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Insilico Approach on Selective Bioactive Compounds Identified From Annona Muricata Barkagainsthuman Estrogen Receptor Alpha (Pdb Id: 3ert)

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ABSTRACT
The bark of Annona muricatahas been widely studied for its medicinal properties since the traditional use of the plant for its therapeutic purposes. In our study, we have performed GC-MS analysis of Annona muricata barkto identify the bioactive compounds that contribute to its pharmacological properties. The analysis revealed the presence of 25 compounds of which 1,2-Propadiene,1-(methylthio)-, 9,12-Octadecadienoic acid,Propanedioic acid Hexadecanoic acid and Trans-beta-ionone-5,6-epoxyde were most predominantly present. Molecular docking studies were performed to evaluate the binding affinity of these compounds with the human estrogen receptor alpha, which plays a vital role in hormone-dependent cancer therapy.Doxorubicin, a standard anticancer drug was used to evaluate the efficacy of these bioactive compounds identified by the GC-MS analysis. 9,12-Octadecadienoic acidwas found to exhibit the highest binding affinity of -6.00kcal/mol when compared to the standard drug. This study underscores the therapeutic potential of Annona muricatabark extract highlighting the need for further bioactivity assays and clinical investigations to validate the efficacy and the safety of the natural compound from the plant.

Keywords: Molecular docking,phytochemicals, anticancer, standard drug, herbal remedies, contemporary medicine.

INTRODUCTION

Medicinal plants have been traditionally used for treating various diseases by many people around the globe. Around 80% of developing nations haven been using medicinal plants for treating primary diseases which contain bioactive compounds which plant a vital role in treating the disease. Approximately 21,000 species of medicinal plants have been used to treat various diseases around the globe with many of them being targeted for drug resistance (Gedlu2024;Chaudhari 2020). The second most common cause of death worldwide is cancer, which is life-threatening illnesses. More potent anticancer drugs are desperately needed as evidenced by rising risks of drug-resistant cancers (Talib 2022). In the fight against cancer, herbal remedies provided a very viable alternative to contemporary medicine. Newer biologically dynamic compounds with unique assemblies and pathways can be found and expanded through the study of natural products (Manojkumar S 2024).

The Annonaceae family is also known as custard apple family, it comprises of tropical and subtropical plants with over 2400 species and 130 genera. The plant in this family has aromatic leaves, fruits and floral structures with significant medicinal and nutritional value(Moghadamtousi 2015). Annona muricata is commonly known as soursop or graviola a tropical plant known for its diverse medicinal properties. The plant grows upto 5-10 meters height with dark green broad leaves. Acetogenins, alkaloids and flavonoids are broad class of phytochemicals (Matsushige 2012) which exhibit various pharmacological properties such as anti-inflammatory (Abdul Wahab 2018), antimicrobial (Silva 2021), antioxidant (Florence2014) and anti-cancer activities (Ilango 2022; Zubaidi 2023).

In recent times, computer-based tools have raised as advanced methodology in drug discovery, an efficient strategies for scrutinizing bioactive compounds from medicinal plants (Sliwoski 2014). Computational models facilitate accurate evaluation of toxicological, pharmacokinetic and pharmacological properties, which enhances pharmaceutical research (Lin 2020). Molecular docking is a low-cost and effective method for developing and testing medications. This method provides information on drug-receptor interactions that can be used to predict how the drug model will attach to the target protein resulting in dependable binding at ligand binding sites (Lee 2019).

MATERIALS AND METHOD

Preparation of extract

1g of powdered plant sample was weighed and used for cold extraction by means of maceration in hydro-ethanolwith 70% ethanol and 30 % water. The extract was kept in intermittent shaking for extraction of the crude followed by filtration using Whatman No 1 filter. The filtrate was stored until further use (Sasidharan 2011).

GC MS Analysis

Shimadzu 2010 plus with an AOC-20i auto sampler GCMS instrument was used from the chromatography analysis of the crude extract with an interface of mass spectrometer. The RTX 5Ms column was used with helium gas of 99.999% at an electron impact mode of 70eV with constant gas flow of 1.63 ml/minute. The injection volume used was 0.5 microliters at 270 degrees with ion source of 200 degrees temperature. The scan range was set to 40 to 450 Da with an interval of 0.5 seconds with a run time of 51.25 minute. The peak area and the relative percentage of each compound was calculated with the software Turbo Mass Ver 5.2.0 to obtain the mass spectrum and the chromatograms (Baeshen 2023).

Identification of components

The NIST library was employed to analyze the spectrum of each compound obtained from the GC-MS analysis. A tabulation was obtained with the name, structure and molecular weight of the compounds obtained from the plant extract (Simón-Manso 2013).

In silico Molecular docking

Molecular docking analysis was carried out by PyRx software with AutoDock Vina inbuilt in them. Based on the binding affinity of the compounds to the protein human estrogen receptor alpha the bioactive compound virtually illustrates the anticancer efficacy (Vincentet al., 2020).

Ligand and protein preparation

The structure of the ligands9,12-Octadecadienoic acid, Trans-beta-ionone-5,6-epoxyde, malonic acid or propanedioic acid, palmitic acid (or) Hexadecanoic acid, 1,2-Propadiene, 1-(methylthio) and standard anticancer drug Doxorubicin were retrieved from the PUBCHEM database. We used OpenBabel tool to convert the ligand structures into pdbqt format for molecular docking and the structure of Human estrogen receptor alpha was obtained from PDB database with the PDB ID: 3ERT. Further preparation of the ligand and the receptor was focused on the removal of water molecules that could hinder the docking process (Khanum 2024).

