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Molecular docking of phytochemical compounds in *Cucurbita maxima* with anti-prostate cancer activity

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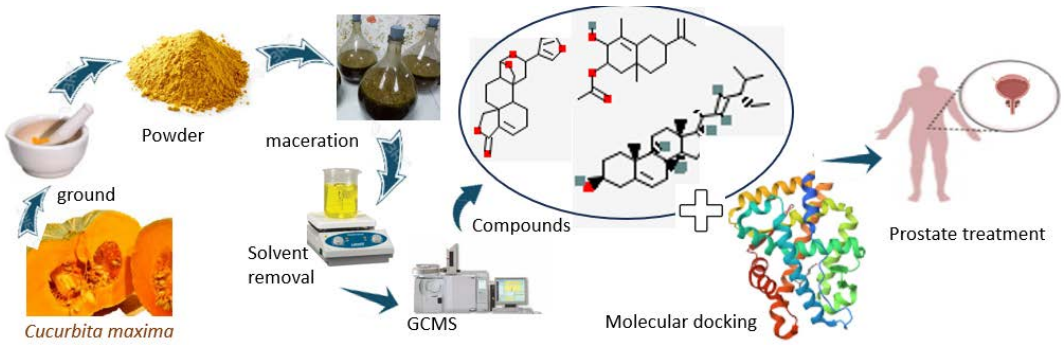
Research Article

ABSTRACT



Cucurbita maxima seeds are used in traditional medicine for the treatment of urinary disorders, blood pressure regulation and prevention of constipation, also for wound healing with dermal application, and recently for prostate cancer treatment. The objective of present study was to identify the medicinal compounds present in chloroform extract of the seeds of *Cucurbita maxima* using Gas chromatography mass Spectroscopy (GCMS). The phytochemicals identified were further assayed to determine the compounds associated with anti-prostate activity using *in silico* molecular docking. The GCMS analysis revealed the presence of twenty-three (23) compounds. The molecular docking of the compounds against Human Androgen Receptor Ligand binding showed that the compounds had good binding affinity against the target protein. However, two compounds, Stigmasterol (-8.5 kcal/mol), and Bacchotricuneatin C (-7.7 kcal/mol) had better binding affinity than the control anti-prostate cancer drug, Enzalutamide (-7.6kcal/mol). Consequently, the results validate the use of the seed of *cucurbita maxima* as an anti-prostate cancer agent.

Keywords: *cucubita maxima, stigmasterol, enzalutamide, phytochemicals, prostate cancer*

INTRODUCTION

In nature, many plants and seeds have been sources of medicine since the earlier times. Plants are a significant source of pharmaceuticals and medicines.[1] Plants have great significance to the health of individuals. The medicinal importance of the plants lies in bio-active constituents of plants i.e. chemical substances that produce a distinct physiological action on the body of human.[2] Plants in all aspect of life have served as important material for drug development. Plants are now playing an important role in many medicines, and are cheaper and easier to get for most people especially in the developing countries.[3]

The pumpkin (Cucurbita spp.), one of the most popular vegetables consumed in the world, has been recently recognized as a functional food.[4] Pumpkin seeds, generally considered agro-industrial waste, are an extraordinarily rich source of bioactive compounds with interesting nutraceutical properties.[5-7] In recent years, several studies have highlighted the health properties of pumpkin seed oil against many diseases, including hypertension, diabetes, and cancer. These species possess a higher number of proteins, phytosterols, unsaturated fatty acids, vitamins (like carotenoids, tocopherols) and microelements (e.g., zinc). Fruits, seeds and leaves from various Cucurbita members (pumpkin, watermelon, melon, cucumber squash, gourds, etc.) possess different pharmacological effects.[8-12] The numerous medicinal uses of pumpkin seeds are due to the nutrients composition and functional ingredients that make it to serve as the principal metabolites that sustain life, prevent diseases and promote health in human beings.[13-16] The anti-carcinogenic properties of pumpkin seeds have been observed with people on diets high in pumpkin seeds because of their been associated with lower risk of gastric, breast, lung and colorectal cancers.[17] Oil extracted from pumpkin seeds has been reported to contain Phytochemicals that posses anti-carcinogenic effects.[18-23]

Cancer is a disease in which abnormal cells divide uncontrollably and progressively destroy body tissue. Cancer according to World Health Organization (WHO) is the second

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most factor for number of casualties over the world after coronary heart disease or all stroke.[24]

