Evaluation of *Hemidesmus indicus* Plant Compounds for Anti-Cancer Studies – An *In silico* Approach

Pavan Kumar Tummala¹, Sreeja Nannapaneni², Sumana Pratyusha Durvasula², Supriya Chadalavada², Sunandini Venigandla², Srikanth Vemuru¹, Suryanarayana Veeravilli³ and Maheswara Reddy Mallu⁴*

¹Department of Computer Science and Engineering, Koneru Lakshmaiah Education Foundation, Andhra Pradesh, India.
²Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Andhra Pradesh, India.
³Department of Humanities and Basic Sciences, Aditya Engineering College, Surampalem, Andhra Pradesh, India.

**Authors’ contributions**

This work was carried out in collaboration among all authors. Authors PKT and SN taken responsibility in the conception and design of the study. Authors SPD and SC contributed substantially in development of methods and its optimization. Authors S. Venigandla and S. Vemuru have provided critical revision of the article for important intellectual content. Author S. Veeravilli has checked the references. Author MRM have given final approval of the version to be published. All authors read and approved the final manuscript.

**ABSTRACT**

**Background:** Phytocompounds in medicinal plants have a wide range of properties and are alternative medicines for those who cannot be helped by conventional medicine.

**Objective:** In this work we have selected bioactive compounds from *Hemidesmus indicus* medicinal plant extracts.

**Methods:** Gas chromatography and Mass spectrum studies were studied to identify the compounds present in the ethanolic extracts based on the retention time and area.

*Corresponding author: E-mail: mahesh_bt@kluniversity.in*
1. INTRODUCTION

Cancer is a global health problem with high morbidity and mortality, as well as economic and psychological costs. As a result, cancer cure and prevention remain top priorities for the scientific community all over the world [1]. Various epidemiological studies encompassing various parameters such as geographical location, ethnicity, sex, age, and trans-migratory populations have collectively revealed that lifestyle is one of the major influencing factors in the etiology of cancer [2]. Environmental factors such as automobile exhaust pollutants, solar UV radiation, occupational exposure to carcinogens and mutagens, bacterial/viral infection, and genetic susceptibility are also factors. Lifestyle factors, which include diet, smoking, caspase-3 activity, alcohol consumption, physical activity, and body mass, are typically classified as modifiable risk factors. Physical activity rather than inactivity, restraint from smoking and alcohol consumption, low body mass, and low fat/calorie diets are recommended for overall good health and have a positive influence on reducing the risk of cancer, particularly breast and colorectal cancers [3]. American Cancer Society has proposed nutrition and physical activity guidelines for cancer prevention, as well as early detection/screening for cancers of specific sites [4]. Epidemiological data show that dietary habits influence cancer risk, considerable scientific interest has been generated in developing various diet-based preventive measures, particularly those involving probiotics [5]. As a result, interventions based on fruits and vegetables are not only “more natural” in lowering cancer risk without causing “any side effects,” but also in maintaining good general health due to their high vitamin, mineral, and fibre content. Here, we used *Hemidesmus indicus* medicinal plant crude extracts for identification of anti-cancer activity.

2. METHODS

2.1 GC-MS Method for Identification of Compounds

GC-MS analysis was carried out on a GC CLARUS 550 PerkinElmer system comprising a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV [6]; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed with split ratio of 10:1 injector temperature 250°C; ion-source temperature 280°C [7,8]. The oven temperature was fixed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

2.2 BCL-2 Active Site Identification

The structure of BCL-2 (PDB: 1GJH) was retrieved from PDB database and active site of BCL-2 of Homo sapiens was identified using CASTp server [9]. A new program, CASTp, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities [10]. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings.

2.3 Docking Method

Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm which allows as partial flexibility of protein and full flexibility of ligand [11]. The compounds identified in GC-MS are docked to the active site of the BCL-2 of Homo sapiens [12,13]. The interaction of the compounds with the active site residues are thoroughly studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of island (1) and niche size (2). Operator parameters for

| Keywords: | Hemidesmus indicus; anti-diabetic; anticancer; docking studies; alpha glucosidase. |
|-----------|--------------------------------------------------------------------------------------------------|
crossover, mutation and migration were set to 100, 100 and 10 respectively [14]. Default cutoff values of 3.0 Å (dH-X) for hydrogen bonds and 6.0 Å for vanderwaals were employed. During docking, the default algorithm speed was selected and the ligand binding site in the targets were defined within a 10 Å radius with the centroid as CE atom of active residues. The number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of a ligand were within 1.5Å RMSD [15]. After docking, the individual binding poses of compounds were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of ligands was selected.