In Silico Docking Studies

AutoDock Vina and user interface friendly tool was used to perform the automated docking following the grid building prior to the process. Energy minimization was carried out using ChemSketch/ACD to remove the hetero atoms which consist of water molecules and other ligands inbuild (Fatoki 2021). A Lamarckian genetic algorithm method, implemented in the program Auto Dock 4.1, was employed. This software used for the estimation of energy during the interaction and identify the best flexible ligand pose with minimum energy. The scoring function is based on the intermolecular interaction of ligand and protein during docking. As per genetic algorithm all the torsions were allowed to rotate during docking (Banu 2024).

The grid map was centered at particular residues of the protein and was generated with grid dimension prepared (center x = 22.53, center y = 5.82 and center z = 22.43). The Lamarckian genetic algorithm (Fuhrmann2010) and the pseudo-Solis and Wets methods were applied for minimization, using default parameters. Complex structures were modeled using modeling software's Pymol (1.1 version, Delano Scientific LLC, San Carlos, CA, USA), Chimera (1.10.1 version UCSF Resources for biocomputing visualization and informatics, NIH, CA, USA) and 2D pose viewed using Discovery Studio Visualizer (Trot and Olson, 2010).

RESULTS

Identification of bioactive compounds in Annona muricata bark by GC MS analysis

A total of twenty-five bioactive compounds were identified from the GC-MS analysis of the hydro-ethanolic extract of Annona muricata bark. The **Table 1** represents the bioactive compound name along with its retention time, molecular weight, molecular formula and its concentration percentage. The compounds identified are Allene, Trans-beta-ionon5,6epoxide, Propanedioic acid, Hexadecanoic acid and 9,12-Octadecadienoic acid. The presence of these compounds would contribute to its pharmacological applications. **Table 2** lists the compounds pharmacological properties from recent studies and **Figure 1** represents the Chromatogram of the GC-MS analysis of the hydro-ethanolic extract of Annona muricata bark.

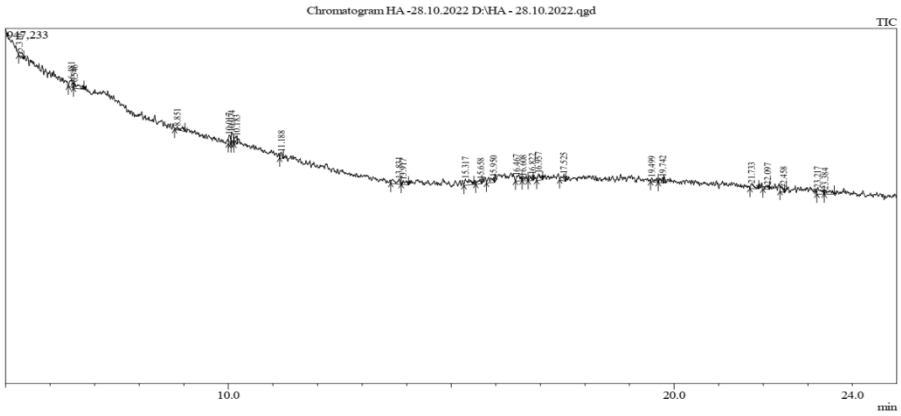


Figure 1: GC-MS Chromatogram of Hydro-ethanolic extract of Annona muricata bark

Table 1: Identification of active compounds in Annona muricata bark extract using GCMS Analysis

Peak	R. Time	Area %	Height %	Molecular Formula	Molecular Weight	Name of the compounds
1	5.300	2.95	2.89	C_3H_4	40	Allene
2	6.483	4.35	5.52	$C_{13}H_{20}O_2$	208	Trans.beta.ionon5,6epoxide
3	6.546	5.01	3.93	C_3H_4	40	1,2-propadiene
4	8.851	3.60	2.58	$C_7H_{11}NO_2$	141	Propanoic acid, 3,3'dithiobis
5	10.017	3.55	6.33	$C_4H_4O_2$	84	But-3-ynoic acid
6	10.074	5.19	8.37	Ar	40	Argon
7	10.183	3.84	5.48	$C_{12}H_{20}O$	180	1,3-dimethyl-adamantan-2-ol
8	11.188	2.87	4.16	$C_5H_9NO_2$	115	5-methoxy-1-aza-6-oxabicyclo (3.1.0)hexane-yloxy)
9	13.831	5.66	3.75	$C_9H_{19}NO$	157	2,2-dimethyl-4-(2-propyl) aminobutanone
10	13.917	3.34	2.92	$C_{16}H_{32}O_2$	256	Hexadecanoic acid
11	15.317	6.24	4.61	C_3H_4	40	1-Propyne
12	15.658	2.95	2.99	$C_8H_{11}NO$	137	7-hydroxy-5,6,7,8-tetrahydroindoliziaine
13	15.950	5.03	3.51	$C_{18}H_{32}O_2$	280	9,12-Octadecadienoic acid
14	16.467	5.06	3.47	$C_9H_{16}NO_2$	170	2,2,6,6-Tetramethyl 4-piperidone-N-oxyl
15	16.608	3.25	3.07	$C_{16}H_{26}O_2$	250	11-carbomethoxy-3,7,7-trimethylspiro[5.5]undec-2-ene
16	16.822	3.41	3.88	$C_8H_{11}NO$	137	7-hydroxy-5,6,7,8-tetrahydroindoliziaine
17	16.957	4.00	4.52	CH_3BO	42	Borane, compd. with carbon monoxide (1:1) (cas) borane carbon
18	17.525	3.48	4.64	$C_{20}H_{21}NO$	291	3,3-dimethyl-2-phenyl-2-(1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)a
19	19.499	3.01	2.78	$C_9H_{10}FEO_4$	238	Tricarbonyl[2-5.eta.-{(e)-2-methyl-2,4-pentadi
20	19.742	3.37	4.16	C_4H_6O	70	3-butyn-1-ol (cas) 3-butynol
21	21.733	4.17	3.25	$C_{22}H_{13}NO_4$	355	Ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinolinecarboxylate
22	22.097	3.00	2.94	$C_{17}H_{26}O_2$	262	1,1'-bibicyclo(2.2.2)octyl-4-carboxylic acid

