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Interaction of Bioactive Component of *Aframomum melegueta* on Molecular Docking

Umoh Esther Udo[1,*], Dibua Redeemed Ihimoya[1], Buraimoh Bose Olaide[1]

Abstract

Computational mimicry of the structure of large complexes created by di- and poly-interaction of molecules has been termed molecular docking. The purpose of molecular docking is the prediction of the three-dimensional structures of interest, this is due to that docking only produces plausible candidate structures. The docking was carried out between the bioactive compounds against 5α-reductase, 3β-hydroxy-steroid dehydrogenase, and 17β-hydroxy-steroid dehydrogenase of returned binding energy, BE (kcal/mol) between -5.9 and -9.0 kcal/mol; -6.0 and -9.4 kcal/mol; and -5.2 kcal/mol and -8.0 kcal/mol respectively. Apigenin and ellagic acid were the compounds with the least binding energy, which translates to high binding affinity to three protein targets. This might be one of the mechanisms in which Aframomum melegueta plays its anti-cancer role in the disease by binding the compounds identified in it to the active site of 5α-reductase, 3β-hydroxy-steroid dehydrogenase, and 17β-hydroxy-steroid dehydrogenase enzymes. Aframomum melegueta has bioactive chemicals that may inhibit three important enzymes involved in human steroid metabolism: 5α-reductase, 3β-hydroxy-steroid dehydrogenase, and 17β-hydroxy-steroid dehydrogenase. This study looked into the potential of these compounds to inhibit these enzymes. The findings of molecular docking tests showed that the binding energies for the different targets ranged from -5.9 to -9.0 kcal/mol, -6.0 to -9.4 kcal/mol, and -5.2 to -8.0 kcal/mol. Interestingly, the lowest binding energies were found for apigenin and ellagic acid, indicating a high binding affinity to all three protein targets. The involvement of hydrogen bonds and hydrophobic interactions between the bioactive chemicals and amino acid residues at the enzyme-binding sites was demonstrated by molecular interaction analysis. These results shed light on the possible mechanism by which chemicals from Aframomum melegueta may impede these enzymes that metabolize steroids to produce anti-cancer effects.

Keywords: Molecular docking, bioactive component, *Aframomum melegueta*, protein-ligand interaction

INTRODUCTION

Herbs play a significant role in life in medicine. Medicinal plants are the "backbone" of traditional medicine, and it has been reported that 3.3 billion people in less developed countries utilize medicinal plants regularly [1]. The reason for this trend is due to the fact that medicinal plants are an excellent source of structurally special chemicals that can be utilized as crude materials for creating new medicaments. Herbal medicine, also known as traditional medicine (TM), is non-injectable, stable at room temperature (25–35°C) and has no observable side effects. The pharmacological treatment of diseases began long ago with herbs [2]. Methods of folk healing worldwide commonly use herbs as part of their traditions. To avoid immune system destruction, which is paramount for systemic

***Author for Correspondence**
Umoh Esther udo
E-mail: estyluv100@gmail.com

[1]Researcher, Department of Biochemistry, Federal University of Technology Akure, Nigeria

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recovery from infectious pathogenicity, herbal combinations can also be used as healthy drinks, healthy food sources, or nutritional supplements (functional foods) [3].

Grains of paradise (*Aframomum melegueta*) are also known by many names in many cultural backgrounds indigenous to Nigeria and many other African countries. In Yoruba, it is called Ewe Atare; in Hausa, it is called Chitta or Guinea pepper leaf. Its seeds have many healing properties that are beneficial to the human population. Grains of paradise, a herbaceous perennial seed-bearing plant, are indigenous to swampy ecological habitats along the coast of West Africa in Nigeria. Its trumpet-shaped, purple blooms transform into pods 5–7 cm in length, holding a plethora of tiny, reddish-brown seeds. Belonging to the ginger family Zingiberaceae, this plant can reach heights of up to 1.5 m, adorned with orange-hued lips and pinkish-orange upper flowers that mature into fleshy, non-splitting pods. It thrives predominantly in Ghana, Liberia, the Ivory Coast, Togo, and Nigeria [4–6]. In Nigeria and West Africa, *Aframomum melegueta*, commonly known as Grains of Paradise, has been utilized for treating infectious diseases, including urinary tract infections caused by various bacteria. It has the potential to manage fat mass in doses commonly consumed in food and medicinal preparations. Additionally, it serves as a spice for enhancing the flavors of meat, sauces, and soups. Traditionally, *Aframomum melegueta* has been combined with other herbs to treat common ailments such as body pain, diarrhea, sore throat, catarrh, congestion, and rheumatism in West Africa, particularly in Nigeria [7].

