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Insilco Anticancer Assessment of the Bioactive Compounds of *Afromomium Melengueta* on HER2 Protein Associated with Breast Cancer

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Abstract

The consequences of the side effects of conventional cancer therapies have been of great concern; hence, the paradigm shift to the use of phytocompounds (medicinal plants) as better medication with little or zero side effects. The concept of medicinal plants as better sources of inhibitors of cancer-causing proteins has been reported as an alternative source of drugs for cancer treatment. The human estrogen receptor (HER) protein is one of the proteins that elevate breast cancer. The aim of this study is to evaluate the inhibitory activity of the compounds of Aframomum melegueta on human estrogen receptor protein (HER2). The molecular docking was done using PYRX. The bioactive compounds were analyzed using GC-MS Methods. Phytochemical analyses were also carried out using standard procedures. Phytochemical screening of the ethanolic extract of the plant revealed a rich amount of flavoniods (14.57mg/l), alkaloids (11.93mg/l), terpenes (0.31 mg/l), and phenolics (0.13mg/l) in the plant. The GC-MS analyses of the ethanolic extract of the plant showed the presence of at least 52 bioactive compounds that were docked with other compounds from the literature. Molecular docking revealed that Caryophyllene oxide, Humulene, isolongifolene-9-hydroxyl, and 6-paradol have binding affinities of -8.6 kcal/mol, -8.4 kcal/mol, -8.1 kcal/mol, and -6.4 kcal/mol, respectively, which are greater than the binding affinity of the standard drug (Fulvestrant), which is -5.8 kcal/mol. The other compounds, however, showed lower binding affinities and, as such, were not further assessed. The results from this study shows that Caryophyllene oxide, Humulene, isolongifolene-9-hydroxyl, and 6-paradol possess anti-cancer effects due to their higher binding affinity for the target protein HER than the standard drug (Fulvestrant), which mitigates the expression of HER.

Key words: Breast Cancer, Treatment, Phytocompounds, Molecular docking, HER2.

Citation
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Introduction

Breast cancer is a disease that develops in the cells of the breast. Is prevalent primarily among women and less common in men across the globe. It poses a major challenge to global health. Impacting millions of individuals worldwide. This cancer ranks high as one of the most frequently diagnosed types among women and is responsible for a substantial number of cancer-related fatalities annually (American Cancer Society, 2022). Cancer, as reported by the World Health Organization (WHO, 2023), stands as the second major cause behind global mortality today, with an estimated count reaching up to 9.6 million deaths in the year 2018 alone. Consequently, there is a substantial burden associated with this disease globally since it gave rise to nearly eighteen point one million fresh cases last year; we project that this number would escalate even further, possibly surpassing twenty-nine point five million by two thousand forty due to natural population growth coupled with advancing age groups becoming prone to its development. However, it should be noted that cancer distribution does differ from place to place, where high-income economies tend to show higher probabilities for

its occurrence compared to middle- or low-income nations, which bear more substantial numbers of related cancers causing fatality burdens attributable mainly to having less precise diagnostic measures alongside proper therapy provisions within their confines (Rebecca et al., 2023).

When looking at global data on common types of cancer, it becomes clear that lung cancer, breast cancers, colorectal cancers, stomach cancers, colorectal cancers, Throat and Mouth Lymph Node Cancers, and prostate cancers are all highly prevalent. Incidences, though, could also see significant variation based on geography, lifestyle decisions, and level of accessibility to good healthcare. Specifically for patients diagnosed with breast Cancer, factors directly linked to this include: One's age, family history, any parent diagnosed previously with this illness, certain mutations within their genes (BR1 and BRCA2), past record of having at some point experienced Breast cancer, or present evidence indicating possibilities thereof. Onset menstruation during years too early or menopause at too late an age is directly associated with the risk of developing BC. The following factors have also been found to be associated with BC in women: Hormonal replacements, obesity, and consumption of alcohol. Breast cancer is associated with lumps or thickening in the breast or underarm, changes in breast size or shape, nipple changes or discharge, and skin changes on the breast (Centers of Disease Control (CDC), 2023).

Aframomum melegueta, also known as Grains Paradise, is a species of plant in the ginger family (Zingiberaceae). It is found in West Africa and is used as a traditional medicine in the region (Kamal, 2023). In addition to its culinary uses, *Aframomum melegueta* has also been used as a medicine plant for various purposes. It is believed to have anti-inflammatory, digestive, and aphrodisiac properties (Nebojsa, Barbara, Alexander, and Ilya, 2009). Some studies have suggested that *Aframomum melegueta* may possess certain bioactive compounds with potential anti-cancer properties. For example, research has indicated that extracts from *Aframomum melegueta* seeds may exhibit antioxidant and anti-inflammatory effects, which could potentially have implications for cancer prevention or treatment. *Aframomum melegueta*, also known as Grains of Paradise, has been used in traditional medicine for various purposes, including its potential medicinal properties, but its specific role in cancer treatment has not been extensively studied.

Materials and Method

Materials

Chemicals/ Reagents Used

Methanol, distilled water, lead acetate, glacial acetic acid, ferric chloride solution, ammonia solution, sulfuric acid, acetic anhydride, H_2SO_4, Folin-Ciocalteu reagent, Gallic acid, potassium ferrocyanide, NaOH.

Equipment

Conical flask, rotary evaporator, weighing balance, measuring cylinder, water bath, round bottom flask, beakers, mucilage cloth, funnel, oven, refrigerator, electric blender, volumetric flask, pippette, shaker, spectrophotometer.