2.4 Gold Score Fitness Function

Gold Score performs a force field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand vander Waals energy (external vdw); 3. Ligand internal vander Waals energy (internal vdw); 4. Ligand intramolecular hydrogen bond energy (internal- H- bond) [16]. The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions. Gold Score = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int), Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand [17].

3. RESULTS AND DISCUSSION

From the PDB databank, the PDB files were collected and the final stable structure of the BCL-2 of Homo sapiens obtained is shown in Fig. 1. The ligands present in the crystal structure were removed along with hetero atoms for docking studies.

![Fig. 1. Structure of BCL-2 retrieved from Protein data bank with seven helices](image)

3.1 Active Site Identification

After the final model was built, the possible binding sites of BCL-2 was searched based on the structural comparison of template and the model build and also with CASTP serverand was shown in Fig. 2. Infact from the final refined model of BCL-2 domain using SPDBV program, it was found that secondary structures are highly conserved and the residues shown below.

![Fig. 2. Amino acids in the active site region (red colour) of the BCL-2protein](image)
3.2 Docking of Inhibitors with the Active Site

GC-MS analysis of plant extract were found as Trans-2-methyl-3-phenyl-2-propen-1-ol, 2,4-bis(1,1-dimethylethyl)-phenol, Isopropyl stearate, Z,E-2-Methyl-3,13-octadecadien-1-ol, Ethanone, 1-[4-methoxy-3-(4-methylphenoxy)phenyl], Butanal,3-methyl-[2,4-dinitrophenyl] hydrazine, Piperazine-2,5-dione, 1,4-(4-methylphenyl), Flavone, E-2-Octadecadecen-1-ol, Geranyl vinyl ether, 4-Amino-1, 5-pentandioic acid, Pipradrol, Cyclopropanebutanoic acid, Methyl 9-methyltetradecanoate, Hexadecanoic acid, Trans-13-Octadecenoic acid, Preg-4-en-3-one, 1,2-diacetoxy-5-idohexane and Methyl-1-Cyclohexane carboxylate. Docking of the compounds with BCL-2 was performed using GOLD 3.0.1, which is based on genetic algorithm [18]. This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this ‘bump map’ are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using CASTp server. The twenty phytocompounds were checked for their anti-cancer activity using insilico method.BCL-2 protein was retrieved from the database and its active site was identified using CASTp server. The twenty phytocompounds were docked to the BCL-2 for their anti-cancer activity. Among the phytocompounds docked, Methyl-1-Cyclohexane carboxylate showed a docking energy of 22.56K.cal/mol with BCL-2. In docking, Methyl-1-Cyclohexane carboxylate (O21) docked to LEU414 with a bond length of 1.733Å [20].

![Fig. 3. Methyl-1-Cyclohexane carboxylate docked to BCL-2 active site](image1)

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![Fig. 4. 1,2-diacetoxy-5-idohexanedocked to BCL-2 active site](image2)

Fig. 4. 1,2-diacetoxy-5-idohexanedocked to BCL-2 active site

In docking studies, 1,2-diacetoxy-5-idohexane showed a docking energy of 20.24K.cal/mol with BCL-2. In docking, 1,2-diacetoxy-5-idohexane (H33) docked to LEU414 with a bond length of 2.559Å [21].

4. CONCLUSION

Based on previous studies [22] and present study, we can conclude that GC-MS analysis identified twenty phytocompounds from Hemidesmus indicus root ethanolic extract. The identified phytocompounds were checked for their anti-cancer activity using insilico method.BCL-2 protein was retrieved from the database and its active site was identified using CASTp server. The twenty phytocompounds were docked to the BCL-2 for their anti-cancer activity. Among the phytocompounds docked, Methyl-1-Cyclohexane carboxylate showed a docking energy of 22.56K.cal/mol and 1,2-diacetoxy-5-idohexane showed 20.24K.cal/mol with BCL-2.From these docking studies we can conclude that among the phytocompounds identified, these two compounds have good BCL-2 inhibitory activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

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