Molecular docking, also called molecular complementarity, is an *in situ* simulation in which two structures interact like a hand in a pair of gloves; this is the basis for molecular docking. The major reason for determining the strict fidelity of two molecular structures for binding and fitting together is to create complementarity initiated by signal transduction, which depends solely on the relationships between biologically essential macromolecules such as proteins, nucleic acids, carbohydrates, and lipids [8].

Proteins form the fundamental framework of living organisms and are involved in a wide array of biological and biochemical processes. To achieve this complex response, proteins are usually combined with other molecules with elevated specificity and complex affinity. The shape of their binding site or combinational site and the physicochemical properties of their amino acid makeup creates a binding cavity that determines how well proteins fuse with other molecules [8].

The relationship between proteins and ligands is crucial for many organic functions within the molecular docking space. Therefore, dual techniques are important. In a corresponding step, the proteins and ligands are seen as complementary surfaces used in one way. The second method clones the docking procedure by determining the ligand-protein pairwise interaction strength, which is followed by the following method [9]. Therefore, the present study aimed to investigate the scientific basis of the bioactive component of *Aframomum melegueta* and its complementary to molecular docking.

MATERIALS AND METHODS

Protein Target Selection and Preparation

The 3-dimensional crystal structures of human's 5α-reductase and 17β-hydroxy-steroid dehydrogenase (PDB IDs of 7BW1 and 1QYX) [10, 11] were retrieved from the protein database (PDB) (https://www.pdb.org/pdb) with a resolution of 2.80Å and 1.69Å, respectively. There was no 3D structure of human 3β-hydroxy-steroid dehydrogenase in the protein database (PDB), so it was modeled using the SWISS-MODEL server, as shown in Figure 1, with a template from Bovine *Bos tarrus* (P14893.1). The protein structures were cleaned by eradicating heteroatoms, such as ions, water molecules, and co-crystallized ligands from the protein using the UCSF-Chimera Software program (ver. 1.31.1) [12].

Additionally, the Dock Prep option in the UCSF-Chimera Software was selected to include charged ions and missing hydrogen atoms.

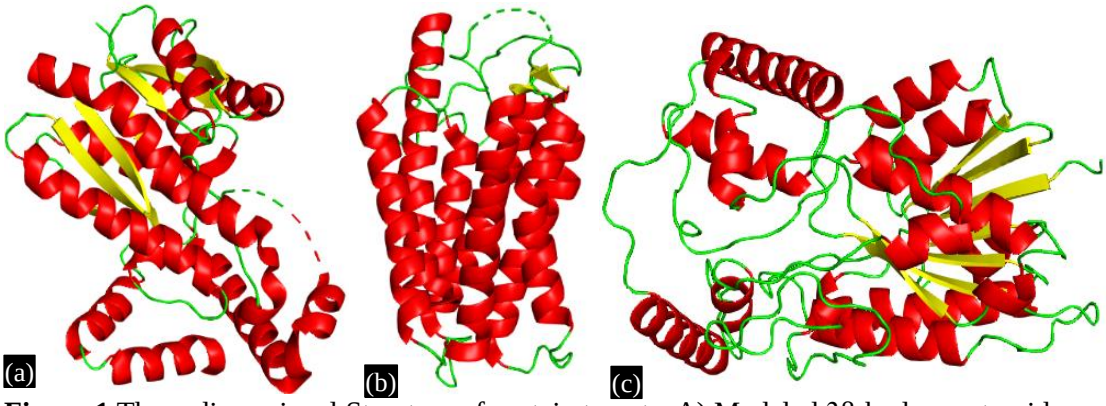


Figure 1 Three-dimensional Structure of protein targets. A) Modeled 3β-hydroxy steroid dehydrogenase using Bovine *Bos tarrus*'s P148931. B) 5α-reductase PDB ID 7BW1. C) 17β-hydroxy steroid dehydrogenase PDB ID 1QYX.

Compounds Selection and Preparation

Apigenin, Ferulic acid, gallic acid, ellagic acid, and vanillic acid were selected from *Aframomum melegueta* based on the HPLC results of this study. The 3D conformers of the compounds in structure data format (SDF) were sought from the National Center for Biotechnology Information (NCBI) PubChem database (https://pubchem.ncbi.nlm.nih.gov/compound/) the world's principal free chemistry database. Before molecular docking, the structures of the compounds were changed to their most stable and energetic conformations using the Merck molecular force field (MMFF94) in the program Open Babel within Python Prescription (PyRx) (Ver. 0.8), as reported [13].

Binding Site Identification in 3β-hydroxy-steroid Dehydrogenase

The binding site of the modeled protein was identified using the Dogsite option of the protein-plus web server (http://proteinsplus.zbh.uni-hamburg.de), based on the drugability score of the identified pockets. However, we have no idea of a putative binding site in the protein.

