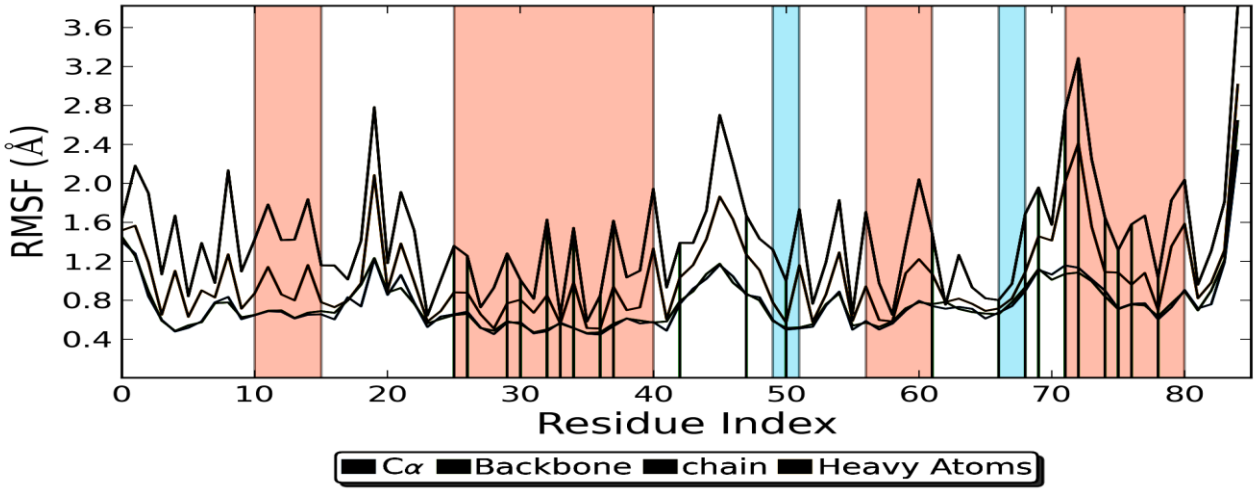


Fig. 5: Root mean square Fluctuations [RMSF] graphs MDM2 protein in complex with Palmirine compound

These results were highly in support to the strong inhibiting and stabilizing potential of Palmirine compound of MDM2 protein when compared with residue fluctuations of MDM2 protein in the presence of no ligand which was shown to having highly fluctuating over 2.5 Å, whereas for MDM2 in presence of the Nutlin was found to be fluctuating around 2.0 at initial 30 residues and at residues located at 80–90 position. 10 ns of simulation time used in this present study is of enough time for the side chain rearrangements in the native as well as protein-ligand complexes in order to facilitate the most stable binding conformation.

Radius of Gyration (ROG) graph (Fig. 6) of the MDM2-palmirine complex has evidenced the protein MDM2 protein has slightly contracted as the simulation progresses in presence of Palmirine compound by maintaining an average of 12.92 Å within a range of 12.60 Å to 13.27 Å.