Collection and Preparation of Plant Materials

The seed samples of *Aframomum melegueta* were purchased from Itoku Market, Abeokuta, Ogun State, and brought to the laboratory. The rhizomes of *Aframomum melegueta* were identified and authenticated at the herbarium section of the Department of Botany, University of Lagos, Nigeria, where their designated voucher specimen numbers (LUH 9747) were deposited. Seeds were promptly removed from the plant materials. The seed samples were then ground to powdery form with an electric blender and packaged as a unit in airtight containers for further analysis.

Preparation of Crude Extract

Five hundred grams (500 g) of the dried pulverized *Aframomum melegueta* were soaked in a 5 L round-bottomed flask containing 0.5L (500 ml) ethanol solvent in shade for 72 hours, with shaking of the extracts in the intermediate time. The extracts were filtered with muslin cloth and concentrated using a rotary evaporated model (RE300), which ensures evaporation of bulky solutions to smaller volume concentrates (semi-solids) at temperatures between 40 and 60^{o}C to obtain crude extracts. The extract was dried at 50°C in a water bath.

The weight of the resulting extract was determined, then it was preserved in glass vials and stored at 4°C until further analysis.

Methods

Qualitative Phytochemical

Test for Flavonoids: To a 1 ml portion of the sample extract with concentrated sulfuric acid, 5 ml of dilute ammonia solution was added; a yellow color indicated the presence of flavonoids (Chukuma & Chigozie, (2016); Iwu *et al*., (2016); Usman, Adamu, Isaac, & Bala, (2022)).

Test for Steroids:

To 1 ml of the extract mixed with H_2SO_4, **2 ml** of acetic anhydride was added, which indicated steroids presence with a color change from violet to green, as described by Chukuma and Chigozie (2016).

Test for Phenolics:

In a beaker with 5 ml of distilled water, the extract was dissolved, and a dark green color was observed after the addition of a few drops of a neutral 5% ferric chloride solution (Chukuma & Chigozie, 2016).

Test for Terpenes:

In a beaker with 5 ml of sample extract mixed in 2 ml of chloroform, the addition of 3 ml of concentrated H2SO4 forms a layer with a reddish-brown coloration interface, establishing the presence of terpenes as described by Chukuma and Chigozie (2016).

Test for Alkaloids:

To 3 ml of acidified ethanol, 5mg of the sample extract was added. This was slightly warmed and filtered. Turbidity was obtained when a few drops of Mayer's reagent were mixed with 1 ml of Dragendroff reagent (Chukuma & Chigozie 2016). Also, the Quantitative Estimation of phytochemicals as described by Chukwuma and Chigozie (2016); Rahman, Umer, Syed, Samiullah, and Nusrat (2017).

Determination of Total Phenolic Content using Folin-Ciocalteu Reagent

Various concentrations of the standard solution (garlic) were prepared as 0.01, 0.02, 0.03, 0.04, and 0.05 mg/ml in ethanol. Two different concentrations of the plant extract were also prepared (0.1 and 1mg/ml) in ethanol. In a test tube containing the mixture of 2.5 ml of a 10-fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate, 0.5 ml of each sample was added and mixed thoroughly; thereafter, paraffin was used to cover the tubes as they were allowed to stand for 30 minutes at 37 ^oC. At a wavelength of 760 nm, the absorbance of the mixtures was obtained as described by Chukuma & Chigozie (2016).

Determination of Total Alkaloid

In a 250-ml beaker, 1 ml of the extract was mixed with 1 ml of 0.025M $Fecl_3$ in 0.5 M HCL and 1 ml of 0.05M 1,10 phenanthroline in ethanol and maintained or incubated at a constant temperature of 70^oC in a hot water bath for 30 minutes. The absorbance of the mixture (a red-colored complex) was measured and compared with the reagent blank at an absorption wavelength of 510 nm. With a quinine standard curve of 0.1mg/ml, the total alkaloid was determined by dissolving 10mg in 10 ml of ethanol and making it up to 100 ml with distilled water as described by Singh *et al*., (2004).

Determination of the Total Flavonoid

In a 10 ml volumetric flask containing 4 ml of distilled water, 1 ml of the standard and 0.3 ml of 5% NaNO2 were added; this was allowed to stand for 5 minutes; thereafter, 0.3 ml of 10% AlCl3 was added; also, 2 ml of 1M NaOH and 10 ml of distilled water were used to make the volume at the six-minute point (the same was done using the extract solutions (20, 40, 60, 80, or 100 mg/l). At 510 nm wavelength, a UV-Visible spectrophotometer was used to determine the absorbance. Flavonoids were obtained as the equivalent of quercetin's, which served as the standard with the quercetin calibration curve (Chukuma & Chigozie, 2016).

GC-MS Analysis

With anHP 5890 series II Gas Chromatograph interfaced to a 5973 Mass Selective Detector (MSD) and controlled by HP Chemstation software (version b.02.05, 1989–1997), the separation of the samples was obtained with

an HP5-MS capillary column (30.0 m x 250 m x 0.25 m) having a stationary phase composed of a 5:95% diphenyl:dimethylpolysiloxane blend.