23	22.458	3.11	2.91	C_{16} H_{14}	206	7-ethylidene-6b,7,8,8a-tetrahydrocyclobut[a]acenaphthylene
24	23.217	5.07	4.26	$C_5H_5BR_2$ CLO	274	2,2-dibromo-1-methylcyclopropanecarbonyl chloride
25	23.384	4.48	3.07	C_3H_4	40	Cyclopropene

Table 2: Biological activity compounds identified in Annona muricata barkusing GCMS Analysis

R. Time	Name of the compounds	Biological activity**
5.300	Allene	Antibacterial effects
6.483	Trans.beta.ionon5,6epoxide	Anti-inflammatory and analgesic effects
8.851	Propanedioic acid	Antimicrobial and anti-inflammatory effects
13.917	Hexadecanoic acid	Antioxidant,Hypocholesterolemic, Anti androgenic, hemolytic,Alpha reductase inhibitor, Antibacterial and antifungal
15.950	9,12-Octadecadienoic acid	Anti-inflammatory,Hypocholesterolemic, Cancer preventive,Hepatoprotective, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor

**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

Molecular docking analysis of GC-MS compounds

The higher negative docking score indicates a stronger binding affinity between the receptor and ligand molecules, reflecting the higher efficiency pf the bioactive compounds. Ligand molecules were selected for further analysis based on their docking energy and favourable interactions with the active site residues. The results obtained are represented in the Table 3. The Figure 4a to 9b represent the docking of 9,12-Octadecadienoic acid (-6.0 kcal/mol), Trans-beta-ionone-5,6-epoxyde (-5.1 kcal/mol), malonic acid or propanedioic acid (-4.2 kcal/mol), Hexadecanoic acid (-5.7 kcal/mol), 1,2-Propadiene, 1-(methylthio) (-3.2 kcal/mol) and standard anticancer drug Doxorubicin (-8.2 kcal/mol). The binding interactions of all compounds have shown hydrogen bonding and hydrophobic interactions with the target protein. The docking studies confirmed the anticancer activity of selected phytochemicalsfrom Annona muricata bark and therebyinhibition of target protein as Human estrogen receptor alpha (PDB ID: 3ERT) through the binding interactions. Among the various compounds, 9,12-Octadecadienoic acid followed by palmitic acid (or) Hexadecanoic acid, Trans-beta-ionone-5,6-epoxyde, malonic acid or propanedioic acid and 1,2-Propadiene, 1-(methylthio). Val 533, Asp 351, Ala 350, Leu 354, Trp 383, Leu 387, Leu 536, Met 522, Leu 525, Tyr 526 and Lys 529 amino acid residues are binding side of the anticancer drug doxorubicin, whose response to the present selected ligand confirms anticancer agents (Figure 2).

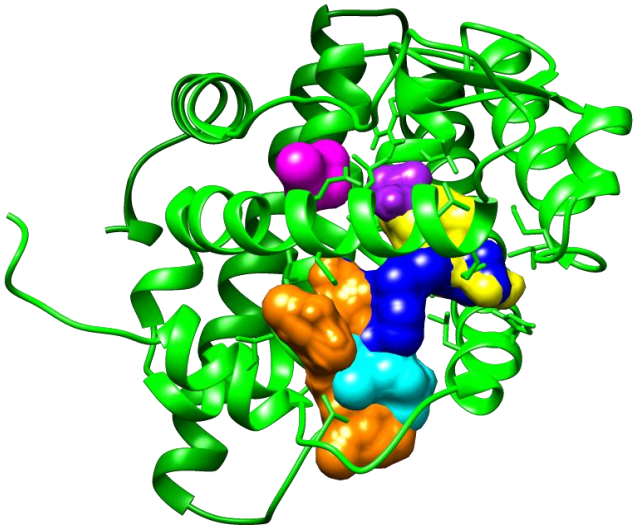
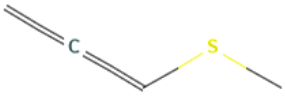
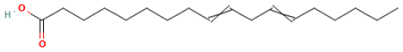


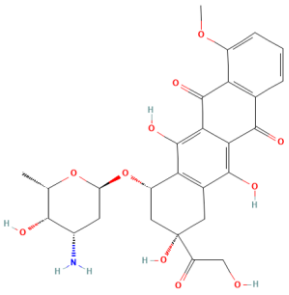
Figure 2: Green colour target and another green colours are anticancer drug interactions with any one of the following amino acid residues are binding sites of the anticancer drug doxorubicin, such as Val 533, Asp 351, Ala 350, Leu 354, Trp 383, Leu 387, Leu 536, Met 522, Leu 525, Tyr 526, and Lys 529 amino acid residues, whose response to the present selected ligand confirms anticancer agents.