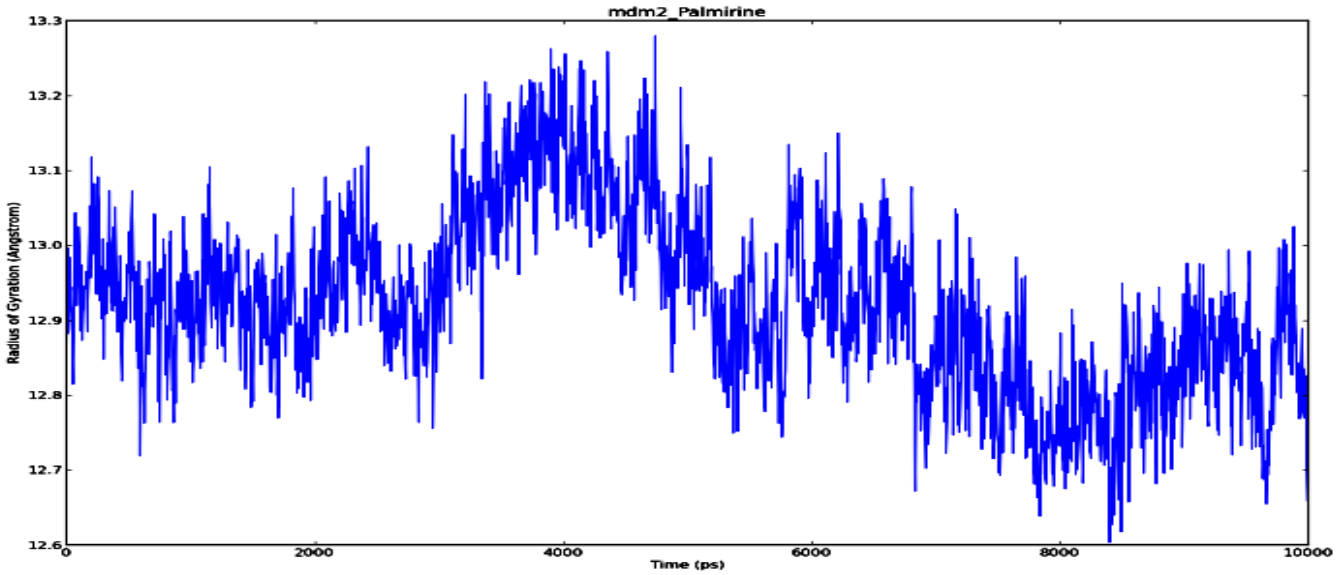


Fig. 6: Radius of Gyration [ROG] graphs of MDM2 protein in complex with Palmirine compound

When this data was compared with MDM2 protein in presence of no ligand and in the presence of Nutlin, it is evident that MDM2 protein is expanding slightly in presence of Nultin similar to this Palmirine compound suggesting this compounds ability to inhibit MDM2 in a similar manner of Nutlin compound.

We were also calculated the intra molecular hydrogen bonds present throughout the simulation time within the MDM2 protein in complex with Palmirine compound and found out that it is maintained an average of 71 intra molecular hydrogen bonds in a range of 60 to 83 throughout the simulation time accounting for its stability and evidenced the increase in protein rigidity in the presence of this ligand in comparison to MDM2's averaged 67 intra molecular hydrogen bonds in presence of no ligands (Fig. 7). However, interestingly, this compound was found to be maintaining similar intra molecular hydrogen bonds 71 as of Nutlin compound.

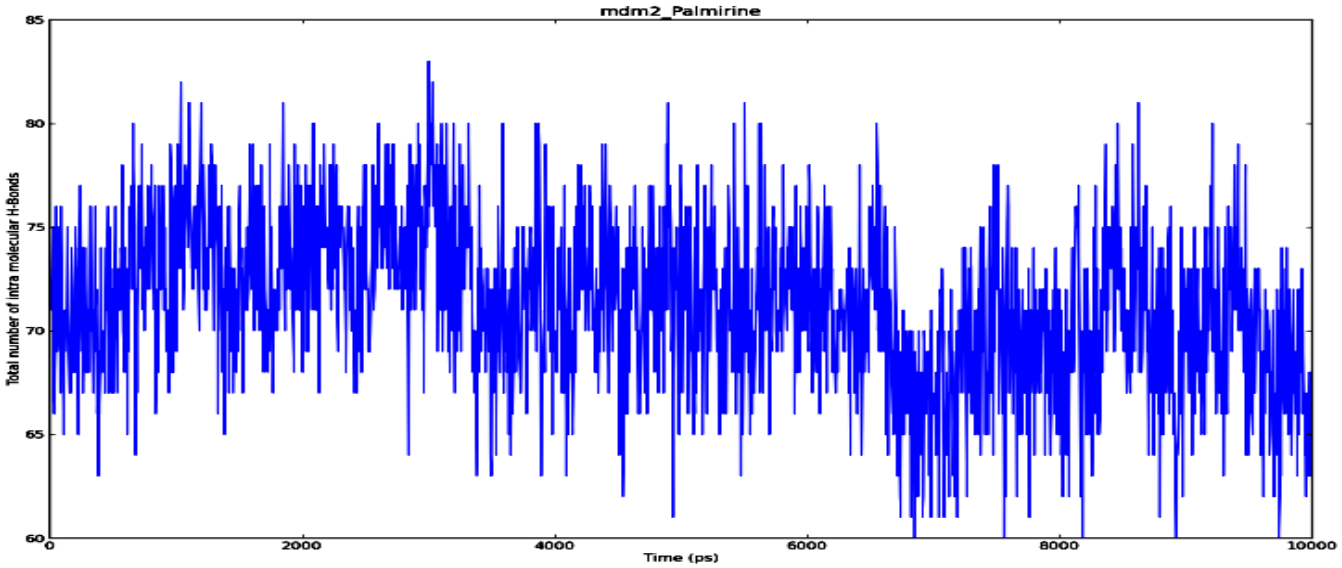


Fig. 7: Total number of intra molecular hydrogen bonds present in MDM2 protein in complex with Palmirine compound