Moleculer Docking

Retrieval of protein and preparation

The protein with PDB ID: 1G3M, 1.5Aº (crystal structure of estrogen protein) was downloaded from the protein data bank. The Pymol toolkit was used to remove non-essential water molecules and heteroatoms to prepare the protein for interaction. With AutoDock tools, the active binding site of the protein was defined within the grid box dimension (Elekofehintia *et al*., 2018)

Ligand selection and preparation

From the PubChem Database, the standard drug and 42 compounds (sdf file) from *Aframomium melegueta* were retrieved. The optimization was done with Open Babel and PyRx to prepare them for docking. The PyRx tool was used to convert the ligand (SDF format) to a PDBQT file in order to generate the atomic coordinates. At a set mmff94 force field, the optimization algorithm was used to optimize the energy to the minimum (Elekofehinti *et al.* (2018); Oyebode *et al.* (2018)).

Molecular docking using PyRx

Based on scoring functions, PyRx and AutoDock Vina options were used to carry out the molecular docking. This software is characterized by a grid box spacing dimension of 252525 and x, y, and z coordinates of 23.3192, 22.3754, and 59.6098, respectively, over the ligand, while other parameters are default. At the active site of the BAK protein, both the compounds and the standard drugs were docked and their interactions compared within the same grid box dimension. The most stable conformation of each compound bonded to the BAK protein-active site was referred to as the best docking pose (Arannilewa et al., 2018).

Validation of Docking Results

Data Warrior software (version 5.5.0) and the Pyrx tool were used to convert the prepared compound (compiled compound) to 2D (in sdf format) and pdbgt format, respectively. The binding affinity of the compounds was determined using a correlation coefficient graph plotted between the compounds (Ambrose et al., 2018). The protein-ligand complexes of the "hit compounds," that is, the compound with the best or highest binding affinity, and the standard drug (obatoclax), were generated. The Discovery Studio and the Pymol software were used to generate the 2D view interaction of the protein-ligand (which shows the distance, bond type, and interacting amino acid residues) and the pocket view of each of the complexes, respectively.

Results

Table 1: Phytochemical screening of the seed extract of *Aframomum melegueta* (both Qualitative and Quantitative result)

PHYTOCHEMICAL	REAGENT USED	SAMPLE EXTRACT	ETHANOLIC	AMOUNT mg/L	PRESENT
Flavonoid	Ammonia	+		14.57	
Steriod	Acetic anhydride	-		0.0	

Phenolic	Ferric chloride	+	0.13
Terpenes	Chloroform	+	0.301
Alkaloid	Mayer's reagent	+	11.93

+ = Present; - = Absent

The results obtained from the quantitative and qualitative phytochemical screening of ethanolic extracts of *Aframomum melegueta a*re revealed that Flavonoid is most abundant, followed by alkaloid then terpenes and phenolic with the least concentration.

Table 2: Compounds identified in the ethanolic extract of *Aframomum melegueta* using GC-MS.

Peak no	**Retention time (min)**	**Compounds**	**Molecular formula**	**Percentage peak (%)**
1	3.609	Hexanal	$C_6H_{12}O$	0.86
2	3.946	1-Octene	C_8H_{16}	0.11
3	4.200	Chloroacetic acid	$C_2H_3ClO_2$	0.04
4	4.510	4-methyl-Benzene	C_8H_7N	0.17
5	4.708	2-Heptanone	$C_7H_{14}O$	0.85
6	5.863	1-Decene	$C_{10}H_{20}$	0.11
7	5.975	Cyclooctane	C_8H_{16}	0.06
8	6.201	Benzenamine	$C_6H_5NH_2$	0.22
9	6.764	1-Hexen-4-yne	C_6H_8	0.01
10	7.468	d-Gluco-heptulosan	$C_7H_{14}O_7$	0.09
11	7.694	2-exo-chloro-Copper	Cl_2CuO^{+2}	0.07
12	8.313	1-Hexen-3-yne	C_6H_8	0.70
13	8.792	1-methyl-2-octyl- Cyclopropane	$C_{12}H_{24}$	0.066
14	9.215	4-acetamido- 3-Cyclopentyl-1-propyne	C_8H_{12}	0.03
15	9.440	Z,Z,Z-4,6,9-Nonadecatriene phenylephrine Cyclononanone	$C_{19}H_{34}$	0.03

16	9.834	Oleic Acid	C18H34O2	0.38
17	10.229	Cyclopentaneundecanoic acid	C17H32O2	0.04
18	10.680	2-Methoxy-4-vinylphenol Phenol	$C_9H_{10}O_2$	2.19
19	11.074	Oxime-3,4-Dihydroxybenzamide	$C_9H_{11}NO_3$	0.38
21	11.412	2- Chloropropionic acid	$C_3H_5ClO_2$	0.72
22	11.527	Gingerol	$C_{17}H_{26}O_4$	9.43
23	11.722	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	0.46
24	12.088	N-[4-bromo-n-butyl]-Oleic Acid	$C_{22}H_{42}O_2$	0.51
25	12.342	3,13-Octadecadien-1-ol Octadecane	$C_{18}H_{34}O$	0.40
26	12.708	(E)-trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	1.29
27	13.046	Butan-2-one	C_4H_8O	24.36
28	14.032	Heneicosanoic acid	$C_{21}H_{42}O_2$	4.09
29	14.257	3,11-Tetradecadien-1-ol	$C_{14}H_{26}O$	1.00
30	14.370	E-9-Tetradecenal	$C_{14}H_{26}O$	1.80
31	14.483	7,11-Hexadecadienal	$C_{16}H_{28}O$	1.65
32	14.764	cis-11-Hexadecenal	$C_{16}H_{30}O$	1.14
33	15.046	2,3-dihydroxypropyl ester	$C_7H_{14}O_4$	2.57
34	15.187	Oxiraneundecanoic acid	$C_{13}H_{24}O_3$	1.73

