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# *In silico* evaluation of potential breast cancer receptor antagonists from GC-MS and HPLC identified compounds in *Pleurotus ostreatus* extracts†

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**Introduction:** Pharmacotherapeutic targets for breast cancer include the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (EGFR). Inhibitors of these receptors could be interesting therapeutic candidates for the treatment and management of breast cancer (BC). **Aim:** This study used GC-MS and HPLC to identify bioactive compounds in *Pleurotus ostreatus* (*P. ostreatus*) extracts and applied *in silico* methods to identify potent EGFR, ER, and PR inhibitors from the compounds as potential drug candidates. **Method:** GC-MS and HPLC were used to identify bioactive chemicals in *P. ostreatus* extracts of aqueous (PO-A), methanol (PO-M), ethanol (PO-E), chloroform (PO-C), and *n*-hexane (PO-H). The ER, PR, and EGFR model optimization and molecular docking of compounds/control inhibitors in the binding pocket were simulated using AutoDock Vina in PyRx. The drug-likeness, pharmacokinetic, and pharmacodynamic features of prospective docking leads were all anticipated. **Result:** The results indicated the existence of 29 compounds in PO-A, 36 compounds in PO-M and PO-E, 42 compounds in PO-C, and 22 compounds in PO-H extracts. With ER, only *o*-tolylamino-acetic acid (4-nitro-benzylidene)-hydrazide ( $-7.5 \text{ kcal mol}^{-1}$ ) from the ethanolic extract could bind to the receptor. PR and EGFR, on the other hand, identified several compounds with higher binding affinities than the control. Ergotaman-3',6',18-trione ( $-8.1 \text{ kcal mol}^{-1}$ ), 5,10-diethoxy-2,3,7,8-tetrahydro-1*H*,6*H*-dipyrrolo[1,2-*a*:1',2'-*d*]pyrazine ( $-7.8 \text{ kcal mol}^{-1}$ ) from the aqueous extract; *o*-tolylamino-acetic acid (4-nitro-benzylidene)-hydrazide ( $-8.4 \text{ kcal mol}^{-1}$ ) from the ethanolic extract had better binding affinity compared to progesterone ( $-7.7 \text{ kcal mol}^{-1}$ ). Likewise, ergotaman-3',6',18-trione ( $-9.7 \text{ kcal mol}^{-1}$ ) from the aqueous extract and phenol, 2,4-bis(1,1-dimethyl ethyl) ( $-8.2 \text{ kcal mol}^{-1}$ ) from the chloroform extract had better binding affinities compared to the control, gefitinib ( $-7.9 \text{ kcal mol}^{-1}$ ) with regards to EGFR. None of the PO-H or PO-M extracts outperformed the control for any of the proteins. Phenols and flavonoids such as quercetin, luteolin, rutin, chrysin, apigenin, ellagic acid, and naringenin had better binding affinity to PR and EGFR compared to their control. **Conclusion:** The identified compounds in the class of phenols and flavonoids were better lead molecules due to their ability to strongly bind to the proteins' receptors. These compounds showed promising drug-like properties; they could be safe and new leads for creating anticancer medicines.

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## 1 Introduction

Mushrooms are high in nutrients such as protein, dietary fiber, vitamins, and minerals, all of which benefit general health.<sup>1,2</sup>

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They contain antioxidants, which help to neutralize damaging free radicals, potentially lowering the risk of chronic illnesses like as cancer.<sup>3,4</sup> Mushrooms have anti-inflammatory properties that may help prevent certain cancers. They also contain immunomodulatory properties, which can help boost the body's natural defenses against cancer cells.<sup>5,6</sup> Studies have yielded positive findings in terms of decreasing cancer cell proliferation, inducing apoptosis, and lowering tumor development.<sup>7-10</sup> Mushrooms are frequently used as a supplement or adjuvant therapy to conventional cancer medications, with the potential to reduce treatment-related side effects and improve general well-being.<sup>11,12</sup>

Breast cancer is a major public health issue worldwide due to its high prevalence, mortality rates, disparities in access to care,



and emotional impact on individuals, families, communities, and healthcare systems.<sup>13–15</sup> The disease is the most often diagnosed cancer among women worldwide, with a high mortality rate.<sup>16–18</sup> It has a significant economic impact, including costs for diagnosis, treatment, care and psychosocial support.<sup>17–19</sup> Tackling breast cancer involves a multifaceted strategy, including prevention, early detection, diagnosis, and treatment such as surgery, chemotherapy, radiation therapy, hormone therapy, targeted therapy, and immunotherapy.<sup>20,21</sup> However, these methods can have side effects and do not always result in complete remission or prevent recurrence due to drug resistance and tumoral heterogeneity.<sup>22</sup>

Breast cancer has several molecular subgroups based on hormone receptor expression and gene profiles. The five primary subtypes are luminal A, luminal B, HER2-enriched, triple-negative breast cancer (TNBC), and basal-like.<sup>23,24</sup> The disease's heterogeneity is continually changing, demanding personalized and focused treatments.<sup>25</sup> Receptors associated with these subtypes, such as estrogen receptors (ER), progesterone receptors (PR), human epidermal growth factor receptor 2 (HER2), are important in cancer initiation, development, and treatment response.<sup>26,27</sup> Targeting these receptors with specific drugs has resulted in significantly improved outcomes, emphasizing the importance of the discovery of natural inhibitors and drug candidates.

Oyster mushrooms, also known as *Pleurotus ostreatus*, have been studied for their potential anticancer properties,<sup>28</sup> including interactions with ER+, PR+ and HER2+ breast cancer receptors.<sup>29,30</sup> Bioactive compounds in *Pleurotus ostreatus* may interact with these breast cancer receptors, either independently or synergistically leading to decreased breast carcinogenesis, survival and proliferation. Therefore this study aims at utilizing gas chromatography mass spectrophotometer (GC-MS) and high performance liquid chromatography (HPLC) techniques to identify and quantify the bioactive compounds in *Pleurotus ostreatus*, and apply *in silico* techniques to evaluate their inhibitory effects on breast cancer receptors.