Finally, we analyzed the total energy involved in the stabilized conformation of this MDM2 protein in complex with Palmirine compound and it was observed to be maintaining an average of -2462.38 Kcal/mol of energy in a range of -2947.56 to -2031.85 Kcal/mol, which was similar to MDM2's averaged energy in its apo state and in the presence of Nutlin compound (Fig. 8).

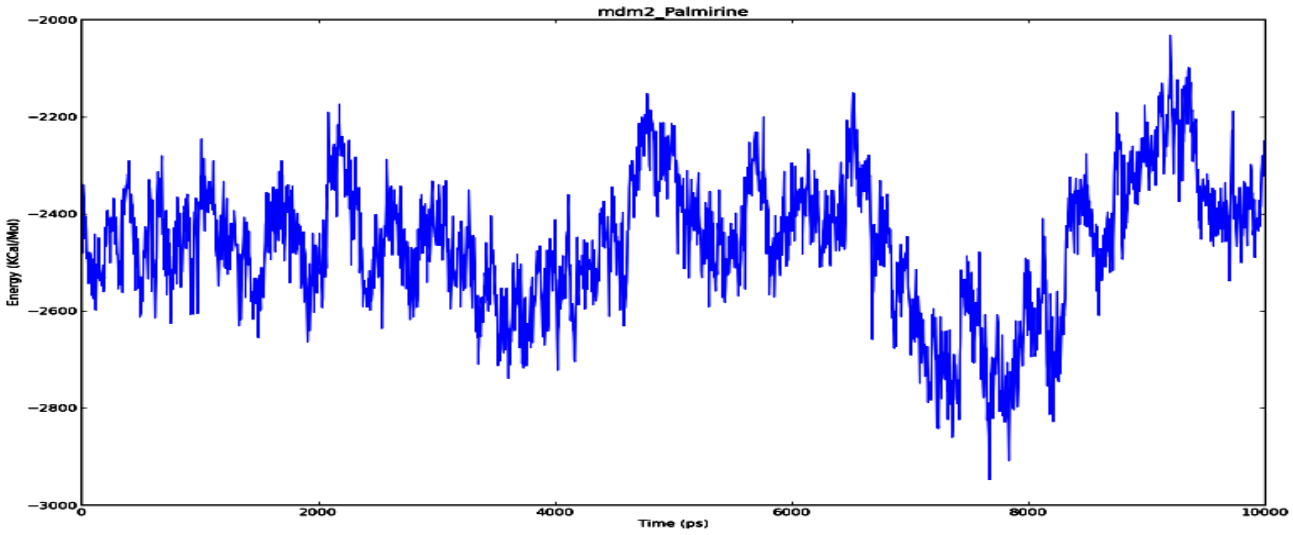


Fig. 8: Total energy of MDM2 protein in complex with Palmirine compound

We have also monitored the effect of Palmirine compound at total Secondary structure elements (SSE) present in the protein MDM2 throughout the simulation trajectory. From the analysis it was revealed that the protein MDM2's SSE composition of helices and strands over simulated time averaged at 50% was similar to that of the SSE composition of MDM2 protein in the presence of Nutlin compound (Fig. 9).