35	15.525	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	4.42
36	15.976	Hexadecenoic acid	$C_{16}H_{30}O_2$	0.93
37	16.285	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	1.46
38	16.652	p-Toluic acid	$C_8H_8O_2$	25.16
39	17.356	Undecyl-ester-(+-)-2-Phenethanamine	$C_{21}H_{39}NO_3$	11.40

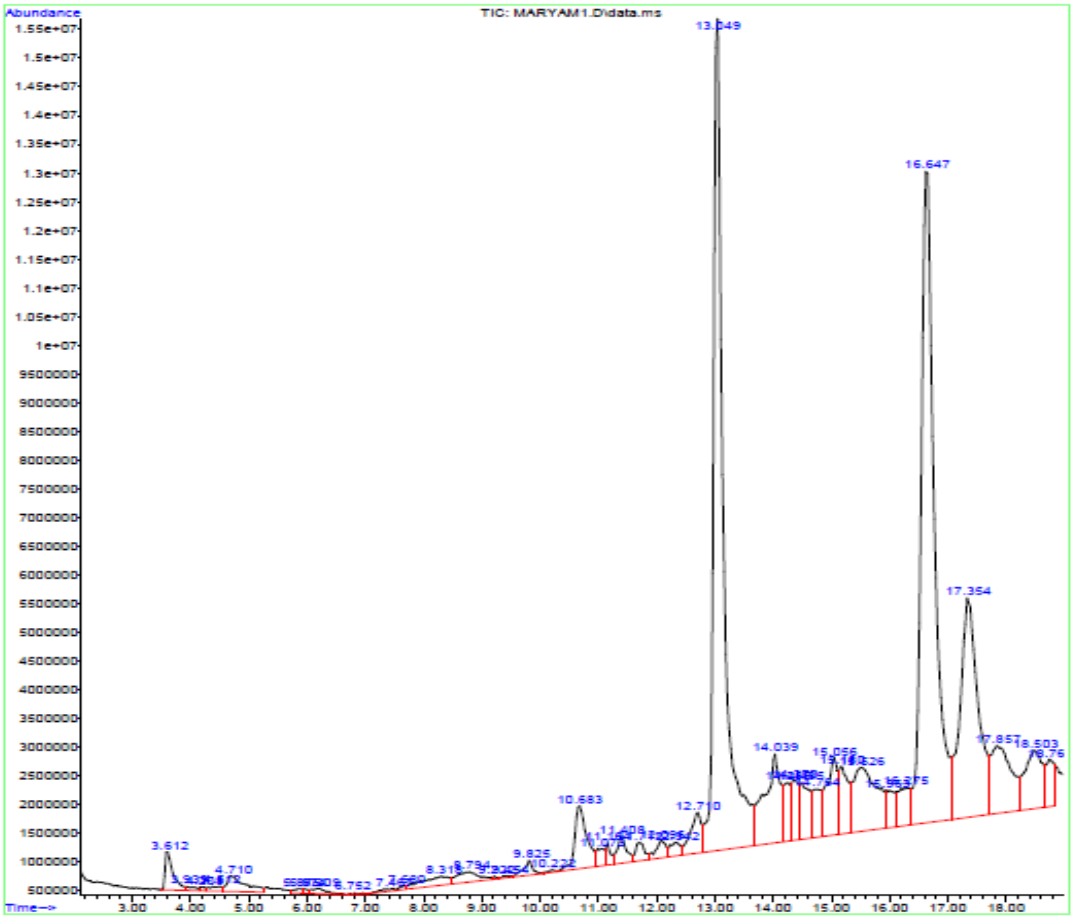

Fig. 1: GC-MS chromatogram of ethanolic extract of *Aframomum melegueta*

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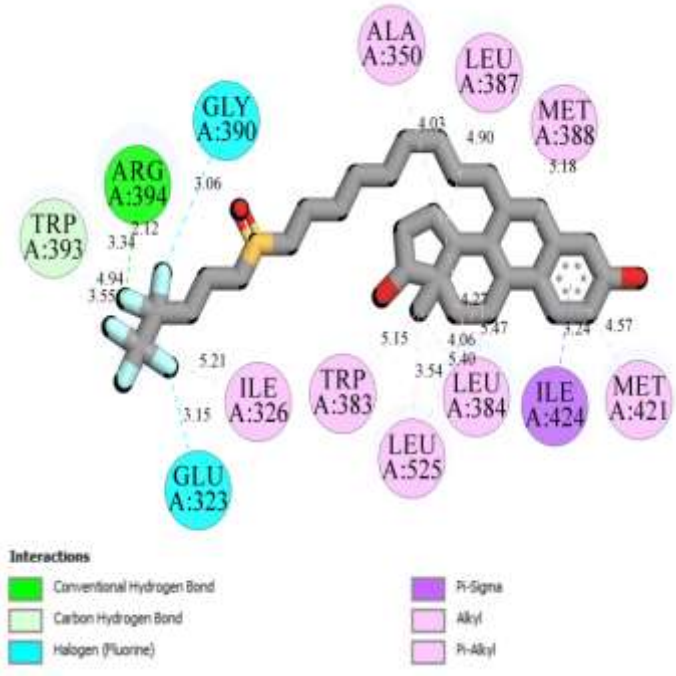

Fig. 2a: The 2D interaction between HER protein and docked Fuvelstrant(standard drug) with the binding affinity of -5.8 K/cal/mol analyzed.

