ISSN 2278 – 540X EISSN 2278 – 5396

DRUG DISCOVERY

GC-MS and in-silico analysis of *Cleistanthus collinus* for its activity against cancer

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Received 26 April; accepted 19 May; published online 25 June; printed 29 June 2012

ABSTRACT

Cleistanthus is a plant genus of the family Phyllanthaceae. *Cleistanthus collinus* plant is well known for its toxicity. All parts of the plants are reported to be highly toxic. This work was done in order to study and predict the medicinal property of the plant. Phytochemical analysis was done to know the presence of components like terpenoids, reducing sugars, steroids, flavonoids etc. GC-MS result of acetone extract shows the presence of a new compound dioctyl phthalate, which is not established earlier. Molecular docking is the in-silico method of predicting the binding site of the ligand with the selected target. p53 is selected as the target and dioctylphthalate was the ligand molecule. By the molecular docking method it was found that the ligand dioctyl phthalate exhibited the ability to bind with the p53 receptor. p53 (also known as protein 53 or tumor protein 53), is a tumor suppressor protein that in humans is encoded by the TP53 gene. p53 is crucial in multicellular organisms, where it regulates the cell cycle and thus, functions as a tumor suppressor that is involved in preventing cancer.

Keywords: Cleistanthin; p53; in-silico methods; dioctyl phthalate; molecular docking; Autodock; Cancer; *Cleistanthus collinus.*

Abbreviations: GC-MS - Gas Chromatography and Mass Spectrometry; DP - Dioctyl phthalate.

1. INTRODUCTION

Cleistanthus collinus is an extremely toxic plant poison (Fig.1). Cleistanthin A and B glycosides are toxins of it (Albert et al., 1994). *Cleistanthus collinus* poisoning usually occurs by intentional ingestion of the leaves with mortality as high as 30%, usually occurs 3-7 days after ingestion (Eswarappa et al., 2000; Ragupath et al., 1992). Gas chromatography-mass spectroscopy (GC-MS) is one of the hyphenated analytical techniques. As the name implies, it is actually two techniques that are combined to form a single method of analyzing mixtures of chemicals. Gas chromatography separates the components of a mixture and mass spectroscopy characterizes each of the components individually (Thomas et al., 1991). By combining the two techniques, an analytical chemist can both qualitatively and quantitatively evaluate a solution containing a number of chemicals. They are used extensively in the medical, pharmacological, environmental, and law enforcement fields (Subrahmanyam et al., 2003). Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for scoring functions (Subramanian and Ramanien, 2010). The objective of this work is to analyze the phytochemicals present in this plant. The work also concentrates on the compounds present in this plant by GC-MS analysis. In-silico analysis also performed to identify the binding site of the newly identified compound (Dioctyl phthalate) against the receptor p53, a tumor suppressor protein.

Dioctyl phthalate: Bis(2-ethylhexyl)phthalate, commonly abbreviated DEHP, is an organic compound with the formula $C_6H_4(C_8H_{17}COO)_2$. It is sometimes called dioctyl phthalate and abbreviated DOP. It is the most important "phthalate," being the diester of phthalic acid and the branched-chain 2-ethylhexanol.

Figure 1

Cleistanthus collinus

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MOLECULAR DOCKING

In the area of molecular modeling, docking is a technique which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism). Therefore docking is useful for predicting both the strength and type of signal produced. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.

2. MATERIALS AND METHODS

2.1. Plant Leaf Collection

Oduvan (*Cleistanthus collinus*) leaves were collected from hillside area near CMC, Vellore. It was then dried and powdered.

2.2. Acetone Extract Preparation

10g of dried plant leaves of *Cleistanthus collinus* was weighed and made into small pieces; this was soaked in a solvent acetone and was incubated at room temperature for 24 hours. After 24 hrs the aqueous extracts were collected and filtered using Whattmann paper. The filtered extracts were stored in refrigerator for future use.

2.3. Phytochemical Analysis

Phytochemical analysis was done to find out the presence of organic compounds in the leaf extracts. The tests performed were; saponins, tannins, carbonyl, flavanoids, phlobatanin, steroids, phenol and reducing sugars.

2.4. Molecular docking

Steps involved in molecular docking

Step 1: Editing the PDB file

Protein data bank files can have a variety of potential problems that need to be corrected before they can be used in Autodock. These potential problems include missing atoms, added more than one molecule, chain breaks, alternate locations etc. The water molecules have to be removed and polar water molecules have to be added and save in 'pdb' format.

Step 2: Preparing the ligand

Before docking partial atomic charges are applied to each atom of the ligand. Ligands are written in files with special keywords recognized by autodock. The TORSDOF for a ligand is the total number of possible torsions in the ligand minus the number of torsions that totally rotate the hydrogen's.

Step 3: Preparing the macromolecule

The receptor file used by autodock must be in "pdpqs" format which is pdb plus 'q' charge and 's' salvation parameters: atval, the atomic fragmental volume the atomic salvation are used to calculate the energy contributions of the macromolecule by ligand binding.