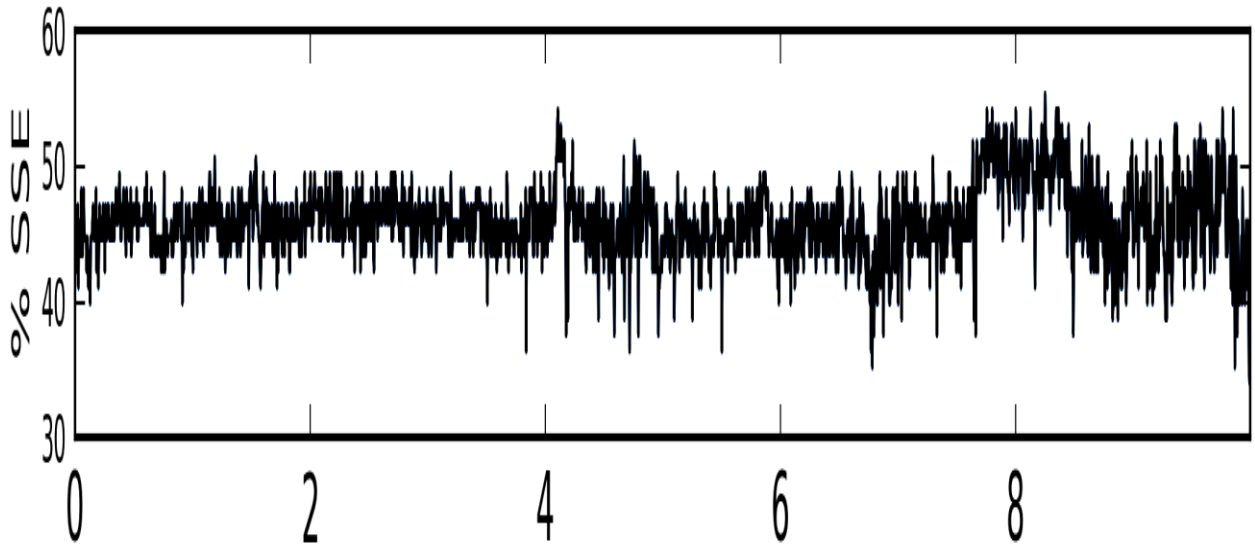


Fig. 9: Secondary structural elements of MDM2 protein in complex with palmirine compound

Molecular interactions of MDM2-palmirine complex during MD simulations

We have used Simulation interactions diagram program integrated within Desmond module of Schrödinger for studying the detailed inter-molecular interactions between MDM2 protein and Palmirine compound. There were about 22 contacts found in between MDM2 protein and palmirine compound in total among which two hydrogen bonds were observed with HIS96 and ARG97 residues and most of other contacts were found to be hydrophobic contacts followed by water bridging and ionic bonds (Fig. 10).