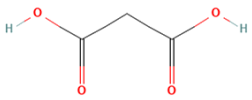
1,2-Propadiene, 1-(methylthio)-



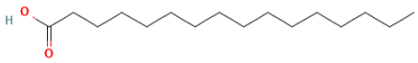
9,12-Octadecadienoic acid



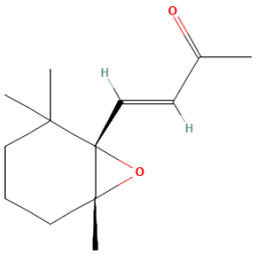
Doxorubicin



Propanedioic acid



Hexadecanoic acid



Trans-beta-ionone-5,6-epoxyde

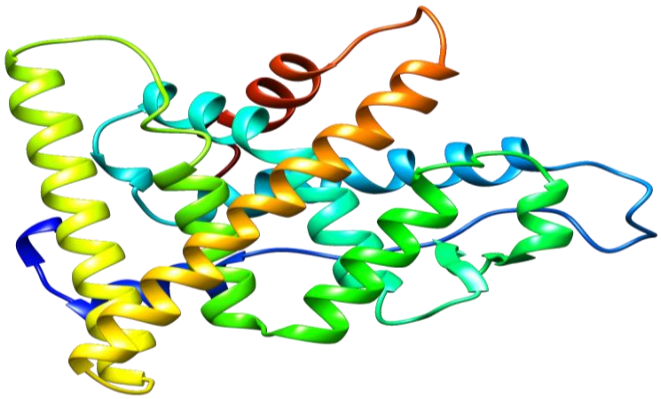


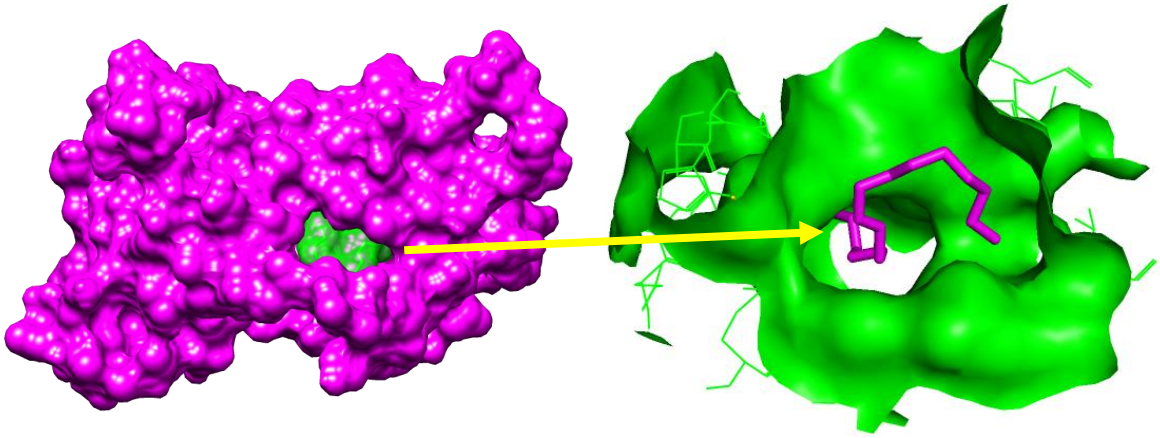
Figure 3: 3D view of Human estrogen receptor alpha (PDB ID: 3ERT)

Table 3: Molecular docking results of phytochemicals with the Human estrogen receptor alpha (PDB ID: 3ERT)

Ligand (CID)	Molecular formula	M. weight (g/mol)	H-bond donors / acceptors	Binding Affinity (kcal/mol)	Ligand binding site of target (Protein ID: 3ERT) Amino acids
9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.40	1/2	-6.00	Met 343, Met 421, Glu 419, Ile 424, Gly 420, His 524, Met 388, Leu 346, Gly 521, **Ala 350**, **Leu 354**, Thr 347, **Leu 525**, **Asp 351**, **Leu 387**, **Trp 383**, **Leu 536**.
Trans-beta-ionone-5,6-epoxyde	$C_{13}H_{20}O_2$	208.30	0/2	-5.10	**Lys 529**, **Tyr 526**, **Trp 383**, **Met 522**, **Leu 536**, **Val 533**, **Leu 525**.
Propanedioic acid	$C_3H_4O_4$	104.06	2/4	-4.20	Arg 394, Glu 353, Leu 349, Leu 346, **Ala 350**, Phe 404, Leu 391, **Leu 387**.
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	1/2	-5.70	Phe 404, Met 388, Arg 394, Leu 428, Leu 384, Leu 349, **Leu 525**, Ile 424, Leu 391, **Ala 350**, Met 421, Leu 346, Met 343, Gly 521, His 524, Gly 420.
1,2-Propadiene, 1-(methylthio)	C_4H_6S	86.16	0/1	-3.20	Met 357, Lys 449, Ile 386, Gly 390, **Leu 387**, His 356, Glu 353, Pro 324, Pro 325.
*Doxorubicin	$C_{27}H_{29}NO_{11}$	543.50	6/12	-8.20	Leu 539, Pro 535, Val 534, **Val 533**, **Asp**

					351, **Ala 350**, **Leu 354**, **Trp 383**, **Leu 387**, **Leu 536**, **Met 522**, **Leu 525**, **Tyr 526**, **Lys 529**.