Virtual Molecular Docking

The prepared ligands were subsequently inserted into specific active sites of our target proteins individually, employing AutoDock Vina within the PyRx software [14]. With the use of the grid box with dimensions (X: 20.8633, Y: 23.1078, Z: 22.066; X: 39.4546, Y: 22.8445, Z: 23.875 and X: 46.0586, Y: 32.5361, Z: 32.9149) Å for 5α-reductase, 17β-hydroxy-steroid dehydrogenase and 3β-hydroxy-steroid dehydrogenase respectively, a target region for the proteins that is analogous to the binding area was tuned, and the respective centers were attuned based on the binding site in 5α-reductase consisting of Tyr91, Arg114, Tyr33, W53, Leu111, Phe118, Leu20, Leu23, Phe219 and Phe223 [11]; 17β-hydroxy-steroid dehydrogenase consist of Leu262, Leu149, Ser142, Tyr155, E282, His221, Val225, Phe259, Phe226, Val188, W51, Val143 and Cys185 (Li et al. 2019) [10]; and 3β-hydroxy-steroid dehydrogenase comprising of Thr9, Gly10, Gly13, Phe14, Leu15, Gly16, Leu35, Asp36, Gly61, Asp62, Ile63, Leu64, His81, Thr82, Ala83, Cys84, Ile85, Ile86, Val88, Phe89, Val100, Asn101, Val102, Gly104, Thr105, Tyr122, Thr123, Ser124, Ser125, Ile126, Glu127, Pro131, Tyr155, Lys159, Ala162, Arg186, Pro187, Met188, Tyr189, Ile190, Ser195, Arg196, Phe197, Leu198, Ser201, Ile202, Glu204, Ala205, Leu212, Asn208, Ser213, Val215, Ser219, Val221, Asn222, Pro223, Tyr265, Asp266, Asn269, Leu272, Leu278, Leu280, Met291, Pro314, Phe315, Ile319, Val320, Thr321, Leu322, Ser323, Asn324, Ser325, Phe327, Thr328, Phe329, Thr353, Trp356 and Val357 based on the Dogsite result (http://proteinsplus.zbh.uni-hamburg.de). Before the molecular interaction analysis, protein-ligand complexes of the compounds were generated using PyMOL Molecular Graphics (version 2.4, 2016, Schrodinger LLC) [15]. The 2D and non-covalent interactions of the protein-ligand complexes were generated using LigPlot^{+} © Roman Lakoskwi (version 2.1.).

RESULTS

Homology Modeling of Human's 3β-Hydroxy-Steroid Dehydrogenase

The 3D protein structure of human 3β-hydroxy-steroid dehydrogenase has not been crystallized or deposited in the popular RCSB protein database. Therefore, a homology modeling approach was employed to build a suitable protein structure for this study [16]. After building the structure, the assessment of the models showed that the modeled 3β-hydroxy-steroid dehydrogenase from Bovine *Bos tarrus* (P14893.1) had 79.28% identity and 97.53% of its amino acid residues were found in the favored region (Figure 2). These results showed that the modeled structure was suitable for molecular docking and molecular dynamics simulations.

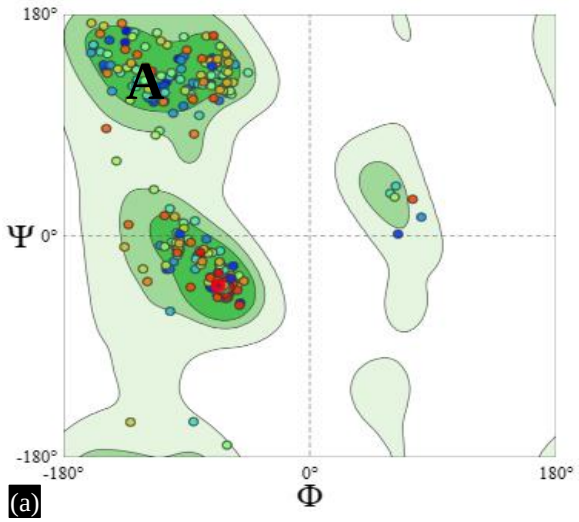
Molecular Docking Analysis

Molecular docking experiments were conducted to elucidate the inhibitory potential of apigenin, ferulic acid, ellagic acid, gallic acid, and vanillic acid against 5α-reductase, 3β-hydroxy-steroid dehydrogenase, and 17β-hydroxy-steroid dehydrogenase in humans. This was achieved through docking of these bioactive compounds against these protein targets following the steps described in the Materials and Methods. Table 1 shows the binding energies (kcal/mol) of the bioactive compounds to the three targets.

The molecular docking between the bioactive compounds against 5α-reductase, 3β-hydroxy-steroid dehydrogenase, and 17β-hydroxy-steroid dehydrogenase returned binding energy, BE (kcal/mol) between -5.9 and -9.0 kcal/mol; -6.0 and -9.4 kcal/mol; and -5.2 kcal/mol and -8.0 kcal/mol respectively (Table 1). Apigenin and ellagic acid were the compounds with the lowest binding energy, which translates to a high binding affinity to the three protein targets. This might be one of the mechanisms in which *Aframomum melegueta* plays its anti-cancer role in the disease by binding the compounds identified in it to the active site of 5α-reductase, 3β-hydroxy-steroid dehydrogenase, and 17β-hydroxy-steroid dehydrogenase enzymes.