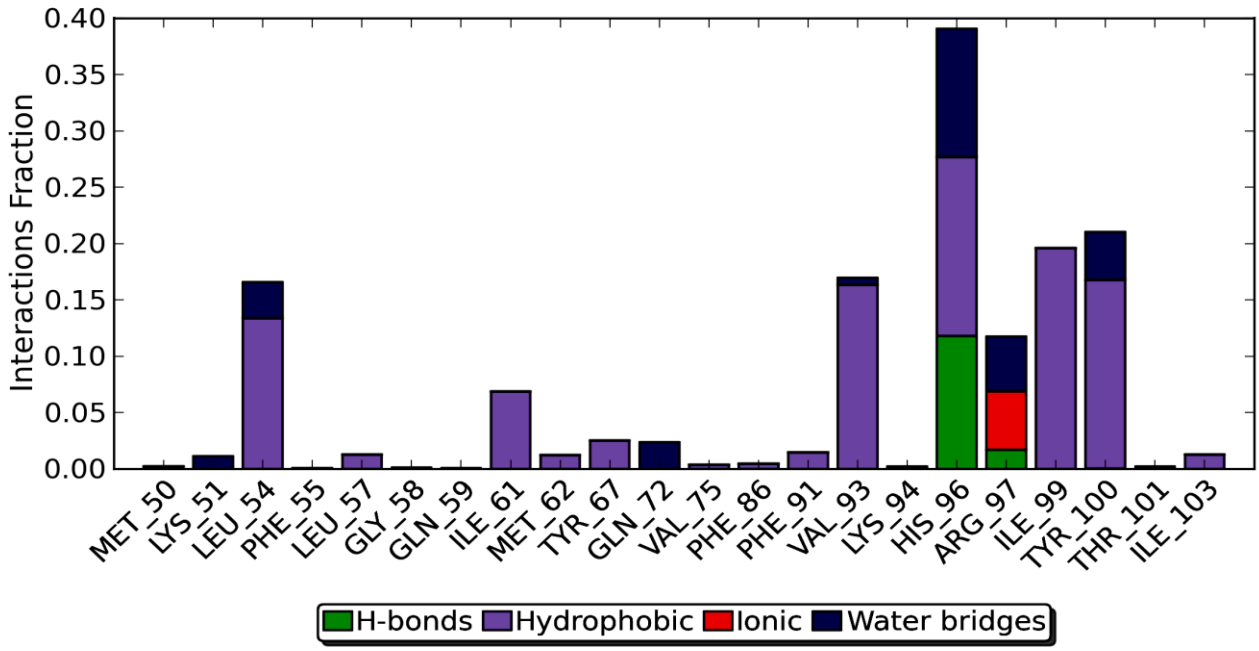


Fig. 10: Protein-ligand interactions (or 'contacts') of MDM2-palmirine complex. The stacked bar charts are normalized over the course of the trajectory. For example, a value of 0.7 suggests that 70% of the simulation time the specific interaction is maintained

Finally, to examine and estimate the ligand torsion dynamics facilitating for the hydrogen bonds along with other interaction between MDM2-palmirine complex; we have analyzed the torsional degree of freedom for the rotatable bonds present in the ligand. For the Palmirine compound, a total of three rotatable bonds have been observed (Fig. 11). From the dial panels it is clear that the above mentioned rotatable bonds are consuming energy of 10.14; 10.00 and 7.83 Kcal/mol of energy respectively.

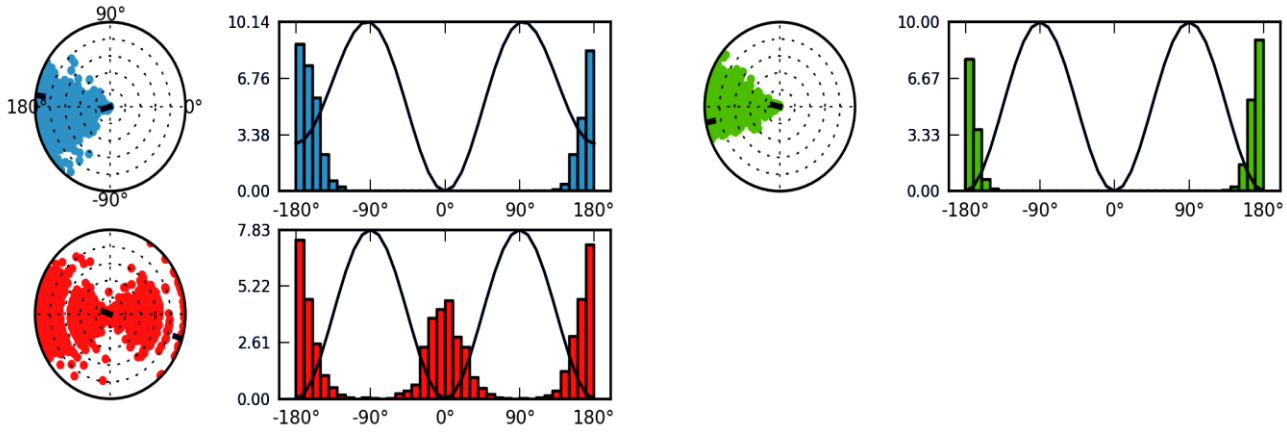
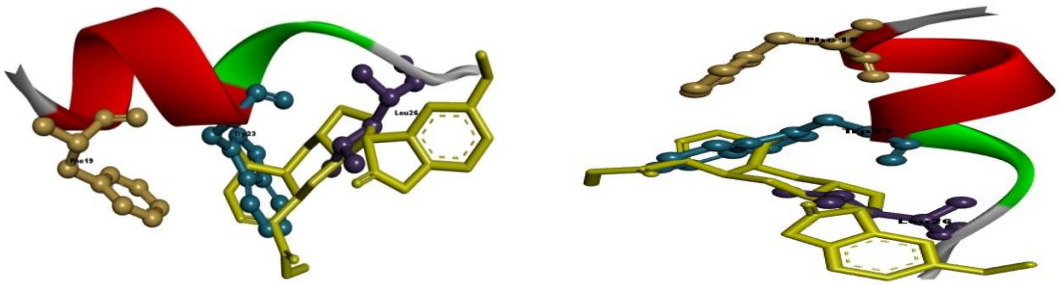