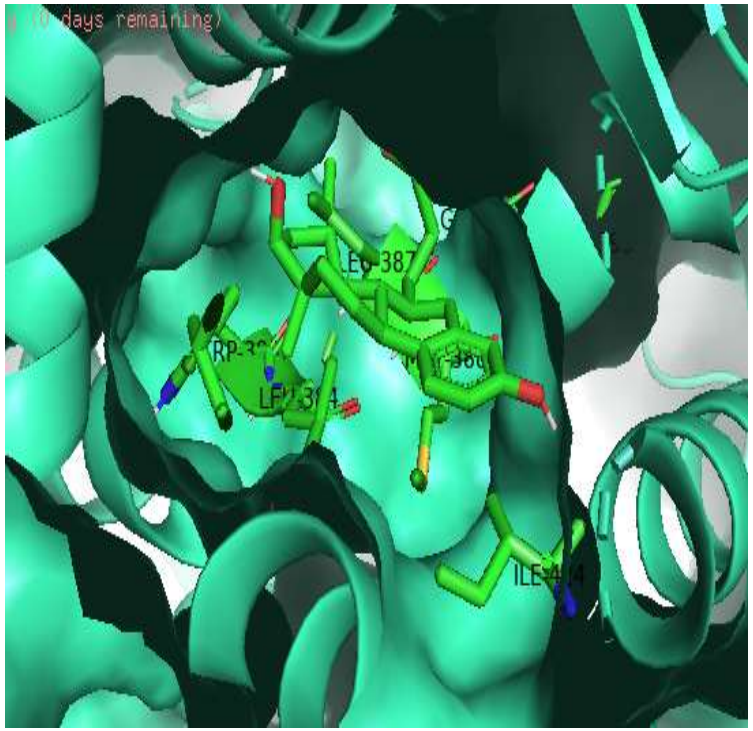

Figure 2b: The 3D interaction between HER protein and docked Fuvelstrant(standard drug) with the binding affinity of -5.8 K/cal/mol using BIOVIA discovery studio analyzer.

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Figure 3a: The 2D interaction between HER protein and docked 6-Paradol with the binding affinity of -6.4 K/cal/molanalyzed using BIOVIA discovery studio analyzer.

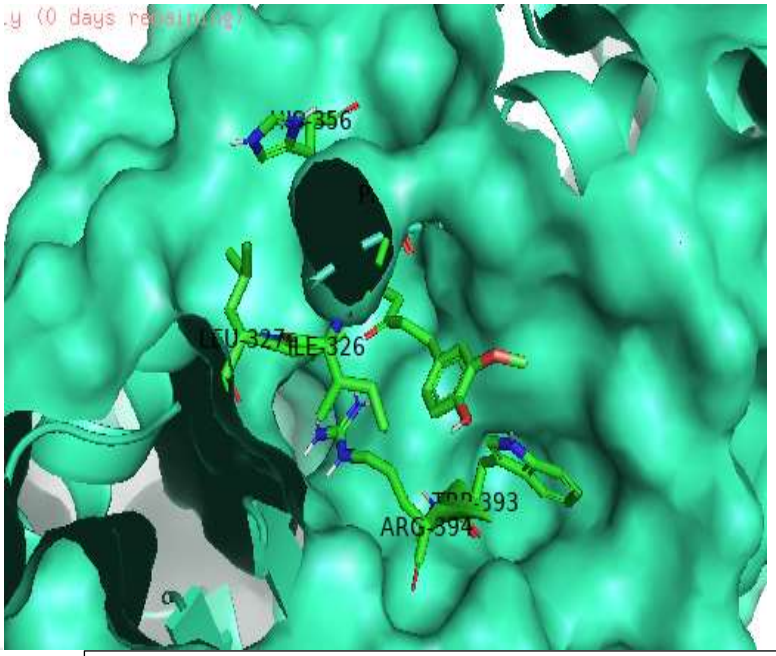

Figure 3b: 3D interaction between HER protein and docked 6-Paradol with the binding affinity of -6.4 K/cal/molanalyzed using BIOVIA discovery studio analyzer.

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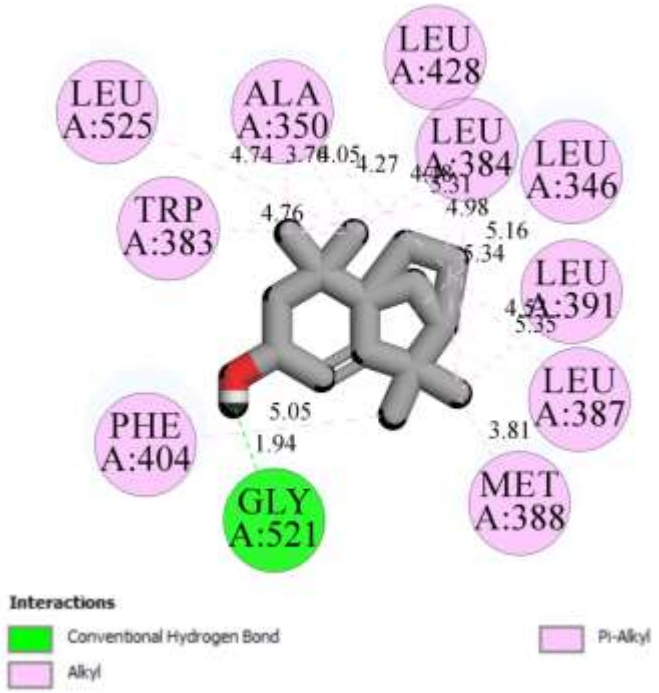

Figure 4a: The 2D interaction between HER protein and docked Isolongifolene-9-hydroxlwith the binding affinity of -8.1 K/cal/molanalyzed using BIOVIA discovery studio analyzer.

