Vol 5, Issue 5, Sep-Oct 2018 ISSN 2349-7041

Research Article

# INSILICO DOCKING ANALYSIS OF DELONIX ELATA LEAF EXTRACTS TO INHIBIT CERVICAL CANCER HPV16 IN ALBINO WISTAR RAT

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**ABSTRACT**

**Objectives:** Cervical cancer is one of the most common forms of cancer diagnosed and causes death among women worldwide. Drug discovery from the medicinal plants has played an important role in the treatment of most human ailments and indeed. Delonix elata belongs to the family of caesalpinoideae, having promising medicinal property. **Methods:** we investigated the anti-cancer potential of the leaf extract of D. elata Linn on Ehrlich Ascites Carcinoma (EAC) tumor model. Tumor was induced in rat by intraperitoneal injection of EAC cells (1 ×106 cells/rat). D. elata was administered to the experimental animals at the dose levels of 250, 500 and 1000 mg/kg/day after 24 hours of tumor inoculation. **Results:** The anti-tumor effect of D. elata was evaluated by assessing the body weight, survival time and hematological parameters ( ). Oral administration of D. elata extended the survival time of the EAC bearing rat. The D. elata normalized the levels of the hematological parameters in a dose-dependent manner in EAC bearing rat when compared to control. The results were comparable to that of the result obtained from the animals treated with the standard drug 5-fluorouracil (20mg/kg/b.w). In this study, we used petroleum ether leaf extract of D.elata and also isolated bioactive compounds namely 2-(4-methoxyphenyl)imino)-4-methyl-1,3-thiazoline-4-yl)methanol,5-(p-aminophenyl)-4-(o-tolyl)-2 thiazolamine and Dodecanoic acid, 2,3-bis(acetyloxy) propyl ester by GC-MS. The 3-D crystal structure of the HPV16 E6 oncoprotein (PDB-ID: 2FK4) responsible for cervical cancer was retrieved from the Protein Data Bank (PDB). The compound 5-(p-aminophenyl)-4-(o-tolyl)-2 thiazolamine shows best docking energy values compare than other compound -10.7013 kcal/mol. **Conclusion:** Thus present study revealed that D. elata possessed 5-(p-aminophenyl)-4-(o-tolyl)-2 thiazolamine has significant antitumor activity.

**Keywords:** Anti cervical cancer, HPV16, Delonix elata, Rat.

## INTRODUCTION

Natural products are the source of synthetic and traditional herbal medicine and are still the primary health care system for underdeveloped and developing countries. The plants are most vital constituents for everyday life on earth. Plants have played an important part in the development of mankind. It provides us with food, medicines and cosmetics. Herbal medicine is the oldest form of medicine known to mankind using plants. Herbs have been used by human being, since antiquity for their extra-ordinary leading abilities and pain relieving properties. Presently man has increasingly started using medicinal plants to overcome various illness and suffering. Today approximately 75% of all prescription drugs are delivered from trees, shrubs on herbs. Medicinal plants have become a major component of human health care as they have no or least side effects [1]. This response localizes and eliminates altered cells, foreign particles, microorganisms, and antigens and paves the way for the return to normal structure and function [2]. Similar plants have so many disease cures and recovery acting traditional and now a day [3].

Cervical cancer is one of the most common forms of cancer diagnosed and one of the most common causes of cancer death among women worldwide. In developed countries, breast cancer [4] is the most common in women, whereas cancer cervix occupies the top rank among cancer in women in developing [5]. Worldwide, cervical cancer is the second most common cancer and the major cause of cancer deaths among women [6]. The rates are generally higher in urban than rural population. Rates are also related to marital status higher in married women than in single and higher in widow or divorced women than in married. In this similar work done by [7] pointed out that the *in silico* docking analysis of marine-derived compounds against oncoprotein of cervical cancer. Quality control is a term that refers to processes involved in sustaining the quality and validity of a manufactured product [8].