Table 1. Binding energy (kcal/mol) of compounds docked against 5α-reductase, 3β-hydroxy-steroid dehydrogenase, and 17β-hydroxy-steroid dehydrogenase using AutoDock Vina.

Compounds	PubChem identity	Binding energy (kcal/mol)		
		5α-Reductase	*3β-HSD*	*17β-HSD*
Gallic acid	370	-6.3	-6.2	-5.2
Ellagic acid	5281855	-8.4	-9.1	-8.0
Ferulic acid	445858	-6.6	-6.8	-5.9
Vanillic acid	8468	-5.9	-6.0	-5.2
Apigenin	5280443	-9.0	-9.4	-7.4



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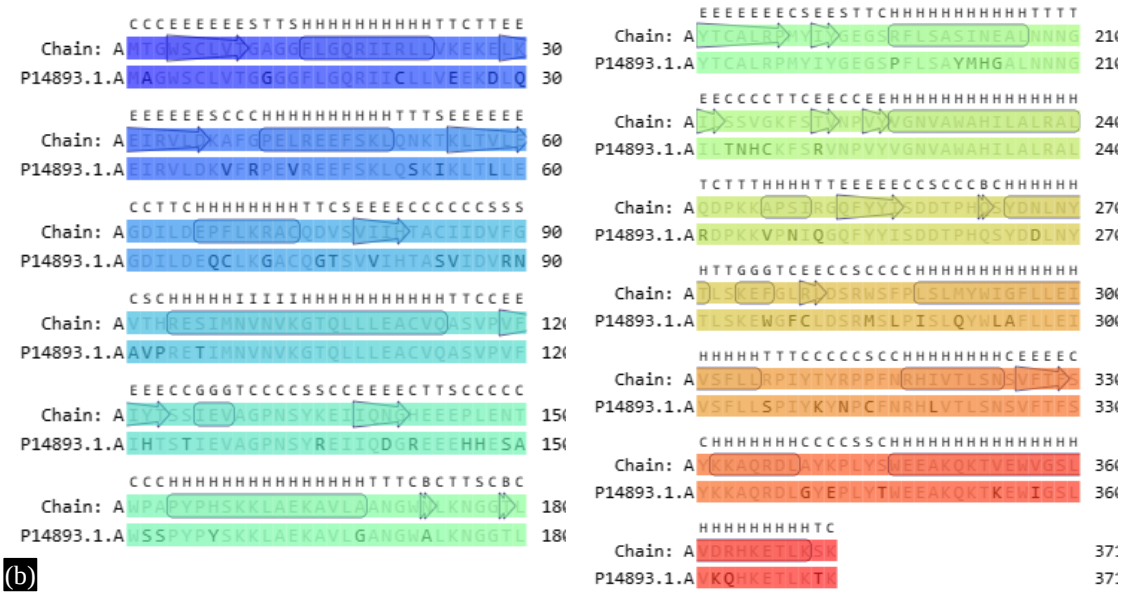


(b)

Figure 2. Homology modeled Protein structures' assessment of human's 3β-hydroxysteroid dehydrogenase. a) Ramachandran plots of 3β-hydroxysteroid dehydrogenase. According to this plot, 97.53% of the amino acid residues were seen to occupy the favored regions. b) Multiple sequence alignments of 3β-hydroxysteroid dehydrogenase against the template from P14893.1 with sequence identities of 79.25%.

Table 2. Phytochemicals detected in extract of *Aframomum melegueta.*

Tests	Result
Steroids	+
Phenols	+
Tannins	+
Alkaloids	+
Flavonoids	+
Terpenoids	+
Cardiac glycosides Saponins	+ +

'+' represent present while '-' represent absent

Table 3. Total contents of major phytochemicals in methanol leaf extract of *Aframomum melegueta*.

Phytochemical	Quantity (µg/g)	Equivalent standard
Phenols	201.03 ± 0.00	Gallic acid
Flavonoids	27.43 ± 0.61	Quercetin
Tannins Saponin	73.56 ± 0.79 39.41±0.69	Tannic acid Terpenoid

Results are expressed as mean ± SD (n=3).

