Anticancer Potential of 
*Rhychosia beddomei*: An *In vitro* and *In silico* Study

M. Lakshmi Madhuri a,b,* and R. Raja Reddy c

a Department of Pharmaceutical Chemistry, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad-500090, Osmania University, Telangana, India. 
b JNTU, Hyderabad, Telangana, India. 
c Department of Pharmaceutical Chemistry, Arya College of Pharmacy, Hyderabad-502285, Osmania University, Telangana, India.

Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJACR/2022/v12i3222

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/94820

Received: 08/10/2022
Accepted: 10/12/2022
Published: 16/12/2022

Original Research Article

ABSTRACT

*Rhychosia beddomei* is widely distributed in tropical and subtropical regions around the world. Traditionally this plant has been used as a medicine for multiple ailments including cancer. The present study addresses the evaluation of *In vitro* anticancer activity by using MTT Assay of methanolic extracts of whole plant of *Rhychosia beddomei* in Human Hepatocellular Carcinoma (HepG2) cell line in comparison with standard drug doxorubicin as well as *In silico* analysis. Doxorubicin acts in the cancer cell by intercalation into DNA and disruption of topoisomerase-II-mediated DNA repair and generation of free radicals and their damage to cellular membranes, DNA

*Corresponding author: Email: madhuri8072@grcp.ac.in;* 

Keywords: Rhychosia beddomei; molecular docking; MTT assay; HepG2 cell lines.

1. INTRODUCTION

“Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are the two major forms of primary liver cancers (PLC), accounting for approximately 90% and 5% respectively” [1,2]. “The incidence of each is the most common widespread cancer in the world. HCC causes high annual mortality rates, particularly in Thailand, Cambodia and Laos, where viral hepatitis is endemic” [3]. “The induction of HCC is preceded by the occurrence of hepatocellular damage via reactive oxygen species (ROS) and the generation of chronic inflammation related to hepatocarcinogenesis” [4]. “Adjunctive therapies such as tumor necrosis factor and melphalan; or cisplatin, epirubicin and 5-FU; doxorubicin, interferon alpha and 5-FU have been used to overcome the HCC” [5]. “However, the main problem of chemotherapy to treat HCC is the cancer resistance mechanism, due to up-regulation of the multi-drug resistance protein (MDR) and a decrease of apoptotic proteins” [6]. “Thus, more effective chemotherapy is needed to control cancer and apoptosis induction, which is the desired effect for successful cancer treatment” [7].

Rhychosia beddomei. (Papilionaceae) is rare medicinal plant found in the Eastern Ghats of Andhra Pradesh. Locally, it is known as adavi kandi and vendaku. The plant parts were used in traditional medicine to cure various diseases viz., leaves for abortifacient, antibacterial, antifungal, antidiabetic [8], hepatoprotective properties [9] and also used for healing wounds, cuts, boils and rheumatic pains. The anti-cancer activity of methanolic extract of Rhynchosia beddomei (Papilionaceae) (MERB) whole plant against MTT assay and In silico docking studies of isolated compounds from the plant have not been reported so far scientifically. Hence, the present study has been carried out to evaluate the anti-cancer activity of MERB.

2. MATERIALS AND METHODS

2.1 Plant Materials

Rhynchosia beddomei Baker whole plant was collected in the month of November 2014 from Sheshachalam hills, Tirupati, Andhra Pradesh, India. The plant was then taxonomically identified and authenticated by the botanist Dr. K. Madhava Chetty, Assistant Professor in S.V. University, Tirupati, India.

2.2 Preparation of Extract

“The pant was dried under shade then coarsely powdered with a mechanical grinder. The powder was passed through a sieve and stored in an airtight container for the extraction, and Extracted with methanol 70% (75-78°C) up to 72 h, as the yield was more for methanol in the selected solvents. After completion of extraction, the solvent was removed by distillation. Dark brown residue was obtained. The residue was concentrated and then stored in desiccators” [10].

2.3 Preliminary Phytochemical Investigation

The extract was subjected to various preliminary phytochemical tests to detect the phytoconstituents present in the plant extract [11].
2.4 Method

The in vitro MTT assay was performed for the test substances on HepG2 (Human Hepatocellular Carcinoma) cell line to determine the level of cytotoxicity.

2.5 Preparation of Test Compound for Cytotoxicity Screening

10 mg of test substances was weighed and dissolved in DMEM-HG medium supplemented with 2% inactivated FBS to obtain a stock solution of 10 mg/mL. Furthermore, serial two-fold dilutions were prepared from the stock solution to prepare lower concentrations for cytotoxicity testing [12].