Global cancer incidence is on the rise and it is predicted that more than 60% of the annual cancer diagnoses by the year 2050 will be individuals residing in Low and middle income countries (LMICs).[25] Prostate cancer is the second most frequently diagnosed cancer in men globally and accounts for 3.8% of all deaths caused by cancer in men as of 2018.[26] Prostate cancer can be treated with multimodal therapy like surgery, controlled drug, antibody therapy, hormonal therapy, radio-therapy, and chemo-therapy though the most commonly used therapy are chemotherapy, surgery, and radiotherapy.[27]

It is well recognized that androgen hormones and their executor androgen receptors control important processes in the development and spread of prostate cancer.[28] Research has shown that androgen receptors contribute to proliferation and differentiation of prostate epithelial cells during development of androgen dependent Prostate cancer. Thus, over the years, androgen receptors has emerged as a potential and attractive target for Prostate cancer therapy through application of androgen receptors antagonists or combined androgen blockade therapy.[29] The most commonly recommended androgen receptor antagonists for prostate cancer are non-steroidal medications like flutamide, bicalutamide, and enzalutamide, as well as steroidal medications like cyproterone acetate.[30] Although a number of gonadotropin releasing hormone (GnRH) agonists and anti-androgens have emerged as the most commonly prescribed chemotherapeutic drugs for Prostate cancer,[31] the problem of cancer recurrence after short period of response and increased cytotoxicity due to drug intake remains a hurdle in the path of effective therapy.[32] The positive correlation with phytochemicals intake and reduced risk of Prostate cancer has attracted interest and thereby focused the attention of research towards phytochemicals as chemotherapeutic agents in Prostate cancer.[33] These plant compounds which exhibit high binding affinity for prostate cells /androgen receptors could possibly lead to effective Prostate cancer treatment. In the current study, we have chosen Cucurbita maxima (pumpkin) for phytochemical analysis study due to its local use for treating prostate cancer. The study aimed at identifying the phytochemicals present in the plant. In silico methods will be used to study the anti-prostate cancer potential of the plant compounds.

MATERIALS AND METHODS

Preparation of crude extract

Maceration is a common procedure for the preparation of extracts. A mass of 200g of the ground plant material was measured and placed in a stoppered container containing 1.5l of the chloroform and allowed to stand at room temperature for a period of 3 days with frequent agitation until the soluble matter had dissolved. The mixture was then clarified by filtration. The solvent was then evaporated to get the crude sample extract.[34]

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis was done at Zaria, kaduna state Nigeria. The compounds in the sample were identified using agilent GC-MS (Agilent 19091-433HP, USA) coupled to a mass spectrophotometer. The initial column temperature was 35 °C with a hold time of 3 minutes. The temperature was programmed to rise by 8°C /min with a final temperature of 280°C. In the process, 1µl of the sample was injected into the port and immediately vaporized and moved down the column with helium as the carrier gas with flow rate of 1 ml/min. The MS Spectrum was taken at 70 eV. The identification of the compounds was done by comparing the spectrum of unknown compounds with the spectrum of known compounds in NIST14 structural library.[35]

Molecular docking

Ligand preparation

The three-dimensional (3D) structure of the identified compounds was downloaded from PubChem database. Hydrogen Bonds were added and the energy minimization was done using the CHARMM force field in open babel software.

Protein target preparation

The 3D structure of the Human Androgen Receptor Ligand Binding was retrieved from Protein Databank (PDB ID: 1E3G). The 3D structure has been prepared by removing water molecules, cofactor and substrate and determination of the active sites using the pymol software. Further preparations include the addition of Kollman charges and polar hydrogen using autodock tools.

Docking studies

Autodock Vina software was used to do docking analysis on the prepared ligand and protein. Based on several scoring functions, the software allows us to virtually screen a library of compounds and anticipate the strongest binders.[36] The docking result was visualized using the accelrys discovery studio software.

RESULTS

Extraction of phytochemicals in the seed of C. maxima

The extraction was carried out in chloroform. The extract was a light-yellow oil, and 7.8% of the extract was collected as yield after recovery of the solvent. The high percentage yield can be attributed to the type of solvent and also the extraction technique.[37]

Gas chromatography-mass spectroscopy (GC-MS)

The result of the Gas chromatography-mass spectrometry has been presented in Table1. In this study, 23 compounds were identified in the sample. The selected compounds were chosen based on their percentage peak area which is described as their concentration.

