In silico DOCKING STUDIES OF BIOACTIVE COMPOUNDS IN CHLOROFORM EXTRACT OF *Annona muricata* LEAVES AGAINST HUMAN ANDROGEN RECEPTOR

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ABSTRACT

It has become a major challenge for clinicians and research scientists to control or treat prostate inflammation due to drug resistance being commonly observed. Crude extracts from medicinal plants could serve as an alternative source for resistance-modifying agents because they contain numerous diverse secondary metabolites. It has been claimed that *Anonna muricata* possess anti-inflammatory, antioxidant, anticancer and antibacterial activities. However, little is still known about the bioactive compounds responsible for this activity. In this study, the plant`s bioactive compounds were extracted using chloroform and analyzed with Gas Chromatography-Mass Spectrometry (GC–MS). The drug likeness and ADME predictions were done with Swissadme webserver, while the molecular docking against human androgen receptor was done using Auto dock Vina. The docking results showed that the binding energy and interactions of 9,12-Octadecadienoic acid (Z,Z)- (-6.7kcal/mol); 1,2-Benzenedicarboxylic acid, butyl octyl ester (-6.1) and 8-Hexadecenal, 14-methyl-, (Z)- (6.1kcal/mol)) were close to the control drug enzalutamide (-7.6 kcal/mol) and the protein native ligand methyltrienolone (-7.4kcal/mol). More so, these compounds showed drug likeness by obeying the limpiski rule qualifying them to be good drug candidate for control or treatment of prostate inflammations.

Key Word: *Anonna muricata*, prostate, inflammation, enzalutamide, androgen.

INTRODUCTION

Prostate cancer which is the highest cause of cancer - associated mortalities in men affects men of middle age and old age [1]. One of the most predisposing genetic risk factors for prostate cancer is family inheritance. Different studies on both hereditary and epidemiological studies have both proven the role of hereditary contributing factor on prostate cancer [2]. Many researchers have investigated the possible role of genetic variation in androgen biosynthesis and metabolism, as well as the role of androgens as possible causes of prostate cancer [3]. However, genomics research has identified molecular processes that result in certain cancer developments, such as chromosomal rearrangements [4] Candidate genes for prostate cancer predisposition are genes that partake in the androgen pathway and metabolism of testosterone. The development of prostate epithelium and prostate cancer cells relies on the androgen receptor signaling pathway and testosterone [5]. Prostate cancer can either be classified as androgen sensitive or androgen insensitive, which is an indicator of testosterone stimulation and the possible treatment option [6]. Several researchers have reported that the

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androgen pathway is one of the most important signaling mechanisms involved in prostate cancer [7] for instance Huggins and Hodges after observing the benefits of castration in prostate cancer patients proposed that prostate cancer growth was driven by androgens [8]. The human androgen receptor (HAR) is a ligand – activated transcription factor that regulates genes important for male sexual differentiation and development [9-10]. The role of androgen receptor (AR) is central to prostate patho-biology, as the prostate is dependent on androgens for normal growth and maintenance [11-12].

Presently, the treatment of prostate cancer mainly includes surveillance at the initial stages, surgery, radiotherapy, chemotherapy, and androgen deprivation therapy (ADT) [13]. However, chemotherapy and radiation therapy exhibit severe toxicity on normal tissues and hormone therapy for prostate cancer also has various unpleasant side effects, such as inducing cardiovascular diseases [14]. Therefore, research by various independent groups suggests the enormous potential of various phytochemicals of plant extracts used in traditional and folk medicine as a potential remedy for deadly diseases like prostate cancer [15 – 16]. Many studies have reported the therapeutic effects of *Annona muricata* leaves for the treatment of different types of cancer-like lung cancer [17], breast cancer [18], colon cancer [19] and prostate cancer [20]. Presently, to explore the potentials of *A. muricata* leaves on treating prostate cancer, a comprehensive approach combining phytochemical screening, pharmacokinetic evaluation, in silico protein-targets investigation and computer experiments validation analysis were used.

MATERIALS AND METHODS

Collection and Identification

The leaves of *Annona muricata* was harvested from a local farm in Logara, Ngor okpala Local Government Area of Imo State Nigeria and was identified by Prof Mbagwu of Plaant Science Department Imo State University Owerri.