2.6 Cell line and Culture Medium

Human Hepatocellular Carcinoma cell line cell line was procured from National Centre for Cell Sciences (NCCS), Pune. Stock cells were cultured in DMEM-HG supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/mL) and amphotericin B (5 μg/ml) in a humidified atmosphere of 5% CO$_2$ at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% Trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm$^2$ culture flasks and all experiments were carried out in 96 well microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

2.7 Determination of Cell Viability by MTT Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 100,000 cells/ml using DMEM-HG containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 mL of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer washed once with medium and different test concentrations were added on to the partial monolayer in the microtitre plates. The untreated cells were maintained as cell control for comparison. The plates were then incubated at 37°C for 24 h in 5% CO$_2$ atmosphere, and microscopic examination was carried out and observations were noted after 24 h, the test solutions in the wells were discarded and 50 μL of MTT is added with DPBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO$_2$ atmosphere. The supernatant was removed and 100 μL of Isopropanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm.

2.8 Molecular Docking Studies

Initially the proteins were downloaded from PDB was prepared by removing extra chains. Attributes of spheres are prepared and noted. Later molecules drawn in molecular and ligand preparation was created. Proteins are uploaded with sphere attributes and the structures were docked against 1DI8 and 1ZXM proteins. Docking indicated that some of our compounds have good binding ability with both CDK-2 and Topo-II proteins. Following are the ligand interactions of compounds present in Rhychosia beddomei whole plant with 1DI8 and 1ZXM proteins [13].

2.8.1 Protein-ligand interactions

Docking stimulations predicts the binding orientation of drug candidates to their protein targets. Mcule was used for generating docking simulation studies.

Docking simulations on Cyclin-dependent kinase-2 (1DI8), Human topoisomerase II (1ZXM).

“Cyclin-dependent kinase 2 (CDK2) drives the progression of cells into the S- and M-phases of the cell cycle. CDK2 activity is largely dispensable for normal development, but it is critically associated with tumor growth in multiple cancer types. Selective CDK2 inhibition may provide a therapeutic benefit against certain tumors, and it continues to appeal as a strategy to exploit in anticancer drug development” [14].

DNA topoisomerases are nuclear enzymes that catalyze the introduction of topological changes to the DNA molecule. Replication and transcription of DNA require the unwinding of the DNA helix, which results in helical tension in the rest of the DNA molecule. Topoisomerases release this tension through the formation of transient single-stranded (i.e., type I topoisomerases) or double-stranded (i.e., type II topoisomerases) breaks in the DNA double helix, which makes topoisomerases essential for proliferating cells.
Hence, we report the molecular docking analysis of CDK 2 and Topo II with phytocompounds.

2.8.2 Ligand preparation

The 2D ligands sketched in Mcule docking in the ligand imported side.

2.8.3 Protein preparation

The x-ray crystallised structure of Cyclin-dependent kinase-2 (PDB ID: 1DI8) and Human topoisomerase II protein (PDB ID: 1ZXM) were retrieved from RCSB protein bank. Attributes of SBD site sphere are obtained from discovery studio visualizer.

2.8.4 Ligand docking and scoring

Protein ligand interactions were stimulated though flexible glide-ligand docking with mCULE Docking allowed. The compounds docked displays a docking score

2.8.5 Visualization and analysis

The resulting docking poses were visualized through discovery studio visualizer. The ligand interactions were visualized to know the binding interactions between ligands and protein. The best docked structures were chosen using glide score function. The more negative the score the more favourable the binding. Additionally, the docked ligand poses were visualized and the different ligand receptor interactions were studied.

3. RESULTS AND DISCUSSION

The plant was collected, authenticated and extracts were prepared using solvents like methanol and the yield of methanolic extract was found to be 63% respectively.