## 2 Materials and methods

### 2.1 Sample collection and preparation

#### 2.1.1 Collection and identification of *Pleurotus ostreatus*.

25 kg of fresh *P. ostreatus* (oyster mushrooms) were purchased from a local mushroom farm in Agbara, Ogun State, South West Nigeria, at coordinates 6.5114°N, 3.1115°E. The Botany Department at the University of Ibadan in Ibadan, Oyo State, validated the acquired specimen (Fig. 1).

#### 2.1.2 Processing and preservation of *Pleurotus ostreatus*.

*P. ostreatus* was thoroughly cleansed to remove pollutants and wiped clean with a sterile towel to remove any water residues from its surface. The washed *P. ostreatus* was dried in an oven with hot air set to 55–65 °C until completely dry. The dried *P. ostreatus* was ground into powder using a blender and weighed. The resulting powder was cooled to room temperature and stored in sealed containers for future use.<sup>32</sup>

#### 2.1.3 Preparation of *Pleurotus ostreatus* extracts.

The bioactive components of the *P. ostreatus* sample were extracted using the method described by ref. 33. To find the optimal solvent, the extracts were prepared in a variety of solvents such as chloroform, ethanol, *n*-hexane, distilled water, and methanol. 20 g of dry powdered material was weighed, combined with 300 mL of each solvent, and stirred for 24 hours. To remove solid particles, the resultant solution was double-filtered with Whatman filter paper. The extracts were created by removing the solvents using a rotary evaporator. The final extracts were weighed, and the percentage extract yield and total dry weight were calculated for each solvent extract. The dried extracts were stored at 4 °C until further evaluation as described by.<sup>33,34</sup>

### 2.2 Gas chromatography-mass spectrometry (GC-MS) analysis of the extracts of *Pleurotus ostreatus*

PO extracts were analyzed by GC-MS on a GCMS-QP2010SE SHIMADZU JAPAN using a fused Optima-5MS capillary column of 30 m length, 0.25 mm diameter, and 0.25 μm film thickness. The GC conditions were pure helium (1.56 mL min<sup>-1</sup> flow rate and 37 cm s<sup>-1</sup> linear velocity), injector temperature (200 °C),

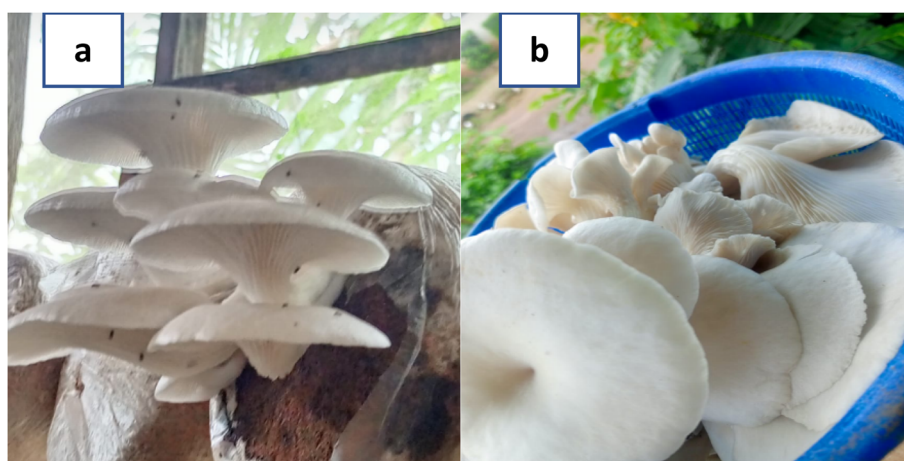


Fig. 1 (a and b): *P. ostreatus* mushroom.<sup>31</sup> (a): *P. ostreatus* growing on its substrate. (b): *P. ostreatus* freshly harvested.



Table 1 Gas chromatography-mass spectroscopy (GC-MS) identified phytochemicals in PO aqueous extract (29 compounds)

S/N	Compound	RT (min)	Area (%)	Formula	Mw	Compounds classification	Structure
1	2,3-Butanediol	5.594	1.50	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>	90	Alcohol	
2	Unidentified	5.688	4.21				
3	Bicyclo[2.2.1]hept-5-ene-2,3-dimethanol	5.860	0.21	C <sub>9</sub> H <sub>8</sub> Cl <sub>6</sub> O <sub>2</sub>	358	Alcohol	
4	1,3-Propanediol	6.238	0.88	C <sub>3</sub> H <sub>8</sub> O <sub>2</sub>	76	Alcohol	
5	L-Lactic acid	7.518	71.51	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>	90	Carboxylic acid	
6	Glycerin	9.084	1.63	C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>	92	Alcohol	
7	Propanamide	9.333	1.01	C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>	89	Fatty amide	
8	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	9.893	0.21	C <sub>6</sub> H <sub>8</sub> O <sub>3</sub>	128	Lactone	
9	2-Pyrrolidinone	10.401	8.60	C <sub>7</sub> H <sub>7</sub> NO	85	Cyclic amines	
10	1,3,4-Thiadiazole	10.624	0.42	C <sub>11</sub> H <sub>11</sub> N <sub>3</sub> O <sub>4</sub> S	281	Heterocyclin	
11	2-Butanol	10.763	0.16	C <sub>5</sub> H <sub>12</sub> O	88	Alcohol	
12	Succinimide	10.942	0.23	C <sub>4</sub> H <sub>5</sub> NO <sub>2</sub>	99	Cyclic imides	
13	4H-Pyran-4-one	11.128	0.96	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144	Pyrones	
14	2(3H)-Furanone	11.363	0.21	C <sub>4</sub> H <sub>6</sub> O <sub>3</sub>	102	Lactone	
15	Isosorbide	12.328	0.49	C <sub>6</sub> H <sub>10</sub> O <sub>4</sub>	146	Alcohol	
16	Unidentified	13.326	0.20				
17	Piracetam	13.760	0.28	C <sub>6</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>	142	Organonitrogen	
18	4-sec-Butoxy-2-butanone	13.881	0.20	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	144	Alkanone	
19	2-Propenamide	14.232	0.86	C <sub>5</sub> H <sub>9</sub> NO	99	Amide	
20	Piperazine	14.365	0.35	C <sub>6</sub> H <sub>14</sub> N <sub>2</sub>	114	Azacycloalkane	
21	3,6-Dimethylpiperazine-2,5-dione	14.434	0.34	C <sub>6</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>	142	Azacycloalkane	
22	±-.Beta.,.beta.-dimethyl-.gamma.-(hydroxy-methyl)-gamma-butyrolactone	14.751	0.16	C <sub>7</sub> H <sub>12</sub> O <sub>3</sub>	144	Alkanone	
23	3-Pyrrolidin-2-yl-propionic acid	17.276	0.90	C <sub>7</sub> H <sub>13</sub> NO <sub>2</sub>	143	Carboxylic acid	
24	Hexahydropyrrolizin-3-one	17.523	0.67	C <sub>7</sub> H <sub>11</sub> NO	125	Cyclic amines	