The *Delonix elata* belongs to the family of caesalpinoideae. It is found in Gujarat, western peninsular and south India. Tree leaves are reported to use by traditional practitioners in cases of inflammatory joint disorders as folklore remedy. Psychosomatic medicinal use related to scorpion bite treatment reported in India [9, 10] find that Anti-inflammatory activity in *Delonix elata* leaf *alcoholic* extract reported [11]. It contributes the beneficial effect on health or plays an active role in interest in bioactive secondary metabolites of plant amelioration of disease. The medicinal properties of the plants have been investigated in the recent scientific developments throughout the world, due to their potential antioxidant activity, no side effects and economic viability. Antioxidant property of the plant mainly presence of phenolic compounds[3]. The importance of medicinal plants and the contribution of Phytomedicine to the well- being of a significant number of the world's population have attracted interest from a variety of disciplines [12].

## MATERIALS AND METHODS

### Samples collection and Preparation of extract

In order to prepare the extract, we used *Delonix elata*, collected from Annamalai University campus, Tamilnadu, India in 2017. The extract was obtained by the hydroethanolic extraction on 80%. To each 100 mL of extract, 80 mL of ethanol and 20 mL of water were added. Next, 64 g of barley (previously grounded using a micro-mill) were added in order to obtain a particle size smaller than 1.5 mm. Subsequently, the sample was sonicated for 30 minutes at room temperature (25 °C ± 1) and filtered through a filter paper of rapid filtration to remove the waste. Subsequently, according to [13], the solvent was evaporated in a rotary-evaporator at 40 °C.

**Animals and treatments**

30 adult male Wistar rats, 50 days old, with initial body weight between 148 to 270 g were used. The animals were separated into three experimental groups; each one with 10 rats. The animals were placed in individual metabolic cages with water bottle, food bowls, and litter box, and were maintained at room (21 ± 3 °C) on a 12:12 hour's light/dark cycle, with free access to food and water.

**GCMS analysis**

The chemical compounds namely 2-(4-methoxyphenyl)imino)-4-methyl-1,3-thiazoline-4-yl)methanol, 5-(p-aminophenyl)-4-(o-tolyl)-2 thiazolamine and Dodecanoic acid, 2,3-bis(acetyloxy) propyl ester were screened from GC-MS analysis of *Delonix elata* leaf. The details of these phytochemical were obtained from PubChem database and their chemical structures were generated from SMILES notation (Simplified Molecular Input Line Entry Specification). ChemSketch (Chemically intelligent drawing interface freeware developed by Advanced Chemistry Development, Inc., (http://www.acdlabs.com) was used to construct the structure of the ligands. Using draw mode of Chemsketch, the ligands were generated and the three-dimensional optimizations were done and then saved in MOL file (a file format for holding information about the atoms, bonds, connectivity and coordinates of a molecule). The 3-D crystal structure of the HPV16 E6 oncoprotein (PDB-ID: 2FK4) responsible for cervical cancer was retrieved from the Protein Data Bank (PDB). Structural and active site enumeration were done by using pymol molecular visualization software and CASTP (Computed Atlas of Surface Topography of Proteins). The molecular docking analysis was performed by widely distributed public domain molecular docking software Argus Lab 4.0.1

**Docking methods**

Argus Lab 4.0.1 was used for docking analysis, which is widely distributed public domain molecular docking software. The inhibitor and target protein were geometrically optimized and docked using docking engine Argus lab. The docking simulations in the active sites of 2FK4 were performed by the Argus lab program, which has been shown to successfully reproduce experimentally observed binding modes in terms of lowest docking energy. The target protein structure of 2FK4 was docked with *Delonix elata* leaf extract compounds which provided excellent results as were seen by the least values of the binding energy.

**Superoxide dismutase (SOD activity)**

Pipette 1.4 ml aliquot of the reaction mixture in a test tube containing 1.1 ml phosphate buffer, 75 Sl methionine, 40 µl Triton X-100, 75 µl hydroxylamine hydrochloride and 100 µl EDTA. 100 Sl of the sample was added followed by preincubation at 37°C for 5 min. 80 Sl of riboflavin was added and the tubes were exposed for 10 min to 200 W Philips fluorescent lamps. The control tube contained equal amount of buffer instead of sample. The sample and its respective control were run together. At the end of the exposure time, 1.0 ml of Greiss reagent was added to each tube and the absorbance of the color formed was measured at 543 nm. One unit of enzyme activity was defined as the amount of SOD capable of inhibiting 50% of nitrite formation under assay condition [14].