The compounds were analyzed based on the retention time present in the total ionic chromatogram of the GC-MS. The percentage area was taken as the relative abundance of the compounds in the sample. Results presented in Table 1 revealed the presence of 23 phytochemical compounds along with their retention time, % abundance, molecular formula, chemical name, and common name. The most abundant phytochemical identified was cis-Vaccenic acid (35.54%). some of the compounds identified in this present study have previously reported medicinal potentials. The most abundant compound, Cis-vaccenic acid for example has been reported to possess

Table 1: Phytochemicals and binding affinity of the chloroform extract of Cucurbita maxima

S/N	Compound	% Area	RT	MW (g/mol)	MF	PUBCHEM CID	BINDING AFFINITY
cnt	Enzalutamide			464.4	C21H16F4N4O2S	15951529	-7.6
1	cis-Vaccenic acid	35.54	31.253	282.5	C18H34O2	46235519	-5.4
2	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthale-2-yl ester	30.09	38.523	278.4	C17H26O3	540542	-6.8
3	8-Hexadecenal, 14-methyl-, (Z)-	6.70	18.805	226.4	C15H30O	6450379	-5.2
4	1-Pentadecene	5.59	18.643	210.4	C15H30	25913	-4.6
5	Cyclohexanecarboxaldehyde, 3,3-dimethyl-5-oxo-	4.65	17.881	154.21	C9H14O2	543470	-6.0
6	2-(4-Methylcyclohexyl)prop-2-en-1-ol	3.89	18.305	154.25	C10H18O	565251	-6.4
7	9,12-Octadecadienoic acid (Z,Z)-	2.96	25.627	280.4	C18H32O2	534410	-6.1
8	Squalene	2.65	35.451	410.7	C30H50	638072	-5.8
9	n-Hexadecanoic acid	1.74	22.476	256.42	C16H32O2	985	-4.7
10	Stigmasterol	1.02	35.782	412.7	C29H48O	5280794	-8.5
11	2-Isopropenyl-5-methylhex-4-enal	1.01	6.912	152.23	C10H16O	93979	-5.7
12	2,4-Decadienal, (E,E)-	0.71	6.399	152.23	C10H16O	5283349	-5.6
13	9-Oxabicyclo[6.1.0]nonane, cis-	0.66	25.019	126.2	C8H14O	533431	-5.5
14	Ethyl palmitate	0.61	21.876	284.5	C18H36O2	12366	-4.8
15	3-Heptafluorobutyroxypentadecane	0.48	16.878	424.4	C19H31F7O2	534410	-6.1
16	2-Decenal, (Z)-	0.41	5.639	154.25	C10H18O	5354834	-5.4
17	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	0.39	20.901	362.5	C22H34O4	15151418	-5.7
18	9,12-Octadecadienoic acid, ethyl ester	0.33	24.887	308.5	C20H36O2	12363975	-5.0
19	Neopentylidenecyclohexane	0.22	23.222	52.28	C11H20	142360	-6.5
20	Sesquirosefuran	0.12	37.281	218.33	C15H22O	5366078	-6.3
21	Bacchotricuneatin C	0.05	16.050	342.4	C20H20O5	101324782	-7.7
22	3-Heptafluorobutyroxytridecane	0.02	16.268	396.4	C17H27F7O2	534408	-6.1
23	3-Chloropropionic acid, heptadecyl ester	-0.01	14.374	347	C20H39ClO2	545757	-4.8

Note: Cnt=controle, RT=retaintion time, MW=molecular weight, MF=molecular formula

antibacterial and hypolipidemic effects in rats. 1-pentadecene has antibacterial activity, sesquirosefuran has insecticide activity. Stigmasterol is reported to be responsible for inhibiting the promotion and growth of apoptosis of cancer cells. Squalene has anti-cancer compound, and benefits cholesterol levels. Oxirane, tridecyl- has bactericidal, fungicidal, and sporicidal disinfectant potentials. Hexadecanoic acid has antioxidants, hypocholesterolemic, nematicide, and pesticidal activity. The identification of these compounds in the pulp extract would serve as the foundation for determining the potential health benefits of this vegetable.

In-silico anti-prostate cancer activity of the chloroform extract of C. *maxima*

Molecular docking was performed for the phytochemicals to evaluate their anti-prostate cancer activity. This was done to identify the phytochemical that have significant androgen receptor (AR) inhibition activity.