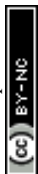
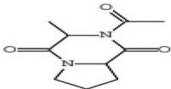

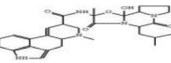
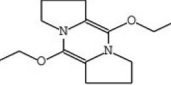



Table 1 (Contd.)

S/N	Compound	RT (min)	Area (%)	Formula	Mw	Compounds classification	Structure
25	3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-dione	19.626	0.53	C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub>	210	Alkanone	
26	Pyrrolo[1,2- <i>a</i> ]pyrazine-1,4-dione	20.297	1.12	C <sub>7</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>	154	Organooxygen-nitrogen	
27	Ergotaman-3',6',18-trione	22.550	0.09	C <sub>30</sub> H <sub>37</sub> N <sub>5</sub> O <sub>5</sub>	547	Ergot alkaloids	
28	5,10-Diethoxy-2,3,7,8-tetrahydro-1 <i>H</i> ,6 <i>H</i> -dipyrrolo[1,2- <i>a</i> :1',2'- <i>d</i> ]pyrazine	22.923	0.31	C <sub>14</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub>	250	Cyclic amines	
29	Tyramine	24.100	1.76	C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>	165	Amine	

column oven temperature (60 °C initially, then increased to 160 °C and later to 250 °C at 10 °C min<sup>-1</sup> with 2 min/increment hold time), and injection volume and split ratio (0.5 μL and 1 : 1, respectively). The MS conditions were: ion source of 230 °C and an interface temperature of 250 °C, a solvent delay of 4.5 minutes recorded in a scan range 50 to 700 amu. Unknown constituents were detected by comparing the retention duration, mass spectrum data and fragmentation pattern of the extracts with established libraries (National Institute of Standards and Technology (NIST) and Wiley libraries).<sup>35,36</sup>

### 2.3 HPLC analysis of the extracts of *Pleurotus ostreatus*

The HPLC identification and characterization of flavonoids, phenols and saponins in the five extracts of *P. ostreatus* was carried out using the method described by ref. 37.

**2.3.1 Sample preparation for HPLC analysis.** An aliquot of the sample extracts (0.1 g) was combined with 10 mL of 70% methanol in a closed test tube and left to stand for 1–2 hours. The extracted material was then decanted, centrifuged using a chilled centrifuge (model: CR21G, serial number: S2025709), and filtered through a micron filter into a 5 mL sample container. The sample filtrate was used to analyze the saponins, phenolic and flavonoid components in the extracts of *Pleurotus ostreatus* using HPLC.<sup>37</sup>

**2.3.2 HPLC analysis for the saponins fractions.** The saponin samples (40 μL) were injected into the HPLC (model: Agilent LC-8518) with acetonitrile/water (70 : 30) mobile phase, 205 nm wavelength, a 14 minutes run duration, sorbent type is C18 silica packed column, 5 μm, 4.6 × 2.50 mm. HPLC analysis was performed using N2000 chromatography software (4.0 version) with a high-sensitivity LC-8518 UV detector, column (150 mm × 4.6 mm) set at 40 °C, and a low-pressure gradient and solvent delivery LC-8518 pump with a high-pressure switching valve to determine flavonoids content and amount in the extracts.<sup>37</sup>

**2.3.3 HPLC analysis for the phenol fractions.** The extracted phenolic samples (40 μL) were injected into the HPLC (model: Agilent LC-8518) running with acetonitrile/water/acetic acid

(19 : 80 : 1) mobile phase, at 272 nm wavelength, and a run time of 25 minutes. To analyze flavonoids in extracts, N2000 chromatography software was used with a high-sensitivity LC-8518 diode array (DA) detector, a column (150 mm × 4.6 mm) set at 35 °C, and a low-pressure gradient and solvent delivery LC-8518 pump with a high-pressure switching valve.<sup>37</sup>

**2.3.4 HPLC analysis for the flavonoids fractions.** The extracted flavonoid samples (40 μL) were injected into the HPLC (model: Agilent LC-8518) running with acetonitrile, water and formic acid (25 : 74 : 1) mobile phase, 210 nm wavelength, and a run period of 25 minutes. To analyze flavonoids in extracts, N2000 chromatography software was used with a high-sensitivity LC-8518 diode array (DA) detector, a column (150 mm × 4.6 mm) set at 40 °C, and a low-pressure gradient and solvent delivery LC-8518 pump with a high-pressure switching valve.<sup>37</sup>

### 2.4 *In silico* studies

**2.4.1 Protein retrieval and preparation.** The three-dimensional structure of proteins involved in breast cancer such as PR (1A28), ER (1ERE) and EGFR (1M17) were obtained from the RCSB Protein Data Bank (PDB) (<https://www.rcsb.org/>). The proteins and their natural substrates/inhibitors are described in Table 1. The proteins were prepared with UCSF Chimera Software version 1.17.3. The co-crystallized ligands and other non-standard residues were removed from the structures; hydrogen atoms and Gasteiger charges were added; the energy of the structure was minimized using the steepest descent method.

**2.4.2 Ligand preparation.** Ligands used in this study were curated from the compounds identified from the plant *via* GCMS and HPLC. The canonical smiles and three-dimensional structure of these ligands were obtained from the PubChem library (<https://pubchem.ncbi.nlm.nih.gov/>). The natural substrates/inhibitors were added to this ligand library to be used as the reference compound.