Identification and Preparation of Ligands

The 3D structure-data files (SDF) of the bioactive compounds in the essential oil were identified and downloaded from the PubChem database. They were minimized in PyRx virtual screening tool, using Universal Force Field at 200 steps. They were then converted to AutoDock ligands (pdbqt) and used for the docking analysis.

Identification and Preparation of the Molecular Target

The 3D structure of human androgen receptor (PDB ID:1E3G) was prepared by removing water molecules, cofactor and substrate and determination of the active sites using the discovery studio 2020 software.

Docking and Post-docking Studies

Multiple docking of the ligands on a specified human androgen receptor (HER) binding pocket was done with AutodockVina in PyRx software (version 0.8). The center grid box and the binding

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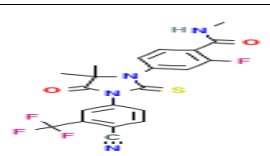
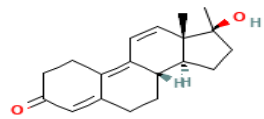
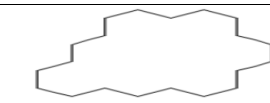
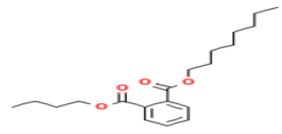
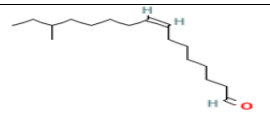
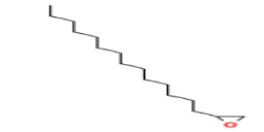
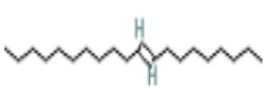
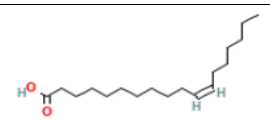
free energies of the compounds on the protein target were obtained after the docking process. Biovia Discovery studio 4.5 was used to visualize the interactions between the protein-ligand complexes after the docking process.

ADME Analysis

Absorption, Distribution, Metabolism and Elimination (ADME) analysis The most potent bioactive compound was chosen and sent to the SwissADME server to examine its drug-like properties.

RESULT AND DISCUSSION

Table 1: THE GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GCMS) RESULT OF THE CHLOROFORM EXTRACT OF *ANNONA MURICATA* LEAVES

S/N	RT	%	COMPOUND	MF	MW	CID	BF	STRUCTURE
Cnt			Enzalutamide	$C_{21}H_{16}F_4N_4O_2S$	464.4	15951529	-7.6	
NL			Methyltrienolone	$C_{19}H_{24}O_2$	284.4	261000	-7.4	
1	5.141	0.44	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	5280450	-6.7	
2	17.286	0.14	Cycloeicosane	$C_{20}H_{40}$	280.5	520444	-6.5	
3	20.940	0.13	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334.4	66540	-6.1	
4	13.378	0.26	8-Hexadecenal, 14-methyl-, (Z)-	$C_{17}H_{32}O$	252.4	5364688	-6.1	
5	18.769	5.46	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240.2	23741	-5.9	
6	17.923	0.81	9-Eicosene, (E)-	$C_{20}H_{40}$	280.5	5365037	-5.5	
7	27.691	0.12	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5	5282761	-5.5	