Step 4: Preparing the grid parameter file

The grid parameter file tells autogrid the types of maps to complete the location and extent of those maps and specifies pair wise potential energy parameters. In general, one map is calculated for each element in the ligand plus an electrostatics map.

Step 5: Starting Autogrid

Autogrid must be run in the directories where the macromolecule, ligand and parameter files are to be found.

Step 6: Preparing the docking parameter file

The docking parameter file sets autodock map files to use which ligand molecule to move. Four different docking algorithms are currently available in auto dock.

Step 7: Run autodock

Autogrid and autodock must be run in the directories where the macromolecule, ligand, gpf and dbf files are found.

Step 8: Analysing the docking results

The key results in a docking log are the docked structures found at the end of each run, the energies of these docked structures and their

Table 1 Phyto-chemical analysis

S.no	Test for Organic Compounds	Presence of Indication	Observation	Results
1	Terpenoids	Reddish Brown color upper face	Reddish Brown color upper face	Presence of Terpenoids
2	Reducing sugars	Orange red color precipitate	Orange red color precipitate	Presence of Reducing sugars
3	Tannins	Dark green or blue color solution	Blue color was formed	Presence of Tannins
4	Carbonyl	Effervescence and yellow color formation	Yellow color solution was formed	Presence of Carbonyl
5	Flavanoids	Yellow to Colorless	Yellow turned to colorless	Presence of Flavanoids
6	Steroids	Green or Brown layer	Brown layer was formed at the bottom	Presence of Steroids
7	Saponins	Creamy mass bubbles	No Creamy mass bubbles	Absence of Saponins
8	Phlobatanin	Red precipitate	No Red precipitate	Absence of Phlobatanin

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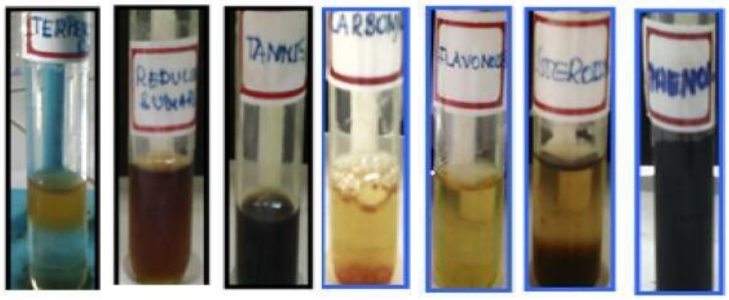
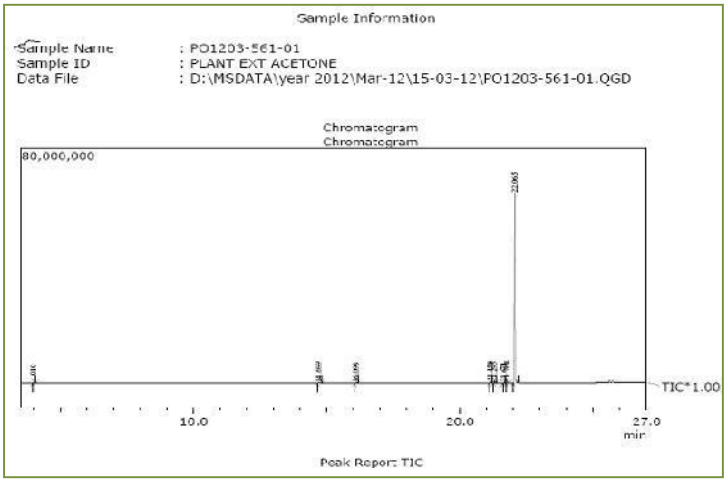


Figure 2

Phytochemical analysis



PEAK#	R.TIME	AREA	AREA%	NAME
1	4.010	858419	0.68	2-Propanol, 1,1,1 trichloro-2-methyl
2	14.699	1149703	0.91	BENZENE, ETHYLPHENOXY-
3	16.098	408929	0.32	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
4	21.158	958825	0.76	Phenol, 2,4-bis(1-phenylethyl)-
5	21.295	976536	0.77	2,4-Bis(1-phenylethyl)phenol
6	21.671	404547	0.32	1,2-BENZENEDICARBOXYLIC ACID, BIS(2-ETHYLHEXYL) ESTER
7	21.768	1290546	1.02	Phenol, 2,4-bis(1-phenylethyl)-
8	22.065	120901477	95.23	DIOCTYL PHTHALATE
		126958982	100.00	