**2.4.3 Molecular docking.** Molecular docking was carried out *via* a blind docking procedure using AutoDock Vina in



Table 2 Gas chromatography-mass spectroscopy (GC-MS) identified phytochemicals in PO methanol extract (36 compounds)

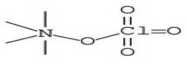
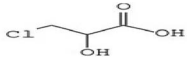


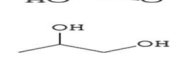
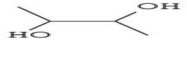

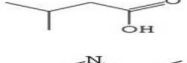
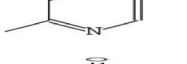
















S/N	Compound	RT (min)	Area (%)	Formula	Mw	Compounds classification	Structure
1	Tetramethylammonium perchlorate	5.496	0.77	C <sub>4</sub> H <sub>12</sub> ClNO <sub>4</sub>	173	Non-metal perchlorates	
2	Propanoic acid	5.666	0.16	C <sub>5</sub> H <sub>5</sub> ClO <sub>3</sub>	124	Carboxylic acid	
3	Acetic acid	5.853	2.39	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	60	Carboxylic acid	
4	2-Propanone	6.199	0.15	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	74	Alkanone	
5	2-Propenoic acid	6.423	0.10	C <sub>3</sub> H <sub>4</sub> O <sub>2</sub>	72	Carboxylic acid	
6	(S)-(+)-1,2-Propanediol	6.732	0.21	C <sub>3</sub> H <sub>8</sub> O <sub>2</sub>	76	Alcohol	
7	2,3-Butanediol	7.085	0.09	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>	90	Alcohol	
8	Unidentified	7.190	0.10				
9	Acetamide	7.282	0.39	C <sub>2</sub> H <sub>5</sub> NO	59	Amide	
10	Butanoic acid	7.758	0.21	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	102	Carboxylic acid	
11	Pyrazine	8.750	0.87	C <sub>6</sub> H <sub>8</sub> N <sub>2</sub>	108	Aromatic heterocyclins	
12	2-Oxepanone	8.893	1.40	C <sub>6</sub> H <sub>10</sub> O <sub>2</sub>	114	Alkanone	
13	2(5H)-Furanone	9.848	2.34	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98	Lactone	
14	1,2-Cyclohexanedione	10.116	1.66	C <sub>6</sub> H <sub>8</sub> O <sub>2</sub>	112	Alkanone	
15	Pentaerythritol	10.525	0.17	C <sub>5</sub> H <sub>12</sub> O <sub>4</sub>	136	Polyol	
16	1H-Pyrrolizine-7-methanol, 2,3,5,7a-tetrahydro-1-hydroxy-	10.660	0.14	C <sub>8</sub> H <sub>13</sub> NO <sub>2</sub>	155	Cyclic amine alcohol	
17	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl	10.866	1.21	C <sub>6</sub> H <sub>10</sub> O <sub>3</sub>	130	Lactone	
18	Pyrazine, 3-ethyl-2,5-dimethyl	11.382	2.93	C <sub>8</sub> H <sub>12</sub> N <sub>2</sub>	136	Aromatic heterocyclins	
19	2-Pyrrolidinone	12.103	16.49	C <sub>4</sub> H <sub>7</sub> NO	85	Cyclic amines	
20	cis-3-Nonen-1-ol, methyl ether	12.595	8.45	C <sub>10</sub> H <sub>20</sub> O	156	Alcohol	
21	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.804	12.07	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144	Pyrones	
22	6-Methyl-1,5-diazabicyclo[3.1.0]hexane	13.426	2.27	C <sub>5</sub> H <sub>10</sub> N <sub>2</sub>	98	Alkane	
23	Isosorbide	13.957	7.84	C <sub>6</sub> H <sub>10</sub> O <sub>4</sub>	146	Alcohol	
24	2-Propenoic acid, 2-methyl, pentyl ester	14.620	0.63	C <sub>9</sub> H <sub>16</sub> O <sub>2</sub>	156	Fatty ester	
25	Isosorbide	14.985	0.94	C <sub>6</sub> H <sub>10</sub> O <sub>4</sub>	146	Alcohol	
26	Piracetam	15.650	2.33	C <sub>6</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>	142	Organonitrogen	



Table 2 (Contd.)

S/N	Compound	RT (min)	Area (%)	Formula	Mw	Compounds classification	Structure
27	Heptane, 2,3-epoxy-	15.942	3.28	C <sub>7</sub> H <sub>14</sub> O	114	Alkane	
28	6-Desoxy-L-altritol	16.095	1.62	C <sub>6</sub> H <sub>14</sub> O <sub>5</sub>	166	Alcohol	
29	2,5-Methylene-D,L-rhamnitol	16.320	3.18	C <sub>7</sub> H <sub>14</sub> O <sub>5</sub>	178	Alcohol	
30	Cyclohexanecarboxylic acid, 4-methylpentyl ester	16.645	4.53	C <sub>13</sub> H <sub>24</sub> O <sub>2</sub>	212	Fatty ester	
31	Linoleic acid ethyl ester	17.095	2.82	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308	Fatty ester	
32	Niacinamide	17.420	1.61	C <sub>6</sub> H <sub>6</sub> N <sub>2</sub> O	122	Amide	
33	Docosanoic acid, ethyl ester	17.745	1.36	C <sub>24</sub> H <sub>48</sub> O <sub>2</sub>	368	Fatty acid	
34	Fumaric acid, ethyl 2-methylallyl ester	19.345	0.12	C <sub>10</sub> H <sub>14</sub> O <sub>4</sub>	198	Fatty ester	
35	3-Methyl-4-phenyl-1H-pyrrole	19.520	0.10	C <sub>11</sub> H <sub>11</sub> N	157	Aromatic heterocyclin	
36	D-Glucitol, 1,4-anhydro	19.820	15.08	C <sub>6</sub> H <sub>12</sub> O <sub>5</sub>	164	Polyols	

PyRx.<sup>38,39</sup> The grid box parameters were set as shown in Table 2 follows: EGFR [centre: size-X(23.5378:93.1492); Y(9.8448:66.2191); Z(59.3929:51.5664)], ER [centre: size-X(42.1102:56.7478); Y(30.3765:38.8400); Z(49.3079:49.8795)], PR [centre: size-X(29.5578:55.0116); Y(8.2756:50.7768); Z(67.8664:53.0039)] and run at exhaustiveness of 8. The protein–ligand interactions were visualized using Discovery Studio 2021 to identify the amino acid residues and the interactions they formed upon binding to the different compounds.