Fig. 11: The ligand torsions of palmirine compound plot summarizing the conformational evolution of every rotatable bond (RB) in the ligand throughout the simulation trajectory (0.00 through 10.00 nsec). A dial/bar plots with the same color also represent these rotatable bonds torsion. Dial gives the manner of torsion formation throughout the course of the simulation. Center of the radial plot denotes the beginning of the simulation against the time, which is plotted radially outwards. The bar plots represent the dial plot by giving approximation of the density of the torsion. The plot also gives the potential of the rotatable bond if available (by adding the potential of the related torsions). Left Y-axis represents the values of the potential in Kcal/mol

For further analysis, the binding mode similarities between the p53 and our proposed compound Palmirine, we have performed the post docking analysis to check for the same via superimposing the docked conformation. From the analysis, it is evident that proposed compound Palmirine mode of inhibition is very much similar to p53 peptide; especially prominent alignments were observed with the p53 residues TRP23 and LEU26 (Fig. 12).

From this analysis, it was evident that proposed compound Palmirine mode of inhibition is very much similar to p53 peptide; especially prominent alignments were observed with the p53 residues TRP23 and LEU26.



(a) **(b)**

Fig. 12: Superimposition of the Palmirine compound (yellow) with p53 peptide showing the alignments of the compound's side chain benzene rings almost in the same orientation as that of TRP23 and LEU26 residues of p53 showing the strong binding association. Above panels depicts the same in various orientations

DISCUSSION

In the present study an investigation was carried out to find out natural plant extract to act as an anti-cancer drug on the mode of interaction between MDM2 and p53 at the molecular level. The p53 is an important tumor suppressor gene with a known role in the later stages of cancer. MDM2 is a p53 responsive gene as its transcription can be activated by p53. Thus, inhibiting the MDM2-p53 interactions has been proven to be the most promising approach for cancer therapy.

The methanolic extract from *H. patens* was subjected to structure based virtual screening approach to identify target specific MDM2 inhibitors by docking studies. The best extracted compound was subjected to molecular dynamic simulations for further validating the docking studies and to reveal interactions during the conformational changes. The identified compounds were compared to that of the FDA drug Nutlin compound that which has already been proven. In this work, we discovered several compounds from our case study with the *H. patens* leaf extract, that are potentially able to inhibit the MDM2-p53 interaction, proving their anti-cancer agents similar to Nutlin. The present study provides a rationalization to the ability of present studied compounds as a valuable small ligand molecule with strong binding affinity towards MDM2 protein for plausible anti-cancer activity. Our computational analysis evidence shows that the large value of binding energy is involved in binding on present investigated five compounds (isopteropodine, rumberine, palmirine, maruquine and alkaloid A) with the MDM2 protein consolidating their complex's thermodynamic stability; moreover, predicted IC50 values further substantiated our hypothesis that these compounds have the potential to inhibit MDM2 protein.

Further, de novo simulations for 10 ns suggest that ligand interaction with the residues of MDM2, all or some of which fall under catalytic active site important residues for its structural stability and/or functionality, could be critical for its inhibitory activity. This knowledge is very important for computational screening of drugs targeting MDM2. A little knowledge was gained through this study, that it would further enhance the discovery of MDM2 target specific drug compounds by understanding the molecular interaction basis between ligand and receptor. This gives the basis of anti-cancer drugs, opening a wide horizon of future opportunities. We have focused particularly on four key steps: target validation and selection; chemical hit and lead generation; lead optimization to identify a clinical drug candidate by using computational techniques. The novel computational techniques have been developed to predict the interaction models of protein- protein (p53-MDM2 interactions) interactions from medium to high resolution. The discovery of new and effective p53 activator/inhibitors opens the broader spectrum of targeted therapy for treating cancers. Collectively, these advances provide new opportunities to use macromolecular structures in pharmaco-genomics and systems pharmacology.

CONCLUSIONS

MDM2 has been identified as a p53 interacting protein, which represses p53 transcriptional activity. Design of non-peptide, small-molecule inhibitors, obtained from secondary plant products, that block the MDM2-p53 interaction has been sought as an attractive strategy to activate p53 for the treatment of cancer and other human diseases. Major advances have to be made in the design of these small-molecule inhibitors of the MDM2-p53 interaction as targeted therapies in advanced preclinical development or clinical trials, justifying the use of plant in traditional medicine practices. It is therefore recommended that more work be conducted to help optimally extract all the bioactive compounds in the plant and formulate into appropriate doses for the treatment.

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