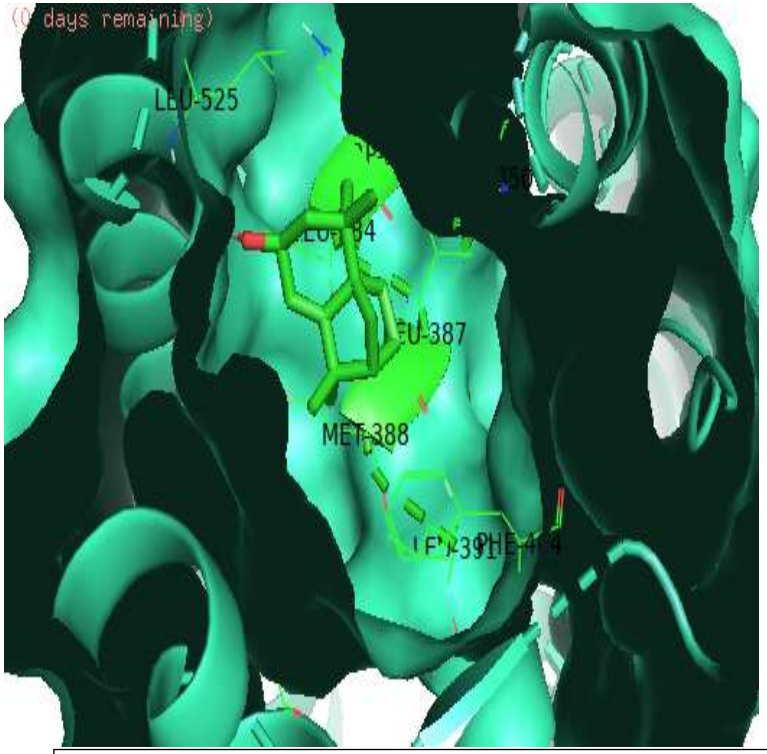

Figure 4b: The 3D interaction between HER protein and docked Isolongifolene-9- hydroxlwith the binding affinity of -8.1 K/cal/molanalyzed using BIOVIA discovery studio analyzer.

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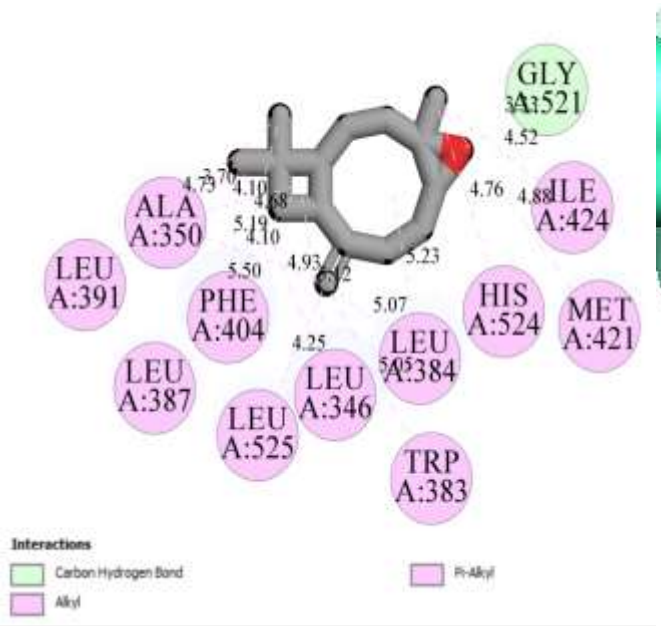

Figure 5a: The 2D interaction between HER protein and docked Caryophyllene Oxidewith the binding affinity of -8.6 K/cal/molanalyzed using BIOVIA discovery studio analyzer.

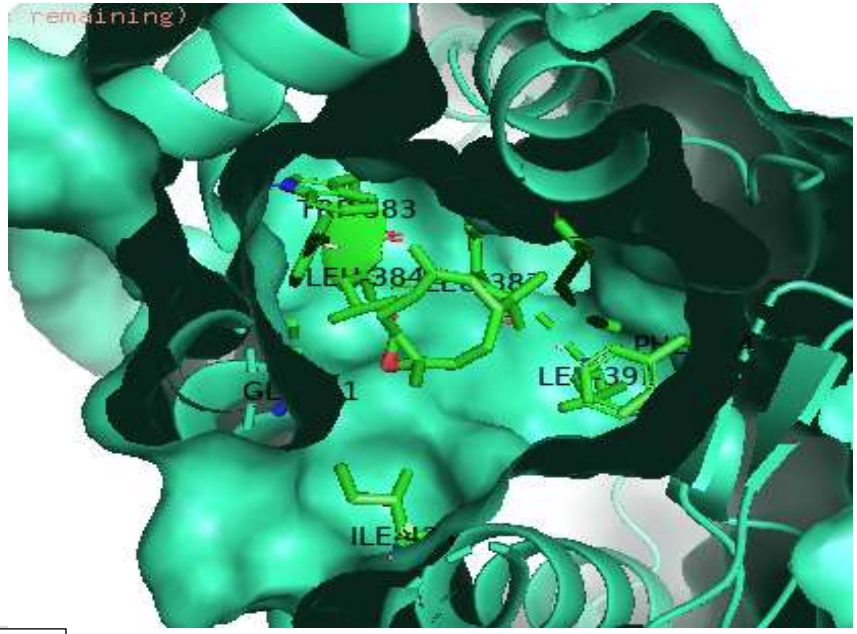

Figure 5b: The 3D interaction between HER protein and docked Caryophyllene Oxidewith the binding affinity of -8.6 K/cal/molanalyzed using BIOVIA discovery studio analyzer.