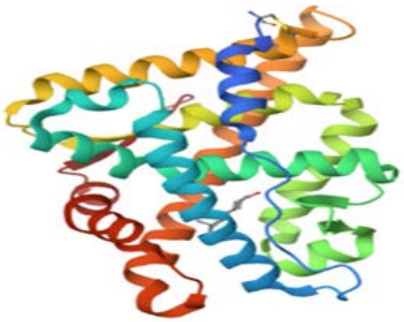


Figure 1. Cartoon display of 3D structure of Human Androgen Receptor Ligand Binding in complex with the ligand metribolone

All the identified phytochemicals were screened against the Human androgen receptor (HAR) ligand binding; however, their binding affinity and interaction against the HAR were recorded. The binding energy of each compound was presented on Table 1. The binding energy with a higher negative value corresponds to a more stable interaction between the compound and target receptor. To predict the binding interactions of active compounds with HAR, the interacting amino acid residues were identified by 2D diagrams of interactions presented in Fig. 1. Out of the 23 compounds, Stigmasterol exhibited the best binding affinity to HAR in terms of a low binding energy of (-8.5) kcal/mol followed by Bacchotricuneatin c (-7.7) kcal/mol; however, their binding energies was also higher than that of the control drug, Enzalutamide (-7.6 kcal/mol). Enzalutamide is the new androgen-receptor targeted treatments that competitively binds to the androgen receptor and inhibits its translocation at several levels.[38] Stigmasterol exhibited strong interaction with the HAR using one conventional hydrogen bond at amino acid residue GLN802. The compound also bonded using alkyl bonds attached to amino acids VAL685, PRO682, and PRO801, unsaturated to saturated pi-alkyl bonding were also observed as attached to residues LEU805, and TRP751. Further interactions are van der Waal's interactions with 10 amino acid residues at the active site of the Human androgen receptor binding (Fig. 2 A-C). Bacchotricuneatin C bonded with the HAR using conventional hydrogen bonding with TYR796. Other interactions were alkyl bonding with amino acid residue LYS 861, and van der Waal's interaction with residues TYR857, GLN858, LEU797, LEU862, GLU793, and SER865. The control compound showed conventional hydrogen bonding with amino acid residue TRP751, other interactions include alkyl and pi-alkyl interactions LEU805, PRO801, and ARG752. The halogens in the compounds bonded with PRO801. Further interactions were van der Waal's interactions.

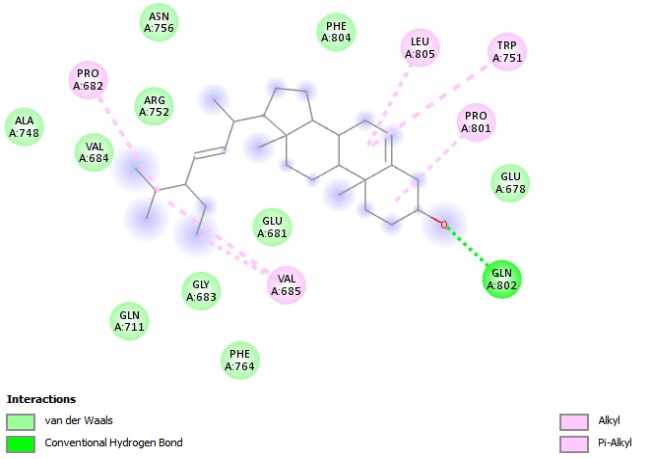


Figure 2A. Human Androgen Receptor Ligand Binding in complex with Stigmasterol

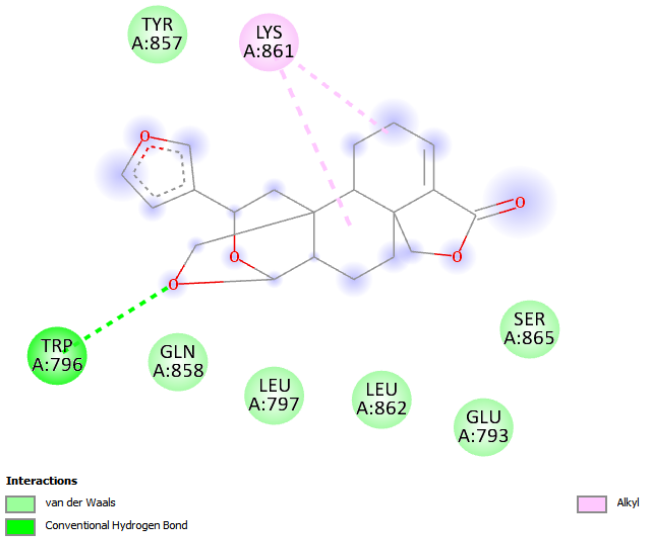


Figure 2B. Human Androgen Receptor Ligand Binding in complex with Bacchotricuneatin C