**Catalase activity (CAD assay)**

To 0.9 ml of phosphate buffer, 0.1 ml of tissue homogenate and 0.4 ml of hydrogen peroxide was added. At 0 seconds and after 60 seconds 2.0 ml of dichromateacetic acid mixture was added. The tubes were kept in boiling water bath for 10 minutes and the color developed was read at 620 nm. Standards in the range of 1.2-6.0 µ mol were taken and processed as test and blank containing reagent alone. The activity of catalase was expressed as Smole of $H_2O_2$ decomposed/min/mg protein or ml of serum [15].

**Glutathione peroxidase (GSH activity)**

To 0.4 ml of buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide, 0.2 ml of reduced glutathione and 0.1 ml of $H_2O_2$ were added to two test tubes labeled as test (T) and control (C). To the test added, 0.2 ml of sample and to the control added 0.2 ml of water was added. The contents were mixed well and incubated at 37°C for 10 minutes. The reaction was arrested with the addition of 0.5 ml of 10% TCA. To determine the glutathione content, 1.0 ml of supernatant was removed by centrifugation. To that added, 3.0 ml of buffer and 0.5 ml of Ellman's reagent were added. The color developed was read at 412 nm. Standards in the range of 40-200 µg of reduced glutathione was taken and treated in the similar manner. The activity was expressed in term of µg of glutathione consumed/min/mg protein or ml of serum [16].

**RESULTS**

Structural elucidations of the compound were done by Gas Chromatography-Mass Spectrum (GC-MS) analysis [17], was shown in result(Table 1 and 2). The spectrum was comparable to the standard report data.

**Structure of protein**

The structures of the target protein (PDB ID: 2FK4) (Figure 2), were obtained from the RCSB Protein Data Bank, http://www.rcsb.org/PDB.

**Protein-ligand docking**

Argus lab is a well-known suite of software for molecular docking. The protein-ligand docking was performed in flexible docking (Figure 1 to 3). The molecular docking predicts the binding ability of the ligand molecule with the receptor molecule.

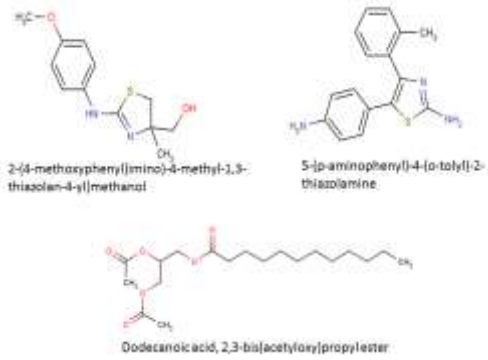


**Figure 1: 2D representation of mangrove-derived compounds linear structure of ligand molecules.**

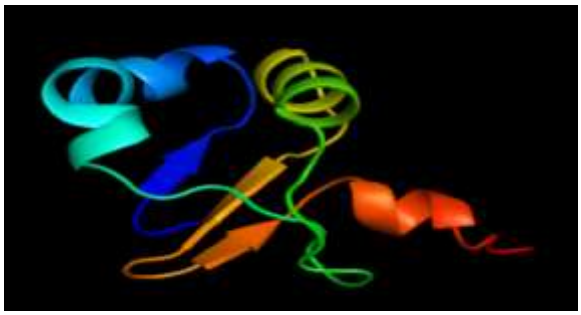

**Figure 2: Structure of Target Protein.**

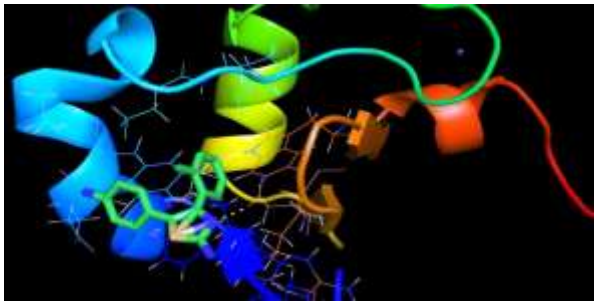

**Figure 3: Protein-ligand docking**

**Table 1: Properties of phytochemical compounds and Properties of Ligand Molecules**

| **Properties** | 2-(4-methoxyphenyl) imino)-4-methyl-1,3-thiazolan-4-yl)methanol | 5-(p-aminophenyl)-4-(o-tolyl)-2 thiazolamine | Dodecanoic acid, 2,3-bis (acetyloxy) propyl ester |
|---|---|---|---|
| **Molecular Formula** | $C_{12}H_{16}N_2O_2S$ | $C_{16}H_{15}N_3S$ | $C_{19}H_{34}O_6$ |
| **Molecular Weight** | 252.333 Da | 281.3754 Da | 358.4697 Da |
| **H bond acceptors** | 4 | 4 | 6 |
| **H donors** | 2 | 2 | 0 |