* Standard anticancer drug Doxorubicin; Amino acid bold residues indicate the binding side of the anticancer drug doxorubicin, whose response to the present selected ligand confirms anticancer agents.



Docked complex **Ligand binding site**

Figure 4a: 3D surface view of Human estrogen receptor alphawith 9,12-Octadecadienoic acid

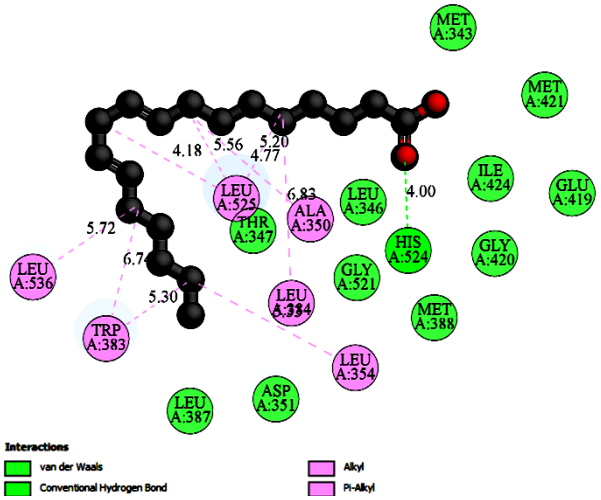
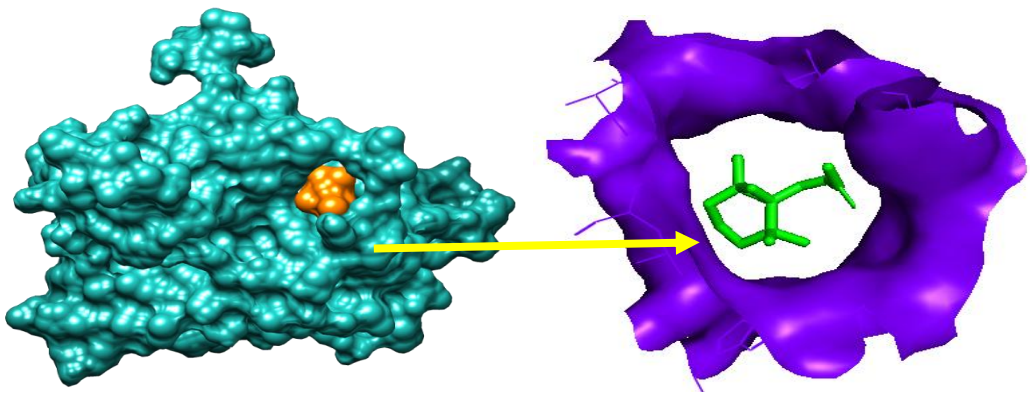


Figure 4b: 2D view of 9,12-Octadecadienoic acid interaction with Human estrogen receptor alpha amino acid residues



Docked complex **Ligand binding site**

Figure 5a: 3D surface view of Human estrogen receptor alpha with Trans-beta-ionone-5,6-epoxyde

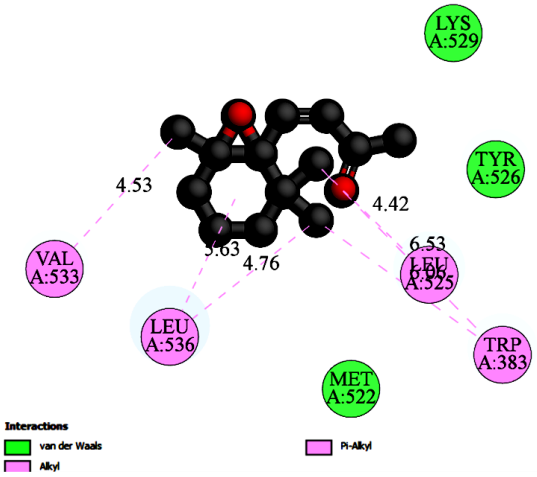
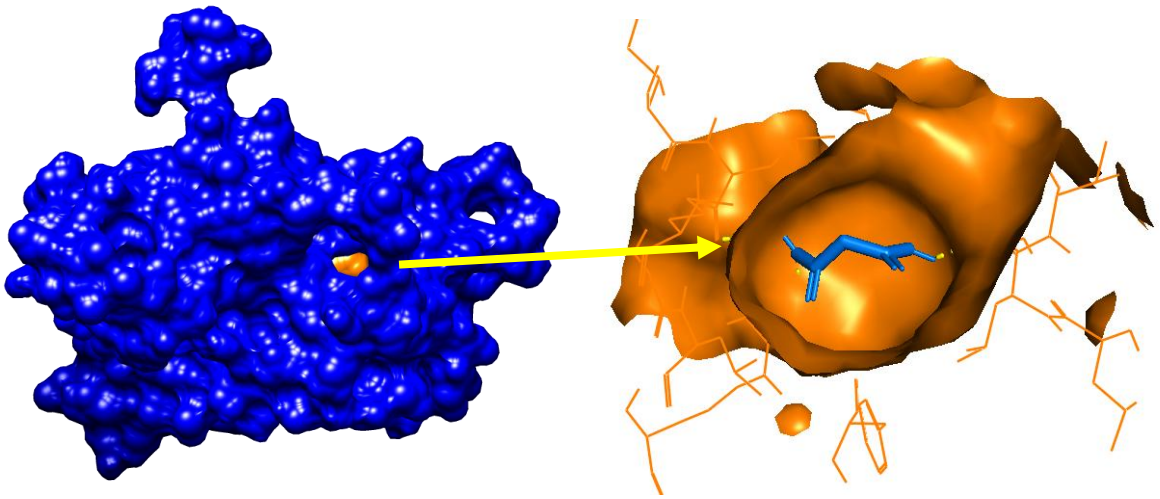