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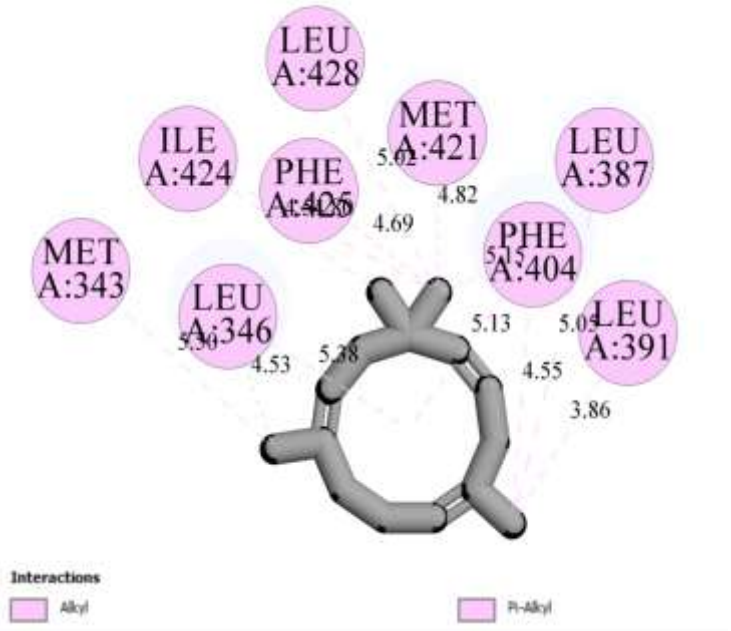

Figure 6a: The 2D interaction between HER (1GWR) protein and docked Humulenewith the binding affinity of -8.4 K/cal/mol analyzed using BIOVIA discovery studio analyzer.

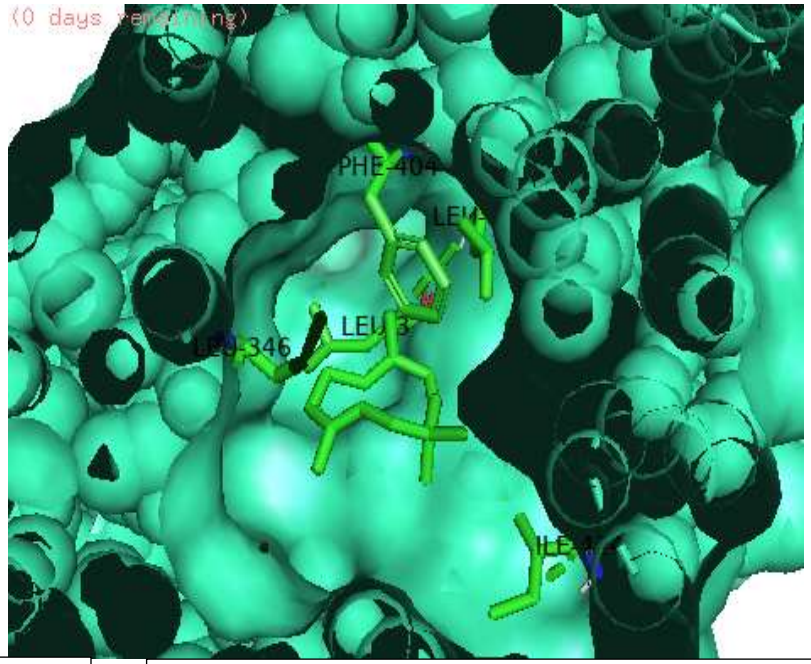

Figure 6b: The 3D interaction between HER (1GWR) protein and docked Humulenewith the binding affinity of -8.4 K/cal/mol analyzed using BIOVIA discovery studio analyzer.

Discussion

In this study's qualitative screening of the ethanolic extract of *Aframomum melegueta*, alkaloids, flavonoids, phenols, and terpenes were found in varying concentrations, but steroids were not. The concentration of flavonoid, alkaloid, terpene, and phenolic compounds was found in ethanolic extracts of *Aframomum melegueta*, with values of 14.47 mg/L, 11.93 mg/L, 0.301 mg/L, and 0.13 mg/L, respectively, according to the findings of a qualitative phytochemical screening. The ethanolic extract of *Aframomum melegueta* had a rather high number of flavonoids, according to the data. Flavonoids have advantageous anti-inflammatory properties and shield cells from oxidative stress, which can cause illnesses. They are also the most prevalent class of polyphenolic compounds in human diets, and due to their stimulant properties similar to those of caffeine and nicotine, morphine and quinine are employed as analgesics and antimalarials, respectively. Polyphenolic compounds are gaining popularity as therapeutic agents for a variety of illnesses, including cardiac and cerebral ischemia problems. They also promote health and protect against several chronic diseases, including cancer and arthritis. It is also claimed to be a strong antioxidant that promotes good blood circulation. It is frequently referred to as phytoestrogens due to its link to the menopausal system's release, the reduction of osteoporosis, and its ability to lower the danger of developing certain hormone-associated malignancies and coronary heart disease (Borekar et al., 2018). Numerous pharmacological functions are supported by the presence of alkaloids in this collection, including antihypertensive effects (various indole alkaloids), antiarrhythmic effects (quinidine, spareien), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine, vinblastine). One instance demonstrating the significant economic significance of this particular study (Iwu et al., 2018) emphasizes the significant economic relevance of this group of plant components. Furthermore, the findings suggest that phenols found in these extracts can function as anti-inflammatory, anti-clotting, antioxidant, immune-boosting, and hormone-regulating agents (Iwu et al., 2018). Terpenoids, such as the sesquiterpenoid antimalarial drug artimisinin and the diterpenoid anticancer drug taxol, are known for their therapeutic properties. Additionally, they possess anticarcinogenic (perilla alcohol), antimalarial (artemisinin), anti-ulcer, hepaticidal, antimicrobial, or diuretic (glycyrrhizin) effects.