**Table 2: Hepatoprotective effect of *Delonix elata* against Atrazine induced toxicity in *Rattus norvegicus* on antioxidant enzymes**

| **Experimental group** | **SOD (U/min/mg protein)** | **CAT (U/min/mg protein)** | **GSH (µg/mg protein)** |
|---|---|---|---|
| **Group I** Control | 7.15 ± 0.51 | 72.62 ± 1.45 | 6.06 ± 0.13 |
| **Group II** Atrazine | 4.68 ± 0.18* – 34.54 | 39.72 ± 0.90* – 45.30 | 3.71 ± 0.15* – 38.77 |
| **Group IV** Atrazine + *D. elata* | 6.40 ± 0.13** – 10.48 | 61.43 ± 0.92* – 15.40 | 5.03 ± 0.19* +16.99 |
| **Group V** *D. elata* alone | 7.28 ± 0.15** + 1.8 | 73.44 ± 1.26** + 1.12 | 6.29 ± 0.18* + 3.79 |

## DISCUSSION

Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain. Several natural product drugs of plant origin have either recently been introduced to the United States market, including arteether, galantamine, nitisinone, and tiotropium, or are currently involved in late-phase clinical trials. Although drug discovery from medicinal plants continues to provide an important source of new drug leads, numerous challenges are encountered including the procurement of plant materials, the selection and implementation of appropriate high-throughput screening bioassays, and the scale-up of active compounds[18].

Plants have been utilized as medicines for thousands of years [19]. These medicines initially took the Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer [20] of all available anticancer drugs between 1940 and 2002, 40% were natural products per and natural product-derived with another 8% considered natural product mimics. The phytochemicals have the cable to arrest the replication West Nile Virus [21]Here we performed an *Insilico* docking method. The natural compounds are considered as 3D ligand molecules and it's docked with cervical cancer responsible protein PDB I.D: 2FK4.

The docked ligand molecules were selected based on docking energy and good interaction with the residues. From the 3 molecules 5-(p-aminophenyl)-4-(o-tolyl)-2 thiazolamine shows higher docking score value is -10.7013 kcal/mol. The compounds were satisfied Lipinski's rule of 5 is a rule of thumb to evaluate drug-likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule describes molecular properties; Not more than 5 hydrogen bond donors (OH an NH groups), not more than 10 hydrogen bond acceptors (notably N and O), molecular weight under 500 g/mol, partition coefficient log P less than 5 and the rotatable bonds less than 10 [22].

In the present investigation the atrazine treated group was decline the activity of SOD, CAT and GSH. Moreover the group III (atrazine along with *D. elata*) augmented the levels of these enzymes. Because the supplementary group of plant having such a wound healing property compounds like pinitol, apigenin, luteolin, chrysoeriol and rutin [23] demonstrated that Hg caused time-dependent decrease in the activities of the enzymes of the glutathione (GSH) metabolism pathway in the rat. Therefore increase in the formation of ROS by atrazine may induce membrane biochemical functional alterations and thus induced liver cell damage.

The elevated level of SOD, CAT and GSH by the influence of *D. elata* compared to the atrazine may have facilitated the conjunction reaction of xenobiotics metabolism and may have increased the availability of non-critical nucleophilic for inactivation of electrophiles and therefore might be playing a major role in metalloprotein. Some of the active constituents of have been reported to possess strong antioxidant activity and provokes free radical scavenging enzyme system. Antioxidants are compounds that can delay or inhibit oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction [24].

## CONCLUSION

Molecular docking analysis was carried out from the natural compound were screened against the Cervical cancer. The ligand molecules have good binding interaction with 2FK4 protein. From this study we conclude the *Delonix elata* leaf extract compounds were binds to HPV16 E6 oncoprotein (PDB-ID: 2FK4) responsible for cervical cancer it inhibits. Therefore, this study states the importance of small molecules from plant sources as docking agents and we suggest that natural derived compounds should be potent drug for cervical cancer. It is like that target prediction method. Further the *in vitro* experiment methods for the foreseeable future.

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