3.1 Preliminary Phytochemical Analysis

The preliminary phytochemical investigation of methanolic extract of whole plant of Rhynchosia beddomei showed the presence of alkaloids, glycosides, carbohydrates, tannins are present in minor quantities. Flavonoids, steroids are present in major quantities. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: + indicates present; - indicates absent

Table 1. Preliminary phytochemical analysis

3.2 In vitro MTT Assay

The results revealed that the cytotoxicity rate was increased when the concentrations of leaf extract increases. MTT assay measured the cell viability based on the reduction of yellow tetrazolium MTT to a purple formazan dye mitochondrial dehydrogenase enzyme. So, the amount of formazan produced reflected the number of metabolically active viable cells" [15]. The CTC_{50} values observed for Cell line HepG2 against methanolic extract of Rhynchosia beddomei was 245.1µg/ml given in Table 2. Test substances were assayed for In vitro cytotoxicity study against HepG2 cells (Human Hepatocellular carcinoma) by exposing the cells to different concentrations ranging from 8 to 1000 µg/mL. MTT assay was employed to test the cytotoxic effect of selected concentrations of the plant extract on the cell viability of HepG2 cells by measuring the metabolic activity through a colorimetric determination. Cell viability is a measure of the proportion of live, healthy cells within a population. The MTT assay is usually carried out to detect the cells with constant mitochondrial activity, thereby; an increase or decrease in the number of viable cells is linearly related to mitochondrial activity. In the present study, the CTC_{50} value of the plant extract was found to be 245.1µg/mL. The plant extract exhibited 75.68±1.44% toxicity in HepG2 cells at the highest concentration tested (1000 µg/mL) and the CTC_{50} value of the Doxorubicin was found to be 0.485 µg/mL given in Table 3.

"The plant has the enormous medicinal properties and its various parts used to cure many diseases. Quercetin exhibits direct proapoptotic effects on tumor cells and thus can inhibit the progress of numerous human cancers. The anticancer effect of quercetin has been documented in numerous in vitro and in vivo studies that involved several cell lines and animal models. On the other hand, the high toxic effect
of quercetin against cancer cells is accompanied with little or no side effects or harm to normal cells” [16]. “Recently, apigenin has been widely investigated for its anti-cancer activities and low toxicity. Apigenin was reported to suppress various human cancers In vitro and In vivo by multiple biological effects, such as triggering cell apoptosis and autophagy, inducing cell cycle arrest, suppressing cell migration and invasion, and stimulating an immune response” [17].

“The naturally occurring flavonoids are reported to possess multiple biological activities” [18,19]. “Flavonoids have been shown to possess a wide variety of anticancer effects: they modulate reactive oxygen species (ROS)-scavenging enzyme activities, participate in arresting the cell cycle, induce apoptosis, autophagy, and suppress cancer cell proliferation and invasiveness. Multiple studies report flavonoids to cause inhibition of the growth factor receptors, and/or multiple downstream signalling pathways, inhibition of activation of carcinogens and inhibition of cyclin-dependent kinases. Among flavonoids, rutin is a common dietary flavonoid that has been found in numerous foods, beverages, and vegetables. Various effects of rutin, including antimicrobial, antidiabetic, antioxidant, anti-inflammatory, anticancer, and neuroprotection effects, have been shown in several studies” [20]. “Also, many studies indicated that it could act as a chemopreventive and chemotherapeutic agent. Its antitumor products are via the suppression of cell proliferation, the induction of autophagy or Apoptosis, and the prevention of metastasis and angiogenesis. In many human cancer cell lines, rutin has caused cell cycle arrest and apoptosis” [21]. Since the presence of flavonoids in the extract might be responsible for the anticancer activity.

3.3 Molecular Docking Studies
Initially the proteins were downloaded from PDB was prepared by removing extra chains. Attributes of spheres are prepared and noted. Later molecules drawn in molecular and ligand preparation was created. Proteins are uploaded with sphere attributes and the structures were docked against 1DI8 and 1ZXM proteins. Docking indicated that some of our compounds have good binding ability with both Cyclin-dependent kinase-2 and Human topoisomerase II proteins represented in Table 4. Following are the ligand interactions of compounds present in Rhychosia beddomei whole plant with 1DI8 and 1ZXM proteins represented in Figs. 1 and 2.

Table 2. MTT Assay- Percentage cytotoxicity of MERB against Hep G2 cell lines

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Concentration (µg/mL)</th>
<th>Percentage Cytotoxicity</th>
<th>CTC&lt;sub&gt;50&lt;/sub&gt; (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic extract of Rhynchosia beddomei</td>
<td>1000</td>
<td>75.68±1.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>67.54±0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>58.22±0.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>5.65±0.18</td>
<td>245.1</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>3.82±0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31.25</td>
<td>2.91±0.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.625</td>
<td>1.60±0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.8</td>
<td>0.55±0.57</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. In vitro MTT assay –Percentage cytotoxicity of Doxorubicin against HepG2 cell lines

