International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.2, pp 500-505, April-June 2014

HPLC, GC-MS and *in-silico* analysis of *Cucurbita maxima* methonolic extract for its activity against Prostate Cancer.

Daisy.P*, Winfan celes.J, Pon Nivedha.R

Bioinformatics Centre (BIF), PG& Research Department of Biotechnology & Bioinformatics, Holy Cross College (Autonomous), Tiruchirapalli-620002, Tamil Nadu, India.

***Corres.author: daisylesslie@gmail.com**

Abstract: Nowadays research on medicinal plants has got more attention and importance globally. A number of evidence has been documented to demonstrate the potential of these plants in pharma industry. In recent years *Cucurbita maxima* an important medicinal plant that has been extensively used in pharmacological analysis and toxicological studies was found and these phytochemicals were considered to be greater importance in pharma industry, To reveal out its medicinal importance the plant was subjected to HPLC analysis. HPLC results helps to isolate a single compound with a retention time of 2.962rt. GC –MS analysis was performed to find the structure of this particular compound and this was reported as Desmosterol. Docking studies were performed to find out the activity of this compound Desmosterol against Androgen receptor which exposes high binding affinity. Desmosterol showed more negative binding energy value of -12.3kcal/mol. This study ruled out a conclusion that Desmosterol may have a very good anticancer action against androgen receptor and future studies are recommended to use this plant and compound for commercial and medical purposes.
Keywords: *Cucurbita maxima*, Desmosterol, Autodock vina.

Introduction

Traditional herbal medicine is a rich source for modern, molecular target specific drug discovery (1). Plants have proven to be the most useful in curing diseases and provide an important source of pharma and medicine. The medicinal importance of these plants lies in some chemical substances that produce a distinct physiological action on the body of human (2). The major importance of these bioactive constitute of plants are Steroid, Terpenoids, Tannins, Carotenoids, Flavonoids, Alkaloids and Glycosides. Plants in all aspect of life have served as important material for drug development (3).Medicinal plants are the foundation of many important drugs of the modern world. Many of these local medicinal plants are used as spices and food items. Plants based drug is the major area of research. According to WHO calculations 80% of the world's population presently uses medicinal herbs drug for their primary health care (4). Many plants are cheaper and more simply to get to most people especially in the developing countries and these plants have lower incidence of side effect after use. Due to this reason they are used worldwide (5).

Prostate cancer is the most common type of cancer occurred in men. Hormones, in particularly the androgens, are essential for the development, growth and maintenance of the prostate. Certain pathological

assaults may trigger the hyper stimulation of androgen and/or growth factors leads to prostate cancer. Androgen ablation therapy is the first step in the prostate cancer treatment(6,7). Other than that various treatment strategies like chemotheraphy, radiation theraphy, hormones and surgery (8). Natural products as chemotherapeutic agent is the recent trends adopted. As natural products causes less side effects it is preferred. It is used as chemoprevention to inhibit or revert carcinogenesis and to suppress the tumor. Current chemotherapeutic drugs are not useful in all cases and have severe side effects on human health. So there is increased demand in identification of new plant based drugs.

Pumpkin is gourd-like squash belongs to genus Cucurbita and the family Cucurbitaceae. It botanical name is *Cucurbita maxima* and its native is North America. They naturally have a thick, orange or yellow shell. Pumpkins are broadly grown for commercial use, and are used both in food and recreation. In India, it is most consuming vegetables. Pumpkins are considered to be a fruit and it contains 90 percent water.

Pumpkins have antioxidant beta-carotene, which help to improve the immune function and can reduce the risk of diseases like heart disease and cancer. (9,10). Cucurbita maxima commonly known as Pumpkin has been used for various ailments cold, benign prostatic hypertrophy etc. This species are believed to have antitumor effect but its mechanism for activity remains to be elucidated. In this study compound present in the methonal extracts of pumpkin was identified through HPLC and Gc/Ms analysis and its mechanism of action was identified through docking analysis.

Materials and methods

Collection of Plant Material

Cucurbita maxima seeds were collected from Tiruchirappalli District Tamil Nadu, India. The species was identified and authenticated at the department of Botany, Holy Cross College, Trichy, Tamil Nadu. The seeds were shade-dried and coarsely powdered.