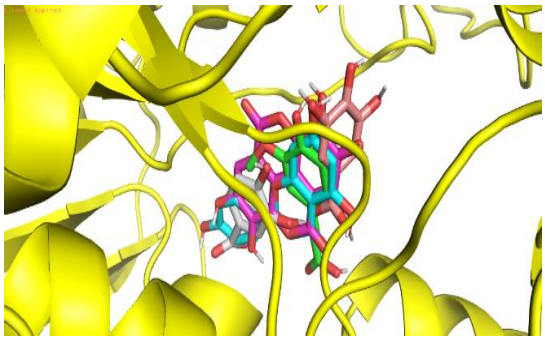
Molecular Interaction Analysis

In addition to the binding energies observed in Tables 2–4, there is a need to examine the mechanism involved in the binding of these compounds to the targeted proteins. Thus, we examined the relationships that result from the protein-compound complexes of these compounds. In this study, hydrogen bonds and hydrophobic interactions were observed between the atoms of the plant compounds and the side

chains of the amino acid residues occupying the binding site of 5α-reductase, 3β-hydroxy-steroid dehydrogenase, and 17β-hydroxy-steroid dehydrogenase. The details are provided in Figures 3–5.

Table 4. High-performance Liquid Chromatography Quantification of Chemical Compounds in Methanol Leaf Extract of *Aframomum melegueta*.

Standard compound	Class of compound	Standard retention time	Methanol Leaf Extract of *Aframomum melegueta*	
			Area (mUA)	Concentration (mg/kg)
Gallic acid	Tannins	1.126	194.124	0.0004 ± 0.00
Ellagic acid	Tannins	2.523	69135696.000	59.4353 ± 0.00
Ferulic acid	Phenolic acid	7.245	191254.406	0.1644 ± 0.00
Vanillic acid	Phenolic acid	1.824	42001893.00	34.0112 ± 0.00
Apigenin	Flavonoids	7.337	8976321.000	7.9326 ± 0.00



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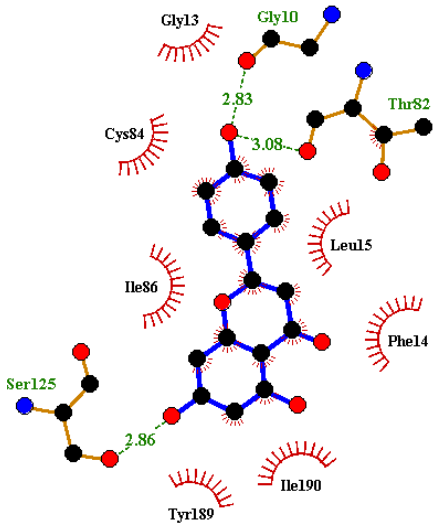

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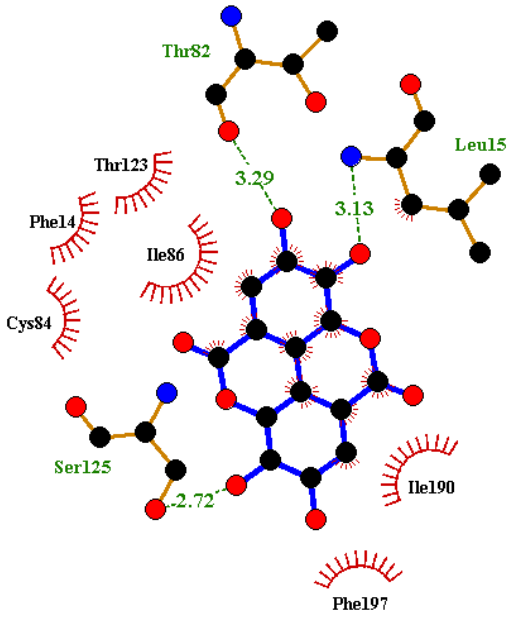

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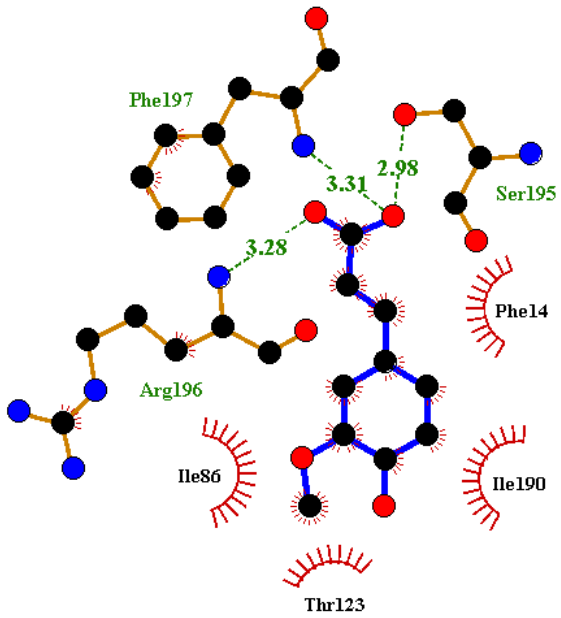