**2.4.4 Pharmacological properties of compounds.** For initial screening, SwissADME (<http://www.swissadme.ch/>), an online web-based platform, was used, which evaluates the pharmaceutical fidelity of the drug candidates. Various attributes such as molecular weight, lipophilicity, number of hydrogen bond acceptors, and donors were analyzed using this tool.

**2.4.5 Bioavailability radar and toxicity.** Drug-likelihood was comprehensively evaluated for candidates, considering six physiochemical properties such as solubility, molecular size, polarity, lipophilicity, saturation, and flexibility and a bioavailability radar was obtained using the SwissADME tool (<http://www.swissadme.ch/>). At the same time, the ADMETlab 2.0 webserver (<https://admetmesh.scbdd.com/service/screening/cal>) was used to predict the toxicity of the ligands.<sup>40</sup>

## 3 Results

### 3.1 Gas chromatography-mass spectroscopy (GC-MS) results

The gas chromatography of *Pleurotus ostreatus* aqueous, methanol, ethanol, chloroform and *n*-hexane extracts revealed the presence of 29, 36, 36, 42 and 22 peaks respectively.

**3.1.1 Gas chromatography-mass spectroscopy (GC-MS) results of PO aqueous extract.** The aqueous extract had 29 bioactive compounds belonging to various compound classes (Table 1). Majority of the bioactive compounds in the aqueous extracts were alcohols.

**3.1.2 Gas chromatography-mass spectroscopy (GC-MS) results of PO methanol extract.** The methanol extract had 36 bioactive compounds belonging to various compound classification (Table 2). Majority of the bioactive compounds in the methanol extracts were alcohols and fatty acids.

**3.1.3 Gas chromatography-mass spectroscopy (GC-MS) results of PO ethanol extracts.** The ethanol extract had 36 bioactive compounds belonging to various compound classification (Table 3). Majority of the bioactive compounds in the ethanol extracts were alcohols and fatty acids.

**3.1.4 Gas chromatography-mass spectroscopy (GC-MS) results of PO chloroform extract.** The GC-MS results of the chloroform extract of PO revealed the presence of 42 bioactive compounds as shown in Table 4. Majority of the bioactive compounds in the chloroform extracts were alcohols and fatty acids.

**3.1.5 Gas chromatography-mass spectroscopy (GC-MS) results of PO *n*-hexane extract.** The GC-MS results of the *n*-hexane extract of PO revealed the presence of 22 bioactive compounds as shown in Table 5. Majority of the bioactive compounds in the *n*-hexane extracts were alcohols and fatty acids.

### 3.2 High performance liquid chromatography (HPLC) profile of *P. ostreatus* extracts

The HPLC profile of *P. ostreatus* extracts revealed the presence of flavonoids, phenols and saponins. The extracts possessed



Table 3 Gas chromatography-mass spectroscopy (GC-MS) identified phytochemicals in PO ethanol extract (36 compounds)


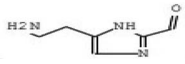
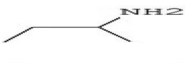









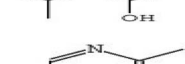
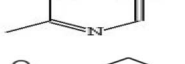
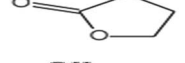

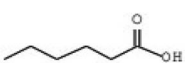
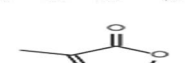
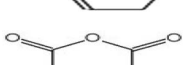



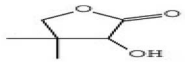

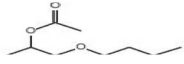
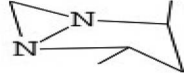
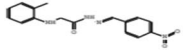
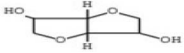



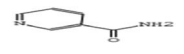
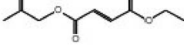
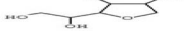

S/N	Compound	RT (min)	Area (%)	Formula	Mw	Compounds classification	Structure
1	Ethanol	4.568	1.20	C <sub>2</sub> H <sub>6</sub> O	46	Alcohol	
2	Unidentified	4.759	0.27				
3	2-Formylhistamine	4.950	0.48	C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O	139	Amine	
4	sec-Butylamine	5.081	1.52	C <sub>4</sub> H <sub>11</sub> N	598	Amine	
5	Acetone	5.170	0.74	C <sub>3</sub> H <sub>6</sub> O	58	Ketone	
6	1-Propanol, 2-methyl	5.235	0.81	C <sub>4</sub> H <sub>10</sub> O	74	Alcohol	
7	Methylamine	5.335	0.60	C <sub>3</sub> H <sub>9</sub> N	59	Amine	
8	Acetic acid	5.537	3.39	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	60	Carboxylic acid	
9	2-Propanone	5.939	0.16	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	74	Ketone	
10	1-Butanol	6.083	2.41	C <sub>5</sub> H <sub>12</sub> O	88	Alcohol	
11	Propylene glycol	6.516	0.28	C <sub>3</sub> H <sub>8</sub> O <sub>2</sub>	76	Alcohol	
12	2,3-Butanediol	7.035	0.19	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>	90	Alcohol	
13	Pyrazine, methyl	7.410	0.09	C <sub>5</sub> H <sub>6</sub> N <sub>2</sub>	94	Aromatic heterocyclins	
14	Butanoic acid	7.593	0.17	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	102	Fatty acid	
15	Pyrazine, 2,5-dimethyl	8.725	0.15	C <sub>6</sub> H <sub>8</sub> N <sub>2</sub>	108	Aromatic heterocyclins	
16	Butyrolactone 2(3H)-furanone	8.858	0.68	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>	86	Ketone	
17	L-Lactic acid	9.369	3.72	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>	90	Carboxylic acid	
18	Hexanoic acid, caproic acid	9.680	0.20	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	116	Fatty acid	
19	2(5H)-Furanone, 3-methyl	9.888	1.03	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98	Alkanone	
20	2H-Pyran-2,6(3H)-dione	10.140	0.51	C <sub>5</sub> H <sub>4</sub> O <sub>3</sub>	112	Alkanone	
21	Pyrazine, trimethyl	10.280	0.22	C <sub>7</sub> H <sub>10</sub> N <sub>2</sub>	122	Aromatic heterocyclin	
22	3-Methyl-3-oxetanemethanol	10.535	0.16	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	102	Alcohol	
23	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl	10.921	0.73	C <sub>6</sub> H <sub>10</sub> O <sub>3</sub>	130	Alkanone	
24	2-Pyrrolidinone	12.163	13.33	C <sub>4</sub> H <sub>7</sub> NO	85	Alkanone	
25	4H-Pyran-4-one	12.830	4.64	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144	Alkanone	



Table 3 (Contd.)