Preparation of Plant Extracts

500gms of seed powder was taken in an aspirator bottle; 1.5 litre of Methanol was used and the mixture was shaken occasionally for 72 hours. Then the extract was filtered. This procedure was repeated three times and all extracts were decanted and pooled. The extract was filtered before drying using Whatmann filter paper no.2 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40°C.

HPLC instrumentation

Shimadzu Model-LC2010 CHT, Serial No. C-21254505638 HPLC instrument was used for the chromatographic separation using C 18 column (250 nm x 4.6 nm). Isocratic elution was carried out with methanol at a flow rate 1.5 mL/min. The detection was performed with a D2 lamp at 223 nm wavelength. L C Solution software was used for integration and calibration. Evaluation was via peak areas with linear regression. Mobile Phase of 65 Volumes of methanol and 35 Volumes of water were used.

GC-MS analysis

The GC-MS analysis was performed on a combined GC-MS instrument (ITQ 900 Model of Thermo Fisher Scientific make) using a HP-5 fused silica gel capillary column. The method to perform the analysis was designed for both GC and MS using the XCaliber Software provided with the machine. A 1 µl-aliquot of sample was injected into the column using a PTV injector whose temperature was set at 275°C. The GC program was initiated by a column temperature set at 60°C for 5 min, increased to 300°C at a rate of 8 C/min, held for 10 min. Helium was used as the carrier gas (1.5 ml/min). The mass spectrometer was operated in EI mode with mass source was set at 200°C. The chromatogram and spectrum of the peaks were visualized using Qual Browser software. The particular compounds present in the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998) library.

Protein Data Bank (PDB)

PDB abbreviated as protein data bank was originally developed by Brookhaven National Laboratory in 1971. From 2003 it was maintained by worldwide Protein Databank (wwPDB). The wwPDB members are RCSB PDB (USA), PDBE (UK), PDBJ (Japan) and the BMRB (USA). It contains archive of information about the 3D structure of biomacromolecules and their complexes determined by X-ray crystallography, NMR spectroscopy and cryo-electron microscopy. The RCSB PDB also provides a variety of tools and resources. Users can perform simple and advanced searches based on annotations relating to sequence, structure and function (11). Three dimensional structure of the androgen receptor was retrieved from database using its id 2PIV.

Protein preparation

The ligand and crystallographic water molecules were removed from the protein, and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. Following the above steps of presentation, the protein was subjected to energy minimization by applying kollman charges.

Pubchem

Pubchem database provides information about the biological activities of small ligand molecule. It comprises three linked database such pubchem compound, pubchem substance and pubchem bioassay. The PubChem Compounds Database contains validated chemical depiction information provided to describe substances in PubChem Substance. Structures stored within PubChem Compounds are pre-clustered and cross-referenced by identity and similarity groups. Additionally, calculated properties and descriptors are available for searching and filtering of chemical structures. (12) Three dimensional structure of the desmosterol phytocompound was retrieved through pubchem text search and the structure was downloaded in .sdf format.

Ligand preparation

The three dimensional structure of phytocompounds saved in .sdf format were converted to .pdb format using open babel 2.3.1. Ligand was prepared using MGL tools by adding hydrogen atom to check the valencies of the heavy atoms. Ligand was minimized by computing gasteiger charges and saved in PDBQT.

Grid generation

Docking program Autodock vina uses a grid based method for energy evaluation of flexible ligand in complex with a rigid protein. Points on a 3D grid, placed to cover the entire inner cavity of the receptor are probed with the atoms that constitue the ligand. The dimensions of the Grid were 17,-8,-3.

Docking

Docking were carried out using Autodock Vina with AMBER force field and Monte Carlo simulated annealing algorithm (13). Throughout the docking studies the protein molecule was kept as rigid and drug molecules as flexible.

Results and discussion

Pumpkin, the most common fruit used in our daily life is been used for various ailments in traditional medicines Inorder to find out its role against prostate cancer docking analysis was carried out with the compound identified through HPLC and GC/Ms analysis.

Analytical HPLC was performed to identify the chemical compounds present in this plant. Totally six compounds were present in this sample. The greater height and the larger area of the peak show the predominantly available compounds in the sample. Hence compound 1 is present in a high concentration (Fig 1). Inorder to isolate compound from the extract, preparative HPLC was performed. Compound 1 which was found to be more in the extract was isolated by the preparative analysis. It was isolated at the retention time of 2.962tr (Fig 2.)