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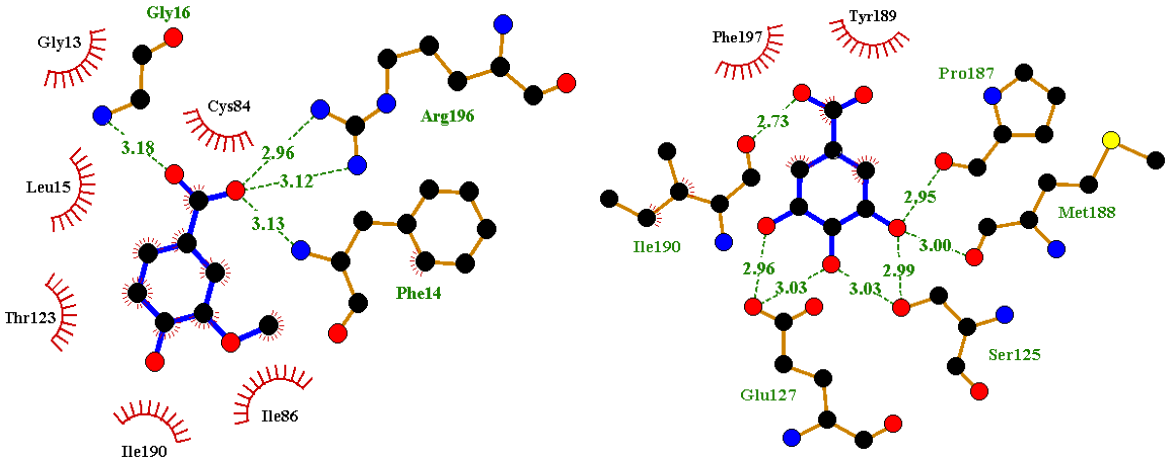
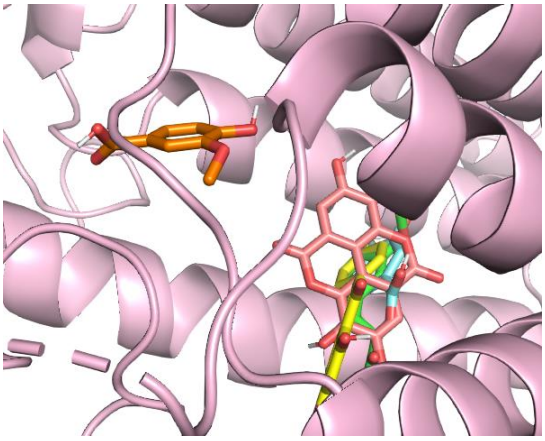


(e) (f)

Figure 3. Molecular docking of bioactive compounds in *A. melegueta* and Human's 3-hydroxysteroid dehydrogenase. (a) 3D binding poses of Apigenin (cyan), ellagic acid (purple), ferulic acid (green), gallic acid (pale pink), and vanillic acid (grey) in the binding pocket of *3β-HSD*. 2D molecular interaction analysis of (b) Apigenin, (c) Ellagic acid, (d) Ferulic acid, (e) Gallic acid, and (f) vanillic acid with amino acid residues in the binding pocket of *3β-HSD*. Compounds are in blue stick, amino acid residues are in brown sticks, green dashes represent hydrogen bonds and red spiked-curve are hydrophobic interactions.



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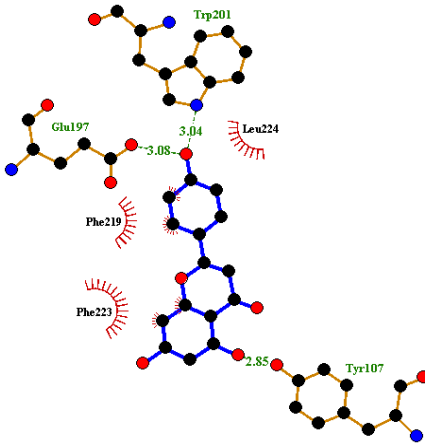

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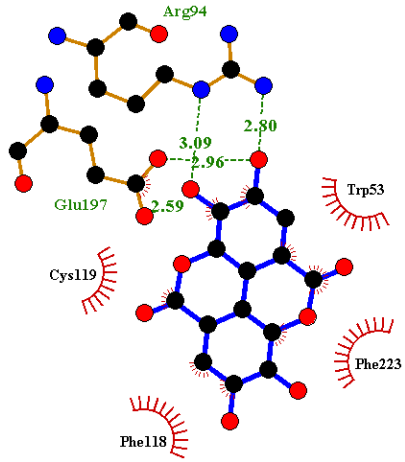