S/N	Compound	RT (min)	Area (%)	Formula	Mw	Compounds classification	Structure
26	1-Butoxy-2-propanol acetate	13.025	4.00	C <sub>9</sub> H <sub>18</sub> O <sub>3</sub>	174	Fatty acid	
27	2,4-Dimethyl-1,5-diazabicyclo[3.1.0]hexane ( <i>trans</i> )	13.603	0.26	C <sub>6</sub> H <sub>12</sub> N <sub>2</sub>	112	Alkane	
28	<i>o</i> -Tolylamino-acetic acid (4-nitro-benzylidene)-hydrazide	13.844	0.12	C <sub>16</sub> H <sub>16</sub> N <sub>4</sub> O <sub>3</sub>	312	—	
29	Isosorbide D-glucitol	14.264	0.09	C <sub>6</sub> H <sub>10</sub> O <sub>4</sub>	146	—	
30	Niacin	15.445	0.64	C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub>	123	—	
31	Heptane, 2,3-epoxy-	16.165	1.24	C <sub>7</sub> H <sub>14</sub> O	114	Alkane	
32	2-Undecanone, 6,10-dimethyl	16.440	0.28	C <sub>13</sub> H <sub>26</sub> O	198	Alkanone	
33	Methoxyacetic acid, 2-tridecyl ester	16.690	0.50	C <sub>16</sub> H <sub>32</sub> O <sub>3</sub>	272	Fatty ester	
34	Niacinamide	17.780	1.38	C <sub>6</sub> H <sub>6</sub> N <sub>2</sub> O	122	Amide	
35	Fumaric acid, ethyl 2-methylallyl ester	19.814	0.20	C <sub>10</sub> H <sub>14</sub> O <sub>4</sub>	198	Fatty ester	
36	D-Glucitol, 1,4-anhydro-	22.289	53.58	C <sub>6</sub> H <sub>12</sub> O <sub>5</sub>	164	—	

a wider array of flavonoid compounds, followed by phenolic compounds and least amount of saponin compounds. Majority of the saponin compounds were unidentified compared to the flavonoid and phenolic compounds (Table 6).

### 3.3 Molecular docking results

**3.3.1 Ligand selection.** Molecules investigated in this study were from a range of compounds in *P. ostreatus* which were identified *via* different GCMS and HPLC extraction methods. Some of these compounds are classified as phenols and flavonoids. In this study, we examined a total of 188 compounds excluding the saponins (ESI File 1†). The natural substrates/inhibitors of the proteins studied were used as control (Table 7).

**3.3.2 Molecular docking.** In the current study, the selected receptors were docked against the screened molecules to examine the binding affinity. Docking scores selected represent the compounds where the upper and lower RMSD = 0, and lower than that of the control and the control, for proteins where the control has the lowest binding affinity, only the control is represented (Tables 8–10). The complete docking score of the ligands against the receptors used in the study is shown in ESI 02.†

#### 3.3.3 Screening of compounds against PR

**3.3.3.1 Aqueous extract.** Among the 29 compounds, 2 compounds, 10531 and 565346 exhibited a low binding affinity lower than the control, (compound 5994) which exhibited a low binding affinity of  $-7.7$  kcal mol<sup>-1</sup>. Compound 10531 exhibited the lowest binding affinity of  $-8.1$  kcal mol<sup>-1</sup> while compound

565346 exhibited a low binding affinity of  $-7.8$  kcal mol<sup>-1</sup>. The interactions formed by compound 10531 with PR include; Conventional Hydrogen Bonds with SER796, Pi-sigma with LEU901, Pi-alkyl with LEU797, Pi-anion with GLU791, and Pi-Pi stacked/Pi-Pi T-shaped with TYR890 (Fig. 2A). The interactions formed by compound 565346 include Conventional Hydrogen Bonds with ARG766, GLN725, and CYS891, Pi-sulfur with MET909, and Pi-alkyl with LEU715 and LEU718 (Fig. 2B).

**3.3.3.2 Chloroform extract.** Among the 42 compounds, none exhibited a lower binding affinity than that of the control, 5994 at  $-11.4$  kcal mol<sup>-1</sup>.

**3.3.3.3 Ethanolic extract.** Among the 36 compounds, compound 6876597 exhibited a binding affinity of  $-8.4$  kcal mol<sup>-1</sup> which was lower than that of the control 5994 at  $-6.9$  kcal mol<sup>-1</sup>. Compound 6876597 formed interactions such as Conventional Hydrogen Bonds with PRO696, ASP697, ARG766, TRP765, and HIS770, Pi-sigma with VAL729, Pi-alkyl with PRO696, Pi-Pi T-shaped with TRP732, Attractive Charge with GLU695 and Carbon Hydrogen Bond with LYS769 (Fig. 3).