The ethanolic extract of *Aframomum melegueta* was analyzed using GC-MS, and the results indicated the presence of at least 42 compounds, as shown in Figure 4.1. This finding is consistent with the studies conducted by Naz et al. (2010) and Arivoli et al. (2019). The GC-MS analysis revealed various bioactive components in the *Aframomum melegueta* extract, which contribute to its diverse properties. The identification of these 42 compounds provides a biochemical basis for the traditional medicinal uses of *Aframomum melegueta* in treating and preventing various diseases and disorders. Table 2 presents the individual names of the compounds, along with their respective peak numbers, retention times, and area percentages. Some of the compounds identified include P-Toluic acid, Butan-2-one, Undecyl-ester (+-)-2-phenethanamine, Gingerol, Cis-13-Octadeconic acid, Heneicosanoic acid, 3 amino-2-methylbutyl ester, 1-Propenyl Indane, 2,3 dihydroxypropyl ester, 2-Methoxy-4-vinylphenol, and E-9-Tetradecanal. Caryophyllene oxide, one of the compounds presents, has been found to have selective agonistic effects on cannabinoid receptor type-2 (CB2) and exhibits significant anti-inflammatory, anti-nociceptive, neuroprotective, anxiolytic, antidepressant, and anti-alcoholism properties (Bahi et al., 2014). The compound palmitic acid, also known as n-Hexadecanoic acid, has various beneficial properties such as antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, and antiandrogenic activities (Bahi et al, 2014). Gingerols have been found to have effects on lowering blood glucose levels as well as anti-inflammatory and antidiabetic activities (Nosiri et al., 2016). Humulene, also called -humulene or -caryophyllene, is a naturally occurring compound that belongs to the monocyclic sesquiterpene group (C15H24). It has anti-inflammatory effects in mammals and shows potential for managing inflammatory diseases. It has also been observed to reduce edema caused by histamine injections and inhibit the production of tumor necrosis factor- (TNF) and interleukin-1 β (IL1B) in rats injected with carrageenan

(Fernandez et al., 2007). 6-Paradol has various properties, including anticancer, anti-inflammatory, anti-obesity, neuroprotective, and blood sugar-reducing activities (Seba, Anatt, Sheeja, & Prasobh, 2020).

Molecular docking studies were conducted to examine the binding interactions between the tested ligands and the HER protein. From the multiple poses obtained for each ligand, the one with the lowest binding energy was selected and visualized using BIOVIA Discovery Studio to assess its reaction binding affinity with the HER-2 protein. The presence of greater hydrogen and hydrophobic interactions with specific amino acid residues indicates a higher binding affinity. Fulvestrant, a well-known anti-cancer drug that targets the HER protein, exhibited a binding affinity of -5.8 kcal/mol, as shown in the results. Interestingly, Caryophyllene oxide, Humulene, isolongifolene-9-hydroxyl, and 6-paradol demonstrated higher binding affinities than Fulvestrant and other selected ligands (-8.6 kcal/mol, -8.4 kcal/mol, -8.1 kcal/mol, and -6.4 kcal/mol, respectively). These compounds have the potential to act as anti-cancer agents against cancer cells that rely on HER protein expression for their progression and proliferation. Their higher binding affinity suggests that they could effectively inhibit the expression of the HER protein. This is supported by the findings of Pruthvish et al. (2021), Soni et al. (2015), Elekofehinti et al. (2018), and Ambrose et al. (2018). The 2D interactions of amino acid residues in different colors were observed using the BIOVIA Discovery Studio visualizer. Among the selected ligands, Fulvestrant (the standard drug) had 12 hydrophobic interactions with specific residues and 1 hydrogen bond with another residue (Figure 2). Caryophyllene oxide had 12 hydrophobic bonds (figure 5) and Humulene had 9 hydrophobic bonds (figure 6), while isolongifolene-9-hydroxyl had 10 hydrocarbon interactions and 1 hydrogen bond (figure 4). Six paradols had five hydrophobic bonds and one hydrogen bond (Figure 3). Caryophyllene oxide and the other compounds had higher binding affinity to the binding pocket of the HER protein compared to the standard drug (Figure 5). This higher binding affinity can be attributed to the medicinal activity of the compounds. In a recent study, it was found that Caryophyllene oxide, which is present in N. nucifera Leaf Extract and N. nucifera Leaf Polyphenol Extract, suppressed the expression of certain enzymes in human breast cancer cells.

Conclusion

To summarize, *Aframomum meleguta* has been found to have minimal or no side effects. Through docking studies, it has been discovered that certain phytocompounds such as 6-Paradol, Isolongifolene, Caryophyllene Oxide, and Humulene have high binding affinities with the HER protein. These compounds have even stronger binding affinities than the standard drug Fulvestrant. Therefore, they have the potential to be effective drug leads for developing anticancer inhibitors that target the HER protein in different types of cancer. These compounds could serve as a viable alternative to the standard drug.

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