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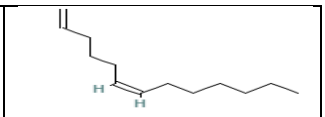
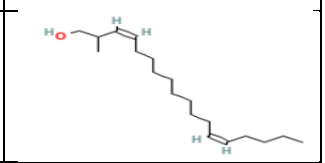
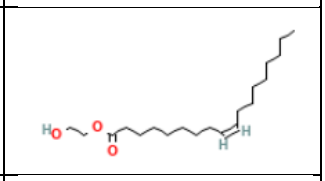
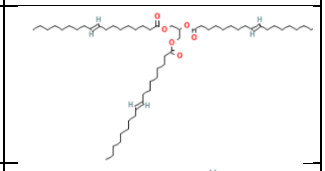
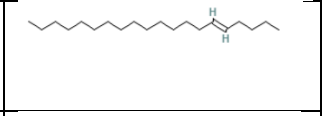
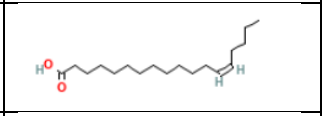
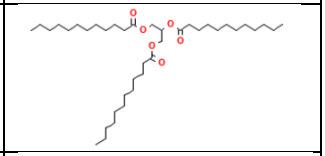
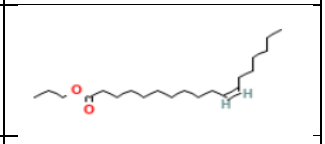
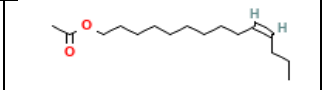
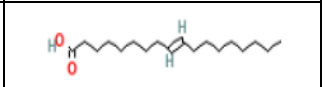
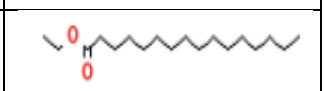
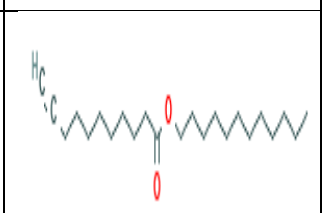
8	25.022	0.40	Z-1,6-Tridecadiene	$C_{13}H_{24}$	180.33	5364461	-5.5	
9	7.796	9.81	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.5	5364412	-5.3	
10	26.537	16.41	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	$C_{20}H_{38}O_3$	326.5	5364420	-5.2	
11	37.121	7.89	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	$C_{57}H_{104}O_6$	885.4	5364673	-5.1	
12	13.406	0.19	5-Eicosene, (E)-	$C_{20}H_{40}$	280.5	5364600	-4.9	
13	27.744	0.20	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	5312441	-4.9	
14	38.310	21.45	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	639.0	10851	-4.6	
15	13.046	0.13	n-Propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324.5	87131822	-4.6	
16	14.851	4.18	Z-10-Tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	254.41	5363221	-4.6	
17	34.904	11.29	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	637517	-4.5	
18	21.887	0.11	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5	12366	-4.2	
19	12.649	0.13	Undec-10-ynoic acid, dodecyl ester	$C_{23}H_{42}O_2$	350.6	91692432	-4.2	

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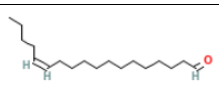
20	24.354	0.02	13-Octadecenal, (Z)-	$C_{18}H_{34}O$	266.5	5364497	-4.1	

GCMS Analysis of Phytochemicals

The compounds identified in the GCMS with their molecular formula, molecular weight (MW), concentration (peak area %), binding affinity (binding scores) and their structures were presented in Table 1. The GCMS result indicated the 18 compounds which include aliphatic fatty acids like Dodecanoic acid, 1,2,3-propanetriyl ester (16.41%), 9-Octadecenoic acid (11.29%), 9,12-Octadecadienoic acid (Z,Z)- (0.44%) etc, one aromatic fatty acid; 1,2-Benzenedicarboxylic acid, butyl octyl ester (0.14%), one *alicyclic* compound; Cycloeicosane (0.14%), heterocyclic compound; Oxirane, tetradecyl (5.46%). Among other aliphatic compounds identified are fatty alcohols, alkanals carboxylic acids and alkenes.

The binding energies were graded based on the highest negative energy. Compounds with preferred binding scores are those that gave higher negative values like 9,12-Octadecadienoic acid (Z,Z)- (-6.7kcal/mol); followed by Cycloeicosane (-6.5kcal/mpl); 1,2-Benzenedicarboxylic acid, butyl octyl ester (-6.1) and 8-Hexadecenal, 14-methyl-, (Z)- (6.1kcal/mol). Some of, the identified compounds have been reported to possess therapeutic properties. The binding affinity of 9,12-Octadecadienoic acid (Z,Z)- (6.7kcal/mol) is closely related to the binding affinity of the commonly use anti-prostate drug enzalutamide (-7.6 kcal/mol) and the protein native ligand methyltrienolone (-7.4kcal/mol) suggesting that 9,12-Octadecadienoic acid (Z,Z)- probably has the potentials of inhibiting the binding of androgen to the targeted protein site of human androgen receptor The compound 9,12-Octadecadienoic acid (Z,Z)- has been reported to possess anti-cancer, anti inflammatory, antioxidant and antibacterial activities[21]. However, compounds like 1,2-Benzenedicarboxylic acid, butyl octyl ester and 8-Hexadecenal, 14-methyl-, (Z)- that gave comparable binding score with the control drug and native ligand have been reported to posses antiinflamatory potentials[22]

Interactions

Four out of the 20 identified compounds were selected for studying their interaction with the human androgen receptor, as they showed binding affinity similar to the cocrystallized ligand and the reference drug enzalutamide.