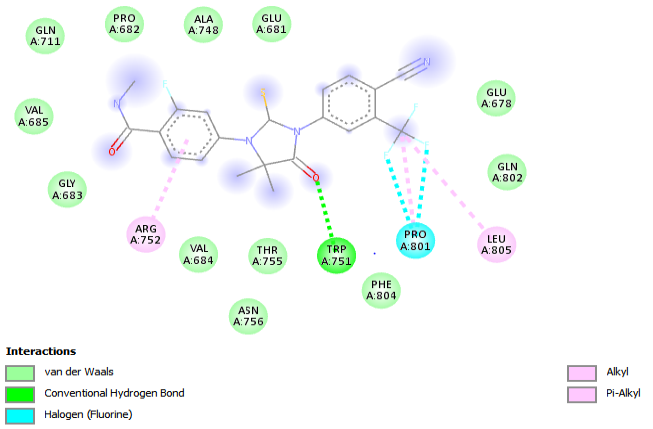
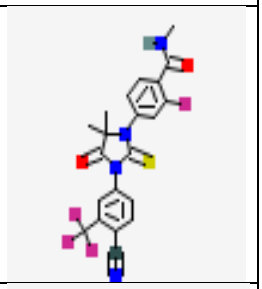
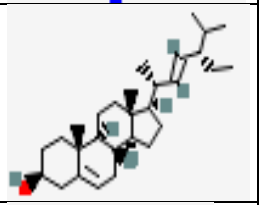
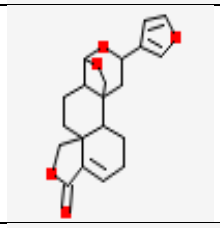
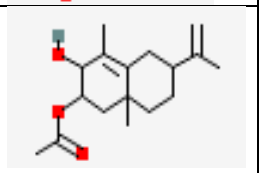


Figure 2C. Human Androgen Receptor Ligand Binding in complex with Enzalutamide

The present study demonstrated that among 23 phytochemicals screened for anti-androgen activity, two

Table 2: The class and structures of the identified compounds with comparable binding activities with the enzalutamide on the protein target

S/N	Name	Class	Binding affinity	Structure
cnt	Enzalutamide	synthetic	-7.6	
1	Stigmasterol	phytosterol	-8.5	
2	Bacchotricuneatin c	bisindole alkaloids	-7.7	
3	3-hydroxy-6-iso propenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	Fatty acid	-6.8	

Cn=controle

compounds had very strong binding affinity and also bonded strongly with the amino acids at the binding site of the human androgen receptor (HAR). However, stigmasterol which has the highest binding affinity to HAR is a class of steroid called phytosterol. Apart from anti-inflammatory properties of stigmasterol, it also has antiviral, antiparasitic, anti-osteoarthritis, antibacterial, neuroprotective, and immunomodulatory properties.[39,40] Followed by stigmasterol in the ligand binding affinity of the identified phytochemicals is Bacchotricuneatin C (an alkaloid) that showed a higher binding affinity and stronger bonds at the active site than that of the proficient therapeutic drug Enzalutamide. The high binding energy of two identified compounds, stigmasterol and Bacchotricuneatin C as compared to the binding energy of the prescribed drug enzalutamide highlights the importance of *Cucurbita maxima* seed in the treatment of prostate cancer.

CONCLUSION

In the present study, twenty-three (23) compounds were identified in the GCMS screening of chloroform extract of

Cucurbita maxima. However, the present study demonstrated that Stigmasterol and Bacchotricuneatin C are potential compounds responsible for the anti-prostate cancer activity of the chloroform extract of *cucurbita maxima* as they showed better binding and better interactions than the control drug. These findings provide more evidence to support the traditional use of the plant. The findings of this study suggest that the extract of *cucurbita maxima* seed may show better result in the treatment of prostate cancer than the use of ezalutamide the regular clinical prescribed drug.

Conflict of Interest

Authors do not have any conflict of interest (financial or academic) for publication of this work.

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