<table>
<thead>
<tr>
<th>µM of Doxorubicin</th>
<th>µg of Doxorubicin</th>
<th>Cytotoxicity %</th>
<th>CTC&lt;sub&gt;50&lt;/sub&gt; (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5</td>
<td>86.28</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>84.79</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.25</td>
<td>70.17</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.625</td>
<td>57.47</td>
<td>0.485</td>
</tr>
<tr>
<td>0.25</td>
<td>0.312</td>
<td>41.63</td>
<td></td>
</tr>
<tr>
<td>0.125</td>
<td>0.156</td>
<td>35.76</td>
<td></td>
</tr>
<tr>
<td>0.0625</td>
<td>0.078</td>
<td>28.59</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Molecular docking studies of isolated compounds against Topoisomerase-2 and CDK-2

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Cyclin Dependent Kinase II (1DI8) Docking Score</th>
<th>Topoisomerase II (1ZXM) Docking Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercitin-7-O-methyl ether</td>
<td>-9.3</td>
<td>-9.7</td>
</tr>
<tr>
<td>Isovitexin</td>
<td>-9.7</td>
<td>-7.8</td>
</tr>
<tr>
<td>5,7,3’4’-tetrahydroxy 6-c-β-D-glucopyranosyl flavone</td>
<td>-9.6</td>
<td>-10.2</td>
</tr>
<tr>
<td>Apigenin</td>
<td>-9.8</td>
<td>-9.3</td>
</tr>
<tr>
<td>Vitexin</td>
<td>10.9</td>
<td>-9.3</td>
</tr>
<tr>
<td>Vicenin</td>
<td>-11.1</td>
<td>-5.4</td>
</tr>
<tr>
<td>Orientin</td>
<td>-10.9</td>
<td>-9.8</td>
</tr>
<tr>
<td>Isoorientin</td>
<td>-10.7</td>
<td>-8.8</td>
</tr>
<tr>
<td>Lucinen</td>
<td>-11.5</td>
<td>-5.5</td>
</tr>
<tr>
<td>Rutin</td>
<td>-9.3</td>
<td>-10.4</td>
</tr>
<tr>
<td>Rhynchosin</td>
<td>-9.7</td>
<td>-10.0</td>
</tr>
<tr>
<td>Biochanin</td>
<td>-8.8</td>
<td>-9.2</td>
</tr>
</tbody>
</table>

The molecular docking in this study shows a vital role in predicting molecular interactions of phytocompounds with targeted proteins. This application is widely used in the pharmaceutical industry as a powerful tool, particularly in the analysis of structure–activity relationship. The analysis of molecular docking outputs, such as binding affinity, are frequently applied in the determination of potential ligands. Molecular docking also has the ability to predict small molecule ligands binding toward appropriate target binding site.

Lucinen, vicenin and orientin have demonstrated remarkable binding affinity towards Cyclin-dependent kinase-2. Lucinen shown highest docking score -11.5 in comparison to other compound topwards CDK-2 [22]. Rutin, 5,7,3’4’-tetrahydroxy 6-c-β-D-glucopyranosyl flavone and Rhynchosin have demonstrated remarkable binding affinity towards Topo II. Rutin shown highest docking score -10.4 in comparison to another compound. Thus, indicating that these compounds are potent inhibitor of the CDK-2 antiapoptotic family of proteins and TOPO-2 proteins [23].
a) Rutin -10.4
b) 7,3',4'-tetrahydroxy 6-c-β-D-glucopyrynosyl flavone -10.2

c) Rhynchosin -10.0

Fig. 2. Docking poses of phytocompounds with Human topoisomerase II protein

The application of molecular docking studies for the compounds that are present in methanolic extract of Rhynchosia beddomei with proteins 1DI8 and 1ZXM are considered very useful and proven anticancer activity. Our findings conclude that all phytocompounds are possibly able to act as potential inhibitors for the targeted 1DI8 and 1ZXM proteins, supported by the high binding affinities.

4. CONCLUSION

The present study scientifically established the in vitro MTT assay and in silico docking studies to predict anticancer activity. The MTT assay was performed and the results showed that the extract has anticancer activity. From docking scores, we can conclude that the isolated compounds from the extract possess anticancer activity through inhibition of CDK-2 and Topo-II can be further preceded to wet lab synthesis.

ACKNOWLEDGEMENTS

The authors are grateful to the Principal and Management of the Gokaraju Rangaraju College of pharmacy, for the constant support and encouragement during the course of the work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


22. Sumirtsundin R, Sungkar S, Hisprastin Y, Sidharta KD, Nurikmah DD. Molecular


© 2022 Madhuri and Reddy; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/94820