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Enzalutamide, the drug that serves as the reference, exhibits higher binding affinity (-7.6kcal/mol) in comparison to the binding affinity of the compounds that have been identified. lt interacted with the human androgen enzyme through four conventional hydrogen bonds THR:755, TRP: 751, PRO:682 and PRO:801, a halogen (fluorine) bond PRO:801, two alkyl and pi alkyl bonds LEU:805 and ARG:752 with ten *vander waal* bonds The cocrystalline ligand (native Ligand) with binding affinity of (-7.4kcal/mol) form *vander waal* bond with nine different amino acids, one conventional hydrogen bond LYS:808 and two alkyl bonds with ARG:752 and LA:748 respectively. 9,12-octadecadienoic acid with affinity (-6.7kcal/mol) interacted with the diseased protein using seven vander waal force and three conventional hydrogen bond with enzymes MET:745, ARG:752 and GLN:711, and eleven alkyl bond. Cycloeicosane though gave good binding affinity (-6.5kcal/mol) showed a very poor interaction with targeted enzyme by interacting with seven *Vander waal* bonds and one Pi-sigma bond with PHE:876. Another extract 1,2-benzenedicarboxylic acid, butyl octylester with affinity (-6.1kcal/mol) interacted with the human androgen protein by forming eleven Vander waals bonds, conventional hydrogen bond with ARG:752, carbon hydrogen bond with TRP:751, and Alkyl and pi alkyl bonds. Phyto compound 8- Hexadecenal, 14-methyl-, with same affinity (-6.1kcal/mo) also interacted with ten vanderwaal bonds, eight Alkyl bonds two conventional hydrogen bonds with enzymes GLN:711 and ARG:752. The interaction results showed that some of the identified compounds like, 9,12- octadecadienoic acid; 1,2-Benzenedicarboxylic acid butyl octylester and 8- Hexadecenal, 14-methyl-, though with lower binding scores as compared with the control and native ligand have significant potentials to block the active site of the human androgen receptor enzyme by having both conventional hydrogen bonds and carbon hydrogen bonds [23- 24]. The binding affinities and the interactions justify the use of the plant as an anti-cancer, anti-inflammatory, antioxidant and antibacterial agents[21- 22].

Pharmacokinetic and Physicochemical Properties

The pharmacokinetic and physicochemical properties of the extract compounds with good binding scores as well as those of the control drug and the native ligand were revealed by their ADME properties as summarized in Table 2. The drug likeliness of all these compounds was assessed from Lipinski's rule of five. Good drug candidates should not violate more than one of the rules [25]. Interestingly, all of the analyzed compounds were found to meet the Lipinski's rule of five, with most of them attaining a good score of bioavailability by having molecular weight < 500, The hydrogen bond donor (5 hydrogen) and hydrogen bond acceptor (not more than 10 hydrogen) of the compounds agreed with