Figure 5b: 2D view of Trans-beta-ionone-5,6-epoxyde interaction with Human estrogen receptor alpha amino acid residues



Docked complex **Ligand binding site**

Figure 6a: 3D surface view of Human estrogen receptor alphawith Malonic acid or propanedioic acid

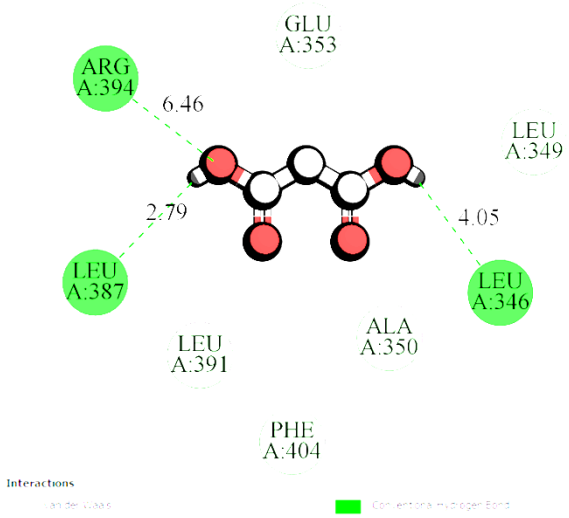
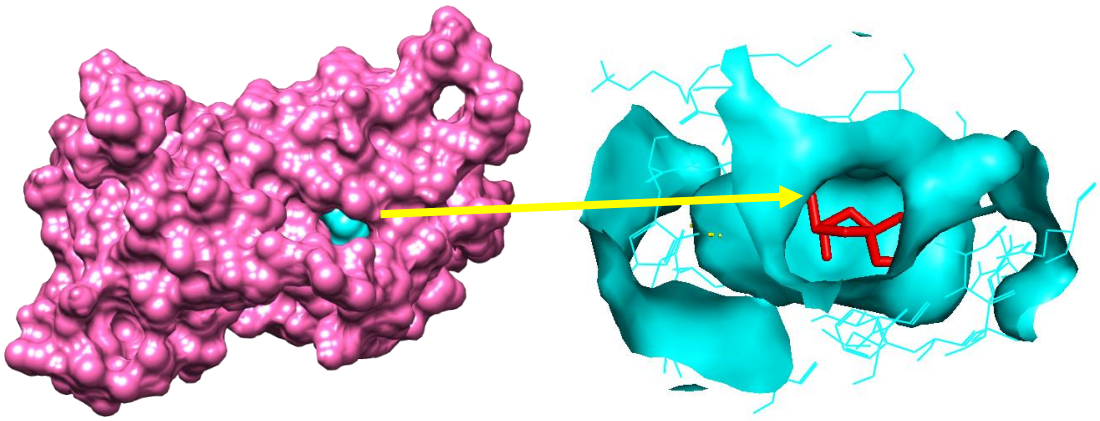


Figure 6b: 2D view of propanedioic acid interaction with Human estrogen receptor alphaamino acid residues



Docked complex **Ligand binding site**

Figure 7a: 3D surface view of Human estrogen receptor alphawith Palmitic acid (or) Hexadecanoic acid