**3.3.3.4 Flavonoids.** Among the 18 Flavonoids studied, three of them possessed binding affinities lower than the control at  $-7.7$  kcal mol<sup>-1</sup>. The first three are 5280445, 5280343, and 439246 at binding affinities of  $-9.6$ ,  $-9.5$ , and  $-9.2$  kcal mol<sup>-1</sup> respectively. The interactions formed by Compound 5280445 include Conventional Hydrogen Bonds with ASN719, LEU887, MET759, and GLN725, Pi-Pi T-shaped with PHE778 and Pi-alkyl with CYS891 and LEU763 (Fig. 4B). Compound 5280343



Table 4 Gas chromatography-mass spectroscopy (GC-MS) identified phytochemicals in PO chloroform extract (42 compounds)





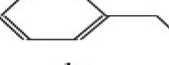

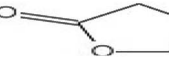
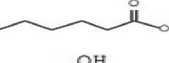
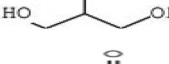



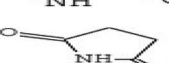


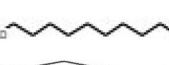



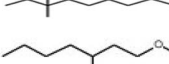
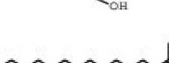


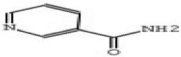

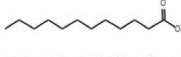
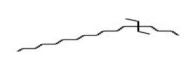
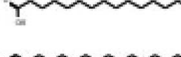
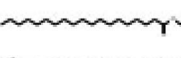
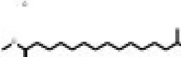


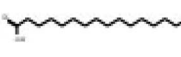
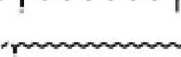
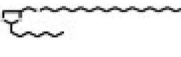

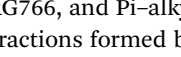
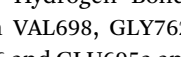

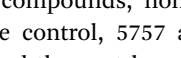
S/N	Compound	RT (min)	Area (%)	Formula	M <sub>w</sub>	Compounds classification	Structure
1	2,3-Butanediol	5.718	0.09	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>	90	Alcohol	
2	Unidentified	5.818	0.29				
3	Hexanal	5.910	0.06	C <sub>6</sub> H <sub>12</sub> O	100	Alkanal	
4	Butanoic acid, 3-methyl	6.336	0.24	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	102	Fatty acid	
5	Butanoic acid, 2-methyl	6.466	0.03	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	102	Fatty acid	
6	Ethylbenzene	6.766	0.05	C <sub>8</sub> H <sub>10</sub>	106	Hydrocarbon	
7	<i>o</i> -Xylene	6.870	0.16	C <sub>8</sub> H <sub>10</sub>	106	Hydrocarbon	
8	Unidentified	7.232	0.08				
9	Butyrolactone	7.618	0.12	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>	86	Hydrocarbon	
10	Hexanoic acid	8.371	0.60	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	116	Fatty acid	
11	Glycerin	8.503	0.33	C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>	92	Hydrocarbon	
12	2(5 <i>H</i> )-Furanone, 3-methyl	8.614	0.31	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98	Hydrocarbon	
13	Cyclohexanol, 1-methyl-4-(1-methylethenyl)	9.292	0.10	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	196	Alcohol	
14	2 <i>H</i> -Pyran-2-one, 5,6-dihydro-	9.566	0.14	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98	Alkanone	
15	2-Pyrrolidinone	10.329	0.73	C <sub>4</sub> H <sub>7</sub> NO	85	Alkanone	
16	Succinimide	10.890	0.23	C <sub>4</sub> H <sub>5</sub> NO <sub>2</sub>	99	Imide	
17	4 <i>H</i> -Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	11.236	0.10	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144	Alkanone	
18	3-Furanol, tetrahydro	11.396	1.22	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	88		
19	1-Dodecanol	11.624	0.44	C <sub>12</sub> H <sub>26</sub> O	186	Alcohol	
20	1,1,2-Trimethyl-3,8,9-trioxo-bicyclo[4.2.1]nonane	11.948	1.20	C <sub>9</sub> H <sub>16</sub> O <sub>3</sub>	172		
21	Benzeneacetic acid	12.694	0.28	C <sub>8</sub> H <sub>8</sub> O <sub>2</sub>	136	Fatty acid	
22	Phenol, 2-(4-diethylaminophenyliminoethyl)	13.294	0.24	C <sub>14</sub> H <sub>22</sub> O	206	—	
23	3-Acetamido-3-methylnonane	13.596	0.41	C <sub>12</sub> H <sub>25</sub> NO	199	Alkanone	
24	1-Methoxy-3-hydroxymethylheptane	14.110	2.44	C <sub>9</sub> H <sub>20</sub> O <sub>2</sub>	160	—	
25	3-Hexadecene, ( <i>Z</i> )	14.543	1.62	C <sub>16</sub> H <sub>32</sub>	224	Alkene	



Table 4 (Contd.)

S/N	Compound	RT (min)	Area (%)	Formula	$M_w$	Compounds classification	Structure
26	Niacinamide	15.102	0.47	$C_6H_6N_2O$	122	Amide	
27	Phenol, 2,4-bis(1,1-dimethylethyl)	16.546	2.15	$C_{14}H_{22}O$	206	—	
28	Dodecanoic acid	17.101	0.33	$C_{12}H_{24}O_2$	200	Fatty acid	
29	1-Pentadecene	17.490	2.86	$C_{15}H_{30}$	210	Alkene	
30	5,5-Dimethylheptadecane	19.790	0.31	$C_{21}H_{44}$	296	Alkene	
31	Pentadecanoic acid	19.901	0.94	$C_{15}H_{30}O_2$	242	Fatty acid	
32	1-Heptadecene	20.265	2.30	$C_{17}H_{34}$	238	Alkene	
33	Nonadecanoic acid, ethyl ester	20.524	0.90	$C_{21}H_{42}O_2$	326	Fatty ester	
34	Pentadecanoic acid, methyl ester	20.762	1.23	$C_{16}H_{32}O_2$	256	Fatty ester	
35	Pentadecanoic acid, 14-methyl, methyl ester	21.334	6.23	$C_{17}H_{34}O_2$	270	Fatty acid	
36	Pentadecanoic acid, ethyl ester	21.690	1.84	$C_{17}H_{34}O_2$	270	Fatty acid	
37	Hexadecanoic acid, ethyl ester	22.154	7.63	$C_{18}H_{36}O_2$	284	Fatty acid	
38	1-Dimethyl(3-chloropropyl)silyloxyoctadecane	22.540	0.37	$C_{23}H_{49}ClOSi$	404	Alkane	
39	n-Hexadecanoic acid	22.856	41.51	$C_{16}H_{32}O_2$	256	Fatty acid	
40	Hexadecanoic acid, 15-methyl-, methyl ester	23.089	18.51	$C_{18}H_{36}O_2$	284	Fatty acid	
41	Heptacosanoic acid, methyl ester	23.507	0.54	$C_{28}H_{56}O_2$	424	Fatty acid	
42	1,3-Dioxolane, 2-heptyl-4-octadecyloxymethyl-	23.550	0.33	$C_{29}H_{58}O_3$	454	Hydrocarbon	

formed interactions which include Conventional Hydrogen Bonds with ASN719, LEU715, MET756, and GLN725, Pi-sulfur with MET801, Pi-Pi T-shaped with PHE778 and Pi-alkyl with CYS891, MET759, and LEU763 (Fig. 4A). Compound 439246 formed interactions such as Conventional Hydrogen Bond with LEU715, Pi-Pi T-shaped with PHE778, and Pi-alkyl with CYS891 and LEU763 (Fig. 4C).