Figure 4

GC-MS analysis showing Dioctyl phthalate

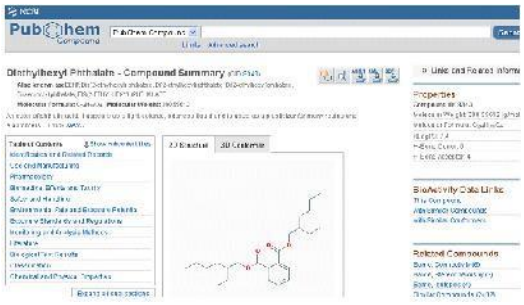


Figure 3

Structure of Dioctyl phthalate

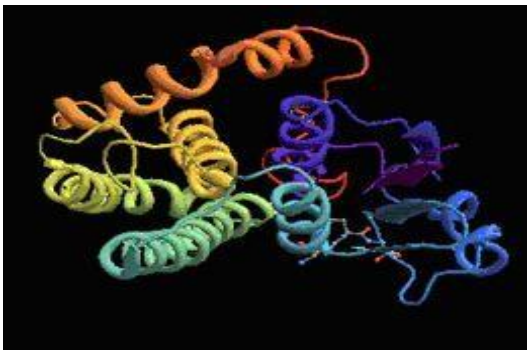


Figure 5

Structure of p53 receptor

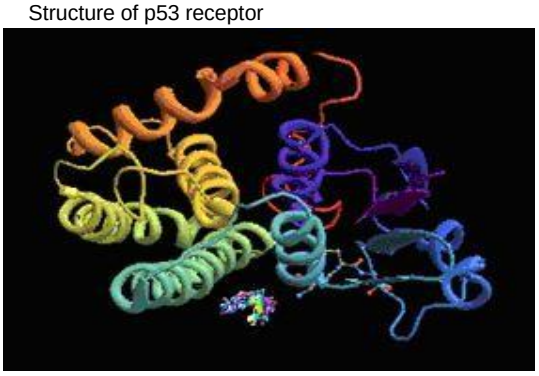


Figure 6

Structure of ligand dioctyl phthalate

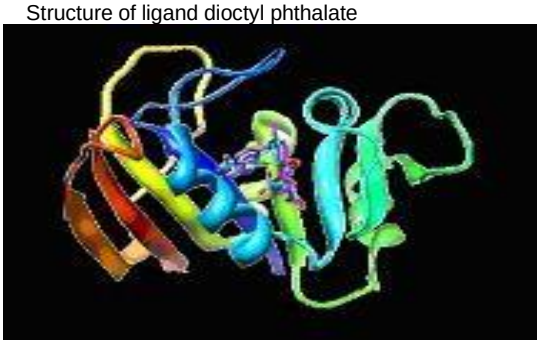


Figure 7

The receptor is successfully bound with the targeted ligand

similarities to each other. The similarity of docked structures is measured by computing the root-mean-square-deviation (rmsd), between the coordinates of the atoms. The docking results consist of PDBQ of the cartesien coordinates of the atoms in the docked molecule, along with the state variables that describe this docked confirmation and position.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Analysis

The results of the phytochemical analysis (Table 1) shows the presence of terpenoids, reducing sugars, tannins, carbonyl, flavanoids, steroids and phenol (Fig.2).

3.2. GC-MS

GC-MS analysis for acetone extract of the plant leaf shows the different types of compounds that

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have been present in the extract. A peak level in the graph indicates the maximum amount of the compound present in the extract. Through this GC-MS result, the high peak compound is selected for the molecular docking technique (Fig.4). The ligand molecule dioctyl phthalate (Fig.3) was successfully bound with the target receptor molecule p53-tumor suppressor protein. Its function is to prevent the human from cancer. The ligand molecule binded with receptor-p53 shows that dioctyl phthalate has ability to suppress the cancer by acting along with the p53 (Fig.5-7).

4. CONCLUSION

The GC-MS results of acetone extract shows the presence of new compound dioctyl phthalate in the plant *Cleistanthus collinus*. The outcome of Molecular docking predicts the anticancer capability of diocytl phthalate based on its strong binding to the p53 receptor. Our Newly discovered drug candidate "dioctyl phthalate" may act as a highly potential therapeutic agent for the people who are suffering from cancer, infected by deadly pathogens etc.

SUMMARY OF RESEARCH

1. *Cleistanthus collinus*, a highly toxic plant leaves were collected, shade-dried and soaked in acetone.
2. Phytochemical and GC-MS analysis was done to identify the presence of any organic compounds in that leaf. The result showed, different hydrocarbons and pharmacological valuable compound especially dioctyl phthalate were present in that extract.
3. Molecular docking is the in-silico method of predicting the binding of target receptor p53 'a tumor suppressor protein' to the ligand dioctyl phthalate.

FUTURE ISSUES

1. All compounds of the plant will be extracted using various solvent and the characterization can be studied.
2. Anticancer capability will be done in cancer cell lines.

DISCLOSURE STATEMENT

There is no financial support for the proposed research work.

ACKNOWLEDGMENTS

I thank my guide for his constant support throughout the research work. I extend my special thanks to my friends and family members for their moral support.

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RELATED RESOURCE

1. Sarathchandra G, Balakrishnamoorthy P. Acute toxicity of *Cleistanthus collinus,* an indigenous poisonous plant in *Caviaprocellus. J of Environ. Biol.* 1998, 145-8.

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