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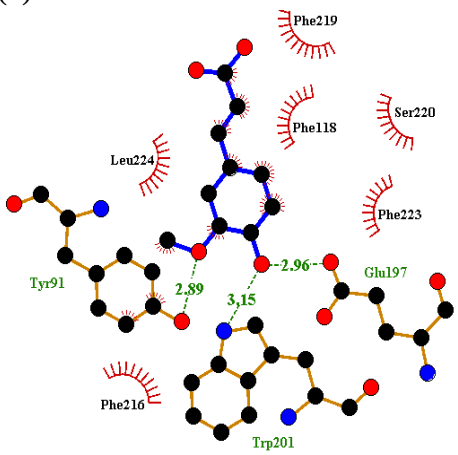

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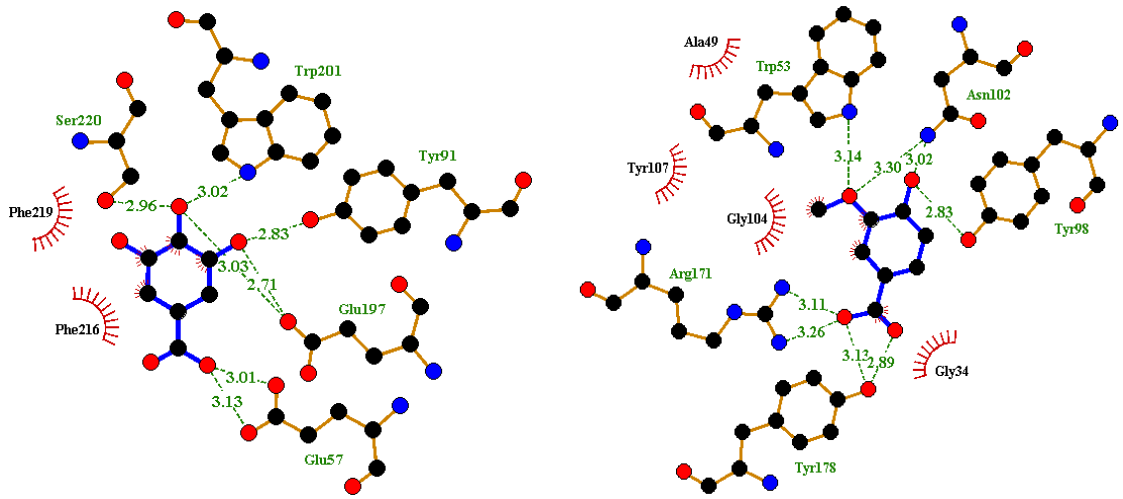
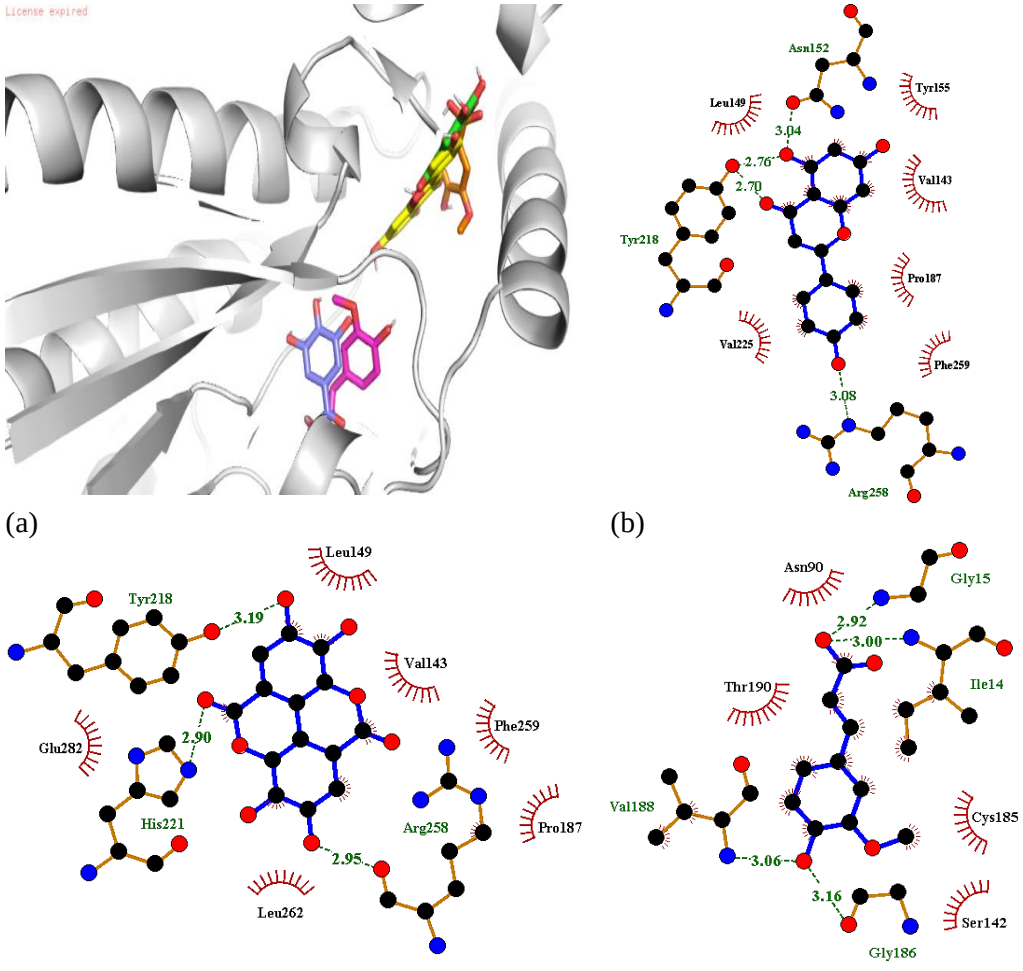