GC-MS analysis was performed to find the structure of particular compound. There was one significant peak in the GC spectrum of the sample. Identification of the peaks was made by retention time and a National Institute of Standards and Technology (NIST) library search. The sharp peak was identified as Desmosterol. (Fig 3)

In order to find the activity of Desmosterol against prostate cancer, docking was performed for Androgen receptor. Androgen receptor plays a key role in the development of prostate cancer. Androgen deprivation therapy is the initial response for the prostate cancer treatment. Inorder to investigate the role of desmosterol against androgen receptor docking studies was performed by using Auto dock vina. Docking is a computational method attempt to predict the noncovalent interaction between macromolecule and the drug. Autodock Vina which was used for docking is recently developed software shows more accuracy than other softwares. It uses genetic algorithm for the docking calculation.

The more negative binding energy values corresponding to the RMSD value of zero were considered as the binding affinity value of the ligand for each docking. Desmosterol showed more negative binding affinity value of 12.3.kcal/mol (Table 1). It showed one hydrogen bond interaction with Arginine 752 involvement of H bonded interaction helped the complex to achieve the established conformation of the complex structure (Fig 4).

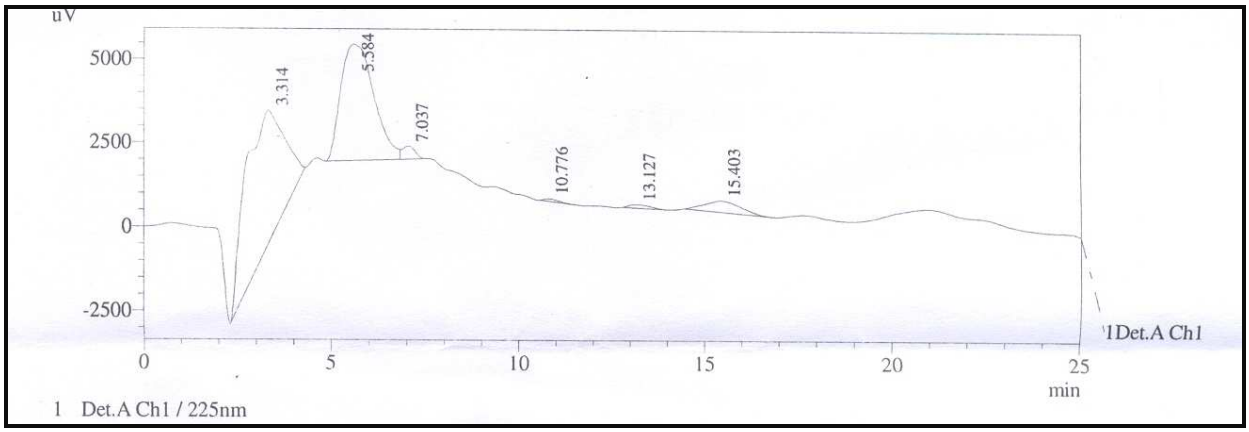

Figure 1: Analytical HPLC chromatogram of *Cucurbita maxima methonolic extract.*

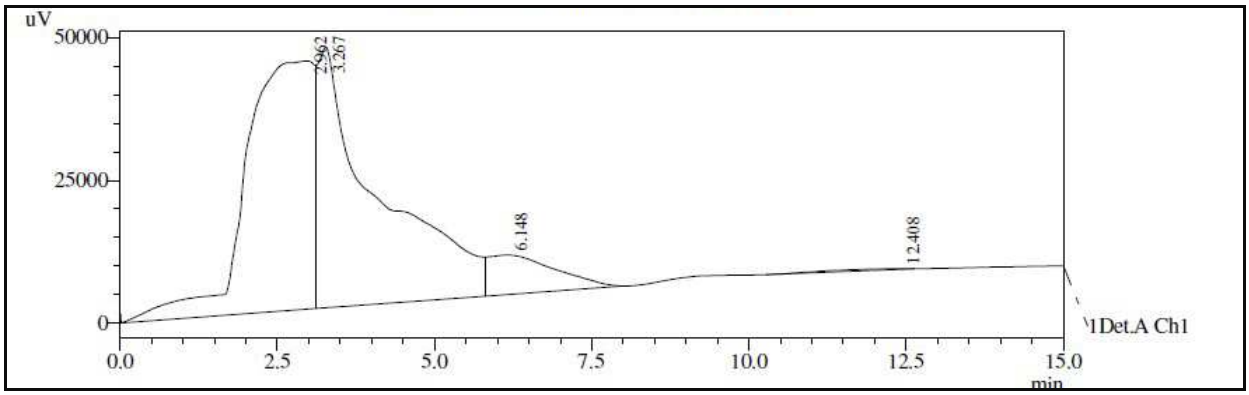

Figure 2: Preparative HPLC chromatogram of *Cucurbita maxima* methonolic extract.