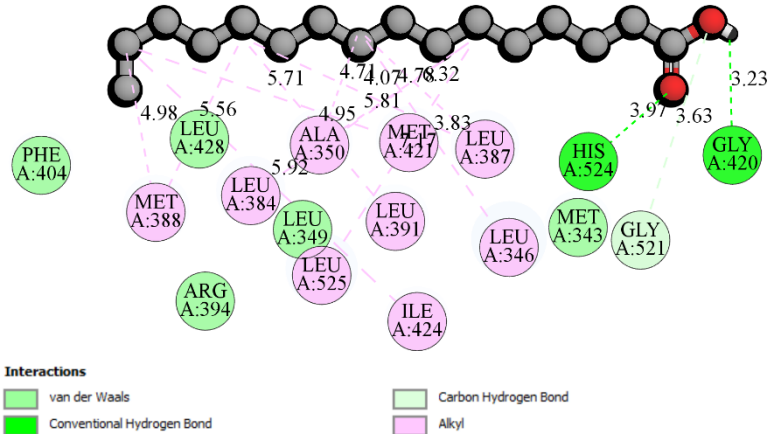
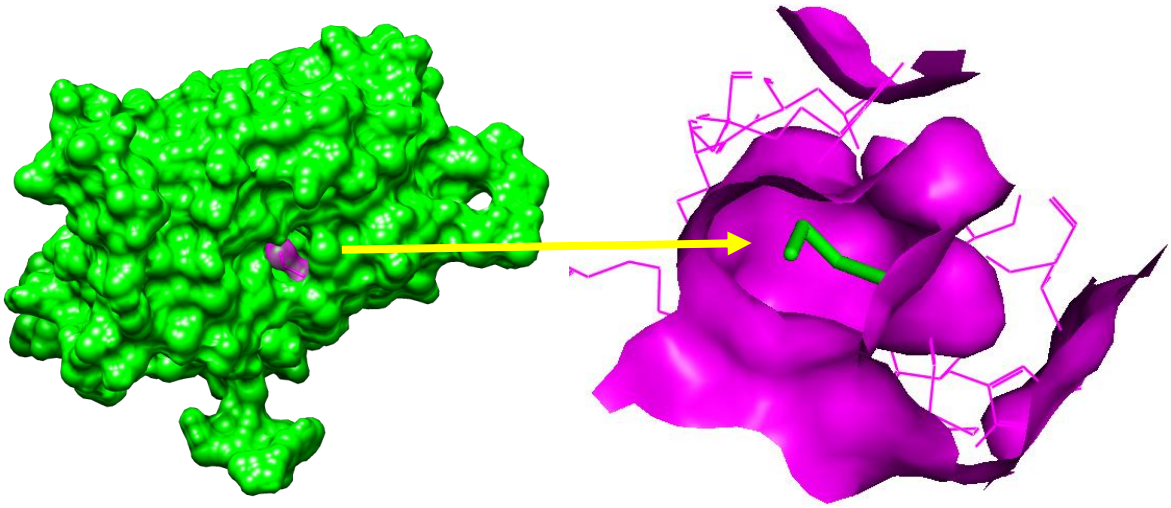


Figure 7b: 2D view of Hexadecanoic acid interaction with Human estrogen receptor alphaamino acid residues



Docked complex **Ligand binding site**

Figure 8a:3D surface view of Human estrogen receptor alphawith 1,2-Propadiene, 1-(methylthio)

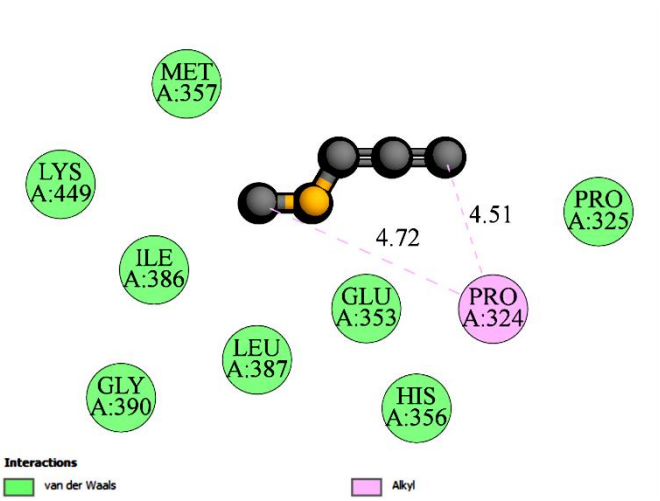
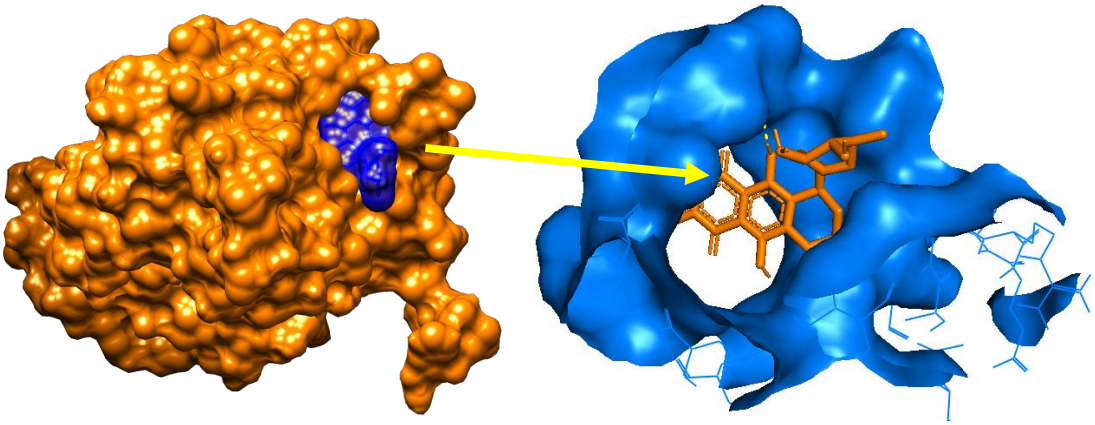