(e) (f)

Figure 4. Molecular docking of bioactive compounds in *A. melegueta* and Human's 5α-Reductase. (a) 3D binding poses of Apigenin (grey), ellagic acid (cyan), ferulic acid (purple), gallic acid (green), and vanillic acid (yellow) in the binding pocket of 5α-Reductase. 2D molecular interaction analysis of (b) Apigenin, (c) Ellagic acid, (d) Ferulic acid (e) Gallic acid, and (f) vanillic acid with amino acid residues in the binding pocket of 5α-Reductase. Compounds are in blue stick, amino acid residues are in brown sticks, green dashes represent hydrogen bonds and red spiked-curve are hydrophobic interactions.



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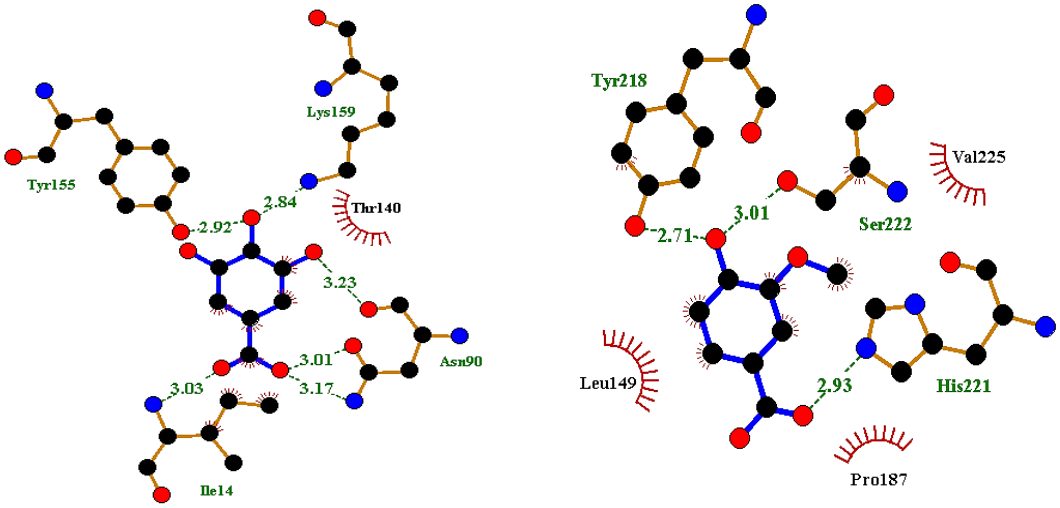

(e) (f)

Figure 5. Molecular docking of bioactive compounds in *A. melegueta* and Human's 17β-HSD. (a) 3D binding poses of Apigenin (yellow), ellagic acid (green), ferulic acid (purple), gallic acid (blue), and vanillic acid (orange) in the binding pocket of 17β-HSD. 2D molecular interaction analysis of (b) Apigenin, (c) Ellagic acid, (d) Ferulic acid (e) Gallic acid, and (f) vanillic acid with amino acid residues in the binding pocket of 17β-HSD. Compounds are in blue stick, amino acid residues are in brown sticks, green dashes represent hydrogen bonds and red spiked-curve are hydrophobic interactions.

CONCLUSION

Molecular docking studies were performed to evaluate the inhibitory capability of different bioactive chemicals present in *Aframomum melegueta* against the three major enzymes involved in human steroid metabolism: 5α-reductase, 3β-hydroxy-steroid dehydrogenase, and 17β-hydroxy-steroid dehydrogenase. The findings, displayed in Table 1, showed that the binding energies for each target ranged from -5.9 to -9.0 kcal/mol, -6.0 to -9.4 kcal/mol, and -5.2 to -8.0 kcal/mol. The two compounds with the lowest binding energies, apigenin, and ellagic acid, showed strong binding affinities for each of the three protein targets. The involvement of hydrogen bonds and hydrophobic interactions between the bioactive chemicals and amino acid residues at the enzyme-binding sites was demonstrated by molecular relationship analysis. These results point to a possible way that the chemicals from *Aframomum melegueta* may work against cancer by preventing these enzymes from metabolizing steroids.

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