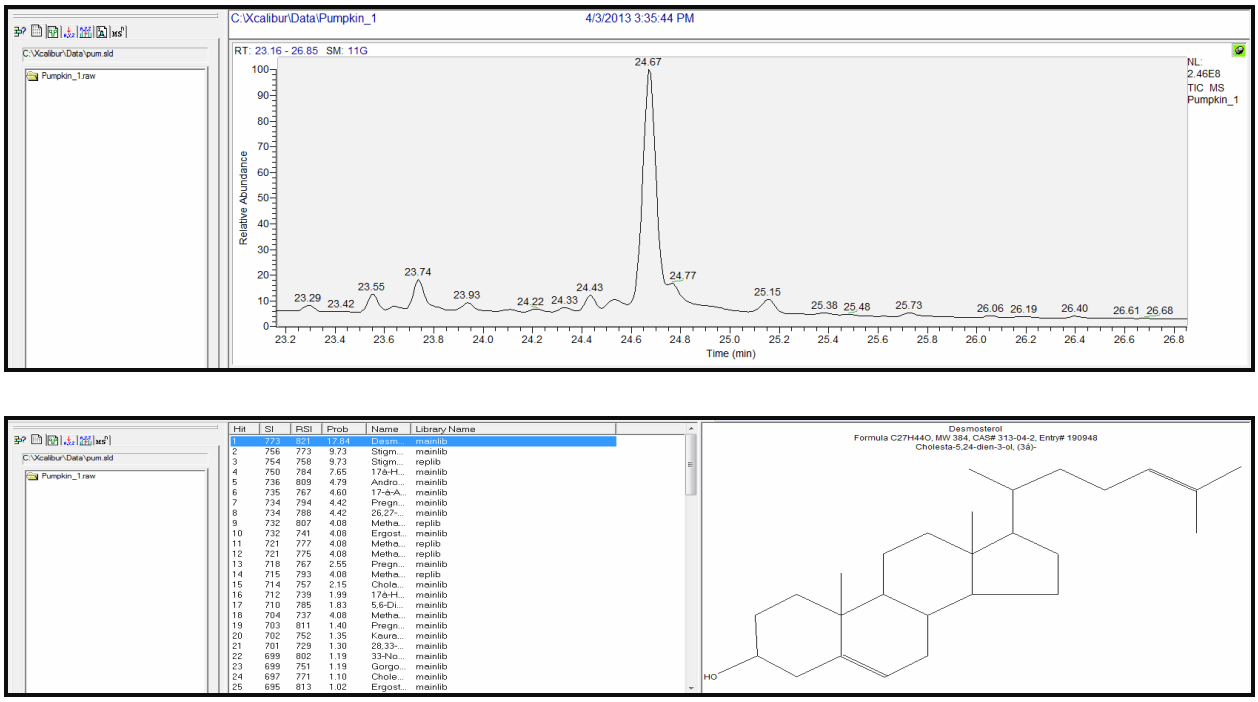

Figure 3:GC-MS chromatogram of isolated compound from *Cucurbita maxima*

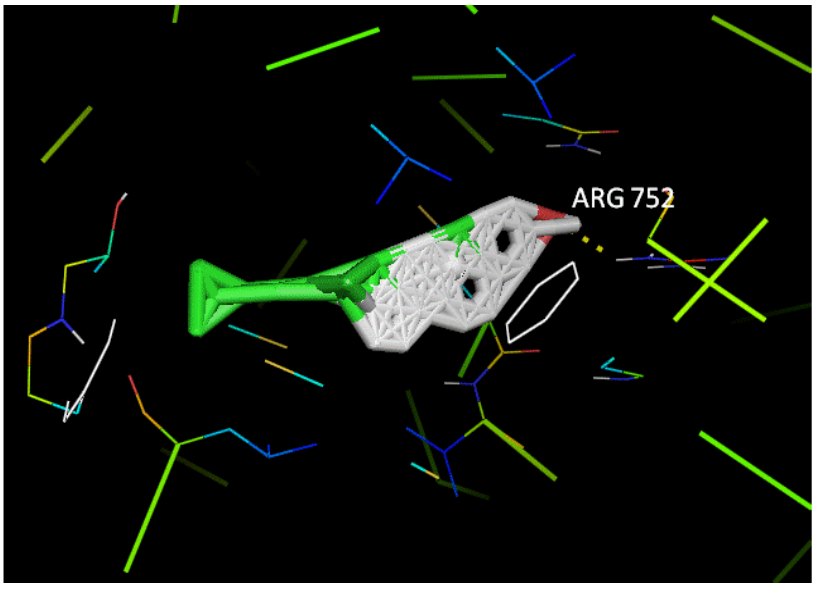

Figure 4: Hydrogen bond interaction between Desmosterol and Androgen receptor

Table 1: Binding affinity score of phytocompounds

S.NO	Ligand	Binding affinity
1.	Desmosterol	-12.3

Conclusion

Desmosterol was identified as a predominant compound present in the methanol seed extract of *Cucurbita maxima*. Docking studies of this compound against androgen receptor shows high binding affinity score. Hence it was concluded that desmosterol compound from this plant may render anticancer activity against Androgen receptor, and it may be used as drug through future studies.

Acknowledgement

The financial support extended by the DBT- Star college scheme, Ministry of Science and Technology, Government of India, is gratefully acknowledged.

References

1. Engel L.w, Straus S.E., Development of therapeutics opportunities within complementary and alternative medicine, Nature reviews: Drug discovery., 2002,1,229.
2. Farnsworth, Wislson E.O., Biodiversity National Academy Press Washington, 1988.
3. Balandrin, N. F, Kinghorn, A.D., Human Medicinal Agents from Plants, ACS Symposium Series, 1993, 534,2-12.
4. Etkin NL, Hausa A., Herbal Pharmacopoeia: Biomedical Evaluation of Commonly used Plant Medicines, Journal of Ethnopharmacology, 1981,4,75–98.