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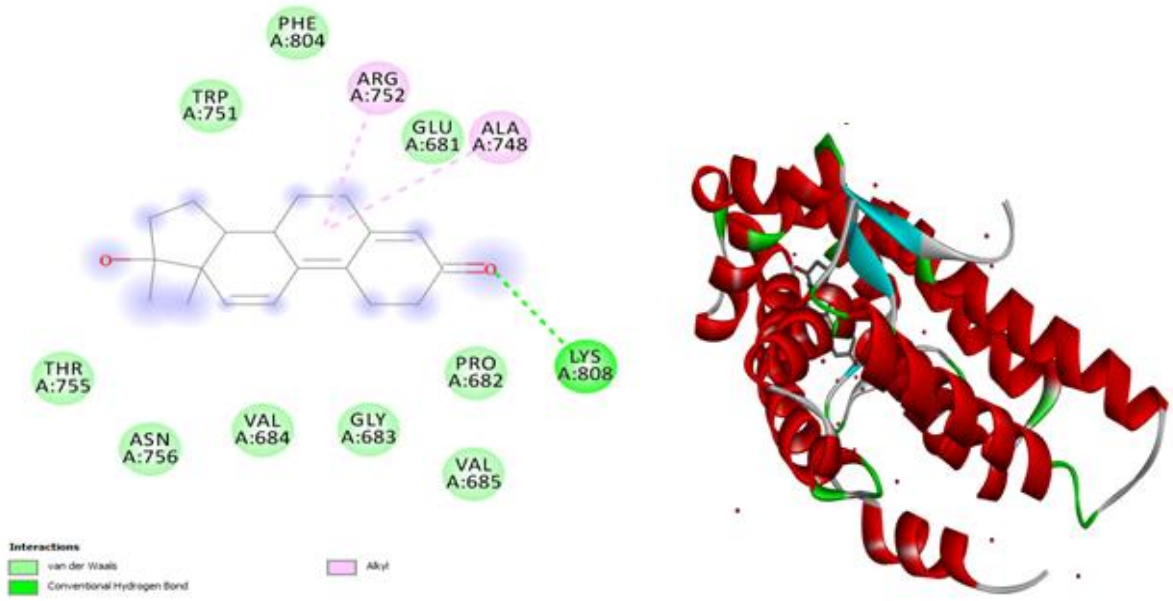
the rule of five. One more important attribute is the solubility for the absorption of the compound and its distribution in the body, which was specified via the value of aqueous solubility. Fortunately, it can be observed in the results that most of the compounds are moderately soluble in water which is acceptable for a drug.



2D diagram of Native ligand (Methyltrienolone): CID: 261000 (BF -7.4) and 3Dview of protein

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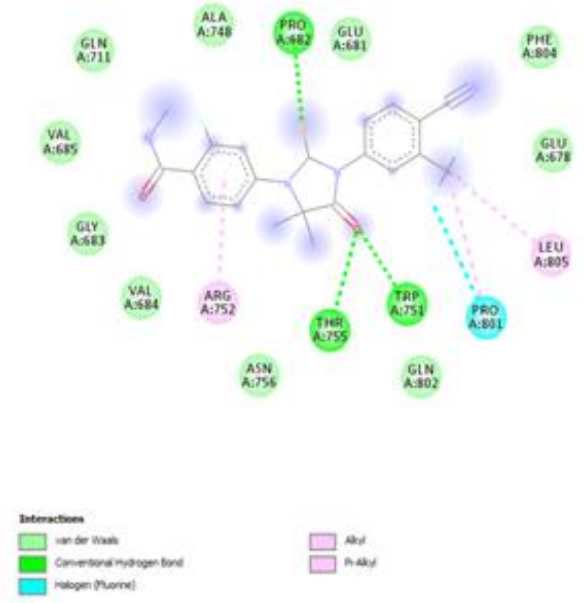

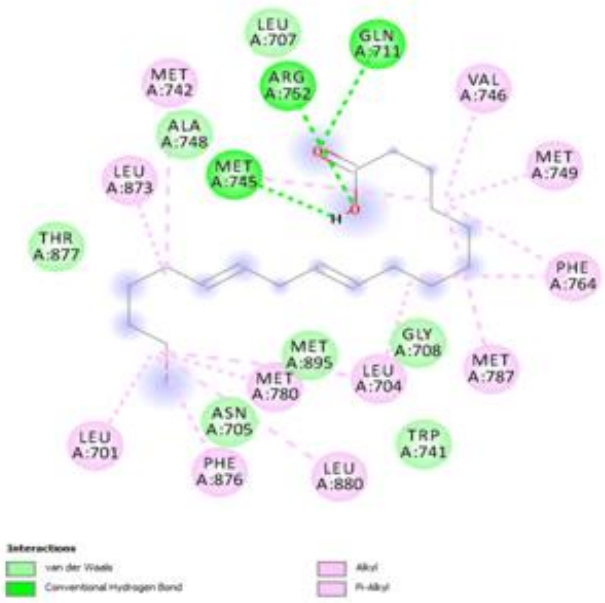

Enzalutamide : (BF -7.6 kcal/mol) and 9,12-Octadecadienoic acid (Z,Z (-6.7kcal/mol)