Figure 8b: 2D view of 1,2-Propadiene, 1-(methylthio) interaction with Human estrogen receptor alpha amino acid residues



Docked complex **Ligand binding site**

Figure 9a: 3D surface view of Human estrogen receptor alpha with Doxorubicin

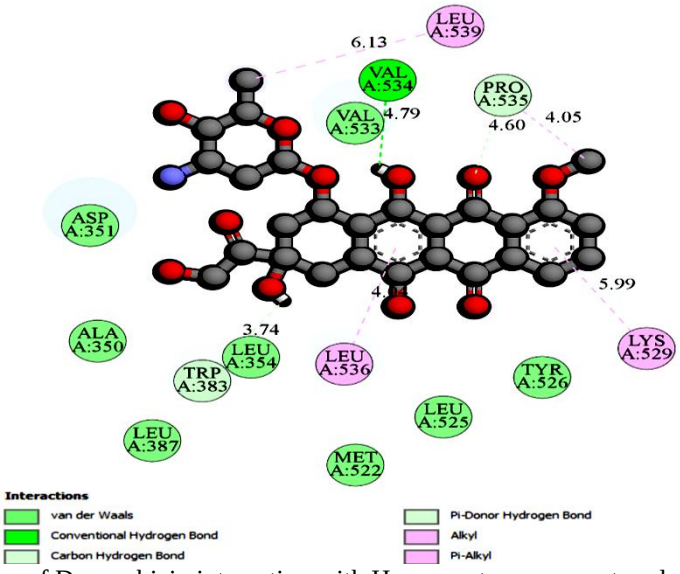


Figure 9b: 2D view of Doxorubicin interaction with Human estrogen receptor alpha amino acid residues

DISCUSSION

Twenty-five compounds were identified in hydro-alcoholic extract of Annona muricata barkby GC-MS analysis. The therapeutic potential of the plant could be due to presence of these bioactive compounds, where GC-MS is

known for its separation technique of volatile compounds. 9,12-Octadecadienoic acid, Trans-beta-ionone-5,6-epoxyde, malonic acid or propanedioic acid, Hexadecanoic acid, 1,2-Propadiene, 1-(methylthio)are the compounds identified through GC-MS analysis of Annona muricata bark. The GC-MS analysis of Amomum nilgiricumleaves and the rhizome extracts have identified over 25 phytocompounds which contribute to the medicinal properties of the plant (Konappa 2020).9,12-Octadecadienoic acid, also known as linoleic acid, is an essential polyunsaturated fatty acid with significant therapeutic potential. It is integral to maintaining cellular membrane integrity and plays a key role in modulating inflammatory responses. Studies have demonstrated its antioxidant properties, which help mitigate oxidative stress, reducing the risk of chronic conditions such as cardiovascular diseases, cancer and diabetes. Additionally, linoleic acid exhibits antimicrobial activity and contributes to skin health by supporting hydration and repair mechanisms. Widely present in plant-derived oils and medicinal plants, it is a bioactive compound of considered interest for its diverse pharmacological applications (Odiase-Omoighe 2022). Propanedioic acid, also known as malonic acid, is a dicarboxylic acid with potential medicinal applications due to its biochemical significance and pharmacological properties. It serves as a precursor in various metabolic pathways and is involved in the synthesis of bioactive compounds. The compound exhibit antimicrobial, anti-inflammatory and antioxidant activities, making them valuable for therapeutic purposes. It plays a vital role in modulating cellular processes and has implications for cancer treatment and metabolic disorder management (Yang L 2021). Hexadecanoic acid, is also known as palmitic acid which is a saturated fatty acid widely found in natural fats and oils. The pharmacological properties exhibited by hexadecenoic acid are antioxidant, antimicrobial and anti-inflammatory properties (Aparna 2012). In a similar study with Multidentiacrassa extract the GC-MS analysis revealed the presence of 58 bioactive compounds which has diverse applications in oral health (Chikowe2024).Another study with Papaver decaisnei methanolic extract revealed the presence of 44 compounds of which alkaloids were the most commonly prevalent followed by phenolics, and fatty acid esters (Jabbar 2022). The molecular docking analysis of the current study revealed that ,12-Octadecadienoic acid had the highest binding affinity with estrogen receptor in par to the standard drug Doxorubicin. In a recent study with the molecular docking analysis of Methyl beta-L-arabinopyranoside 3-n-Hexylthiolane, S,S-dioxide, L-(+)-Ascorbic acid, 2,6-Dihexadecanoate, 1H-Imidazole, 2-(Diethoxy methyl), Oleic acid, Cyclohexane butanoic acid identified from the GC-MS analysis of Parkia timorianarevealed highest binding affinity with the BCL-2 and COX2 proteins thus exhibiting their anticancer activity potential (Ralte 2022).

CONCLUSION

The present study was distinct on the identification of bioactive phytocompounds from Annona muricatabark for the first time by Gas chromatography mass spectrometry analysis, which revealed the presence of twenty-five bioactive compounds of which 12-Octadecadienoic acid had the highest binding affinity with human estrogen receptor compared to the standard Doxorubicin, thus exhibiting its anticancer potential. Our findings of Annona muricata bark suggests their potential to facilitate the development of reliable and effective herbal drugs, further clinical trials analyzing its bioactivity should carried out on animal models for further testing of the efficacy of the compounds in cancer treatment.

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