5. John M. Pezzuto., Plant-derived anticancer agents, Biochemical Pharmacology, 1997, 53,121-133.
6. Jin HJ, Zhao JC, Ogden I, Bergan R., Androgen receptor-independent function of FoxA1 in prostate cancer metastasis, Cancer Research, 2013, 28,111-116.
7. Drudge-Coates L, Turner B., Prostate cancer overview. Part 1: non-metastatic disease British Journal of Nursing, 2012, 21,23-8.
8. Bokhour BG, Clark JA, Inui TS, Silliman RA, Talcott TA., Sexuality after treatment for early prostate cancer: exploring the meanings of “erectile dysfunction” ,Journal of general internal medicine,2001, 10,649–55.
9. Lam Attarde D.L, Kadu S, Chaudhari B, Kale S, Bhamber R.S., In vitro Antioxidant activity of Pericarp of Cucurbita maxima Duch. Ex,International Journal of PharmTech Research, 2010,2, 1533-1538.
10. Gove Kebalka T, Pumpkin therapy, Journal of Cancer Education, 2013, 1:209-10.
11. Sussman JL, Lin D, Jiang J, Manning NO, Prilusky J, Ritter O, Abola EE, Protein Data Bank (PDB): database of three-dimensional structural information of biological macromolecules, Acta Crystallographica section D section Bioligical Crystallography, 1998,54,1078-84.
12. Wang Y, Xiao J, Suzek TO, Zhang J, Wang J, Bryant SH., PubChem: a public information system for analyzing bioactivities of small molecules. Nucleic Acids Research, 2009,1:37.
13. Trott O, Olson A.J., AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, Journal of Computational Chemistry, 2010,31, 455-461.

*****