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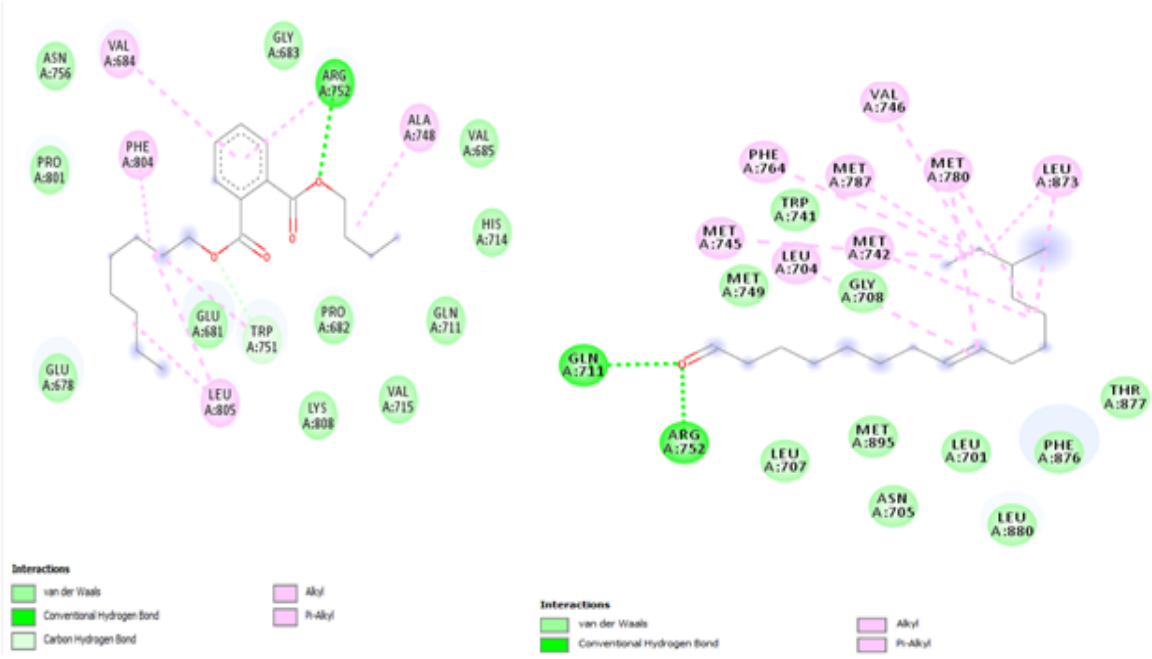

1,2-Benzenedicarboxylic acid, butyl octyl ester : and 8-Hexadecenal, 14-methyl-, (Z)-:
(-6.1kcal/mol) (-6.1 kcal/mol)

TABLE 2: ADME Properties of Enzalutamide, Native Ligand and the Identified Compounds with Good Binding Affinities and Interactions

S/N	COMPOUND	PARAMETERS								
		MW(g/mol)	NA	AA	RB	HBa	HBd	LogS	DL	LV
cnt	Enzalutamide	464.44	32	12	5	7	1	-4.94	yes	0
NL	Methyltrienolone	284.4	21	0	0	2	1	-2.91	yes	0
1	9,12-Octadecadienoic acid (Z,Z)-	280.45g/mol	20	0	14	2	1	-4.91	yes	1 (Mlop>4.15)
2	1,2-Benzenedicarboxylic acid, butyl octyl ester	334.4 g/mol	24	6	14	4	0	-4.91	yes	1 (Mlop>4.15)
3	8-Hexadecenal, 14-methyl-, (Z)-	252.4 g/mol	18	O	13	1	0	-4.56	yes	1 (Mlop>4.15)

MW: molecular weight; NA: number of atoms; AA: aromatic atoms; RB: rotable atoms; HBa: hydrogen bond acceptors: HBd: Hydrogen bond donors; Log S: Water Solubility; ; Log P: Lipophilicity; TPSA: topological surface area; DL: drug likeness; LV:limpiski violation

CONCLUSION

The GC-MS of the chloroform extract of *Annona muricata* leaves showed 20 phyto compounds. However, the present study demonstrated that some of the phyto compounds have the potentials of inhibiting the binding of androgen in the human androgen receptor enzyme socket. The binding scores and interaction of these compounds with the amino acids of the human androgen receptor justifies the traditional use of A. *muricata* leaves to treat or prevent the inflammation of the prostate gland. The ADME analysis of identified compounds with good binding scores and amino acid interactions showed excellent pharmacokinetics and physicochemical properties and therefore is a promising drug candidate for the inhibition of human androgen receptor.

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