**3.3.3.5 Hexane extract.** Among the 22 compounds, none exhibited a lower binding affinity than that of the control, 5994 at  $-7.7 \text{ kcal mol}^{-1}$ .

**3.3.3.6 Methanolic extract.** Among the 36 compounds, none exhibited a lower binding affinity than that of the control, 5994 at  $-11.4 \text{ kcal mol}^{-1}$ .

**3.3.3.7 Phenols.** The Phenols studied showed a low binding affinity with PR. Two compounds namely 5280343 and 5280443 showed a low binding affinity with  $-9.5 \text{ kcal mol}^{-1}$  and  $-8.1 \text{ kcal mol}^{-1}$  respectively which are considerably much lower than the control at  $-7.2 \text{ kcal mol}^{-1}$ . A third compound 5281855 also exhibited low binding affinity at  $-7.2 \text{ kcal mol}^{-1}$ . The interactions formed by compound 5280343 include Conventional Hydrogen Bonds with GLN725 and ASN719, Pi-sulfur with MET801, Pi-Pi T-shaped with PHE778 and Pi-alkyl with MET759, CYS891 and LEU763 (Fig. 5A). The interactions formed

by compound 5280443 include Conventional Hydrogen Bonds with SER728 and GLN725, Pi-cation with ARG766, and Pi-alkyl with PRO696 and VAL698 (Fig. 5B). The interactions formed by Compound 5281855 include Conventional Hydrogen Bonds with ILE699, Carbon Hydrogen Bonds with VAL698, GLY762, and SER728, Pi-anion/Pi-cation with ARG766 and GLU695a and Pi-alkyl with PRO696 (Fig. 5C).

### 3.3.4 Screening of compounds against ER

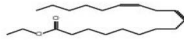


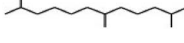



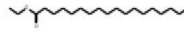

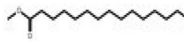
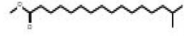
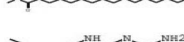
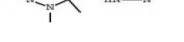
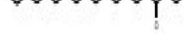
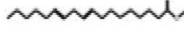
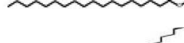



**3.3.4.1 Aqueous extract.** Among the 26 compounds, none exhibited a lower binding affinity than the control, 5757 at  $-10.9 \text{ kcal mol}^{-1}$ . Compound 10531 exhibited the next lowest binding affinity of  $-8.8 \text{ kcal mol}^{-1}$ .

**3.3.4.2 Chloroform extract.** Among the 42 compounds, none exhibited a lower binding affinity than that of the control, 5757 at  $-10.9 \text{ kcal mol}^{-1}$ . In another pose, the control also exhibited a low binding affinity at  $-8.6 \text{ kcal mol}^{-1}$ . Compound 88693 showed a binding affinity of  $-6.8 \text{ kcal mol}^{-1}$  which is considerably higher than that of the control.

**3.3.4.3 Ethanolic extract.** Compound 6876597 exhibited a binding affinity of  $-7.5 \text{ kcal mol}^{-1}$  which is lower than that of the control at  $-7 \text{ kcal mol}^{-1}$ . No other compound showed a lower binding affinity. The interactions formed by compound 6876597 are Conventional Hydrogen Bonds with PRO324 and



Table 5 Gas chromatography-mass spectroscopy (GC-MS) identified phytochemicals in PO *n*-hexane extract (22 compounds)

S/N	Compound	RT (min)	Area (%)	Formula	Molecular weight	Compounds classification	Structure
1	Linoleic acid ethyl ester	8.357	1.90	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308	Fatty ester	
2	Unidentified	8.833	3.63				
3	DLimonene	9.319	3.89	C <sub>10</sub> H <sub>16</sub>	136	—	
4	Nonane	13.013	0.16	C <sub>13</sub> H <sub>28</sub>	184	Alkane	
5	Dodecane	13.679	0.10	C <sub>15</sub> H <sub>32</sub>	212	Alkane	
6	Tetradecane	14.720	0.11	C <sub>14</sub> H <sub>30</sub>	198	Alkane	
7	Hexadecane	16.208	0.26	C <sub>16</sub> H <sub>34</sub>	226	Alkane	
8	Unidentified	16.881	0.11				
9	Octanoic acid	17.671	0.22	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	172	Fatty acid	
10	Eicosane	19.335	0.26	C <sub>20</sub> H <sub>42</sub>	282	—	
11	Octadecanoic acid	20.478	0.43	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312	Fatty acid	
12	Pentadecanoic acid, ethyl ester	20.929	0.44	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	Fatty acid	
13	Pentadecanoic acid, methyl ester	21.875	1.50	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	Fatty acid	
14	Hexadecanoic acid, 15-methyl	22.349	3.55	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	Fatty acid	
15	<i>n</i> -Hexadecanoic acid, methyl ester	22.957	1.90	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	Fatty acid	
16	1,2,4-Triazol-3-amine	23.075	0.61	C <sub>8</sub> H <sub>13</sub> N <sub>7</sub>	207	Amine	
17	Hexadecanoic acid, ethyl ester	23.318	11.96	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	Fatty ester	
18	9,12-Octadecadienoic acid, methyl ester	24.988	15.64	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	Fatty ester	
19	Methyl stearate	25.310	0.68	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298	Fatty acid	
20	9,17-Octadecadienal	25.797	1.29	C <sub>18</sub> H <sub>32</sub> O	264	Fatty acid	
21	Unidentified	26.017	48.49				
22	Octadecanoic acid	26.304	2.88	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312	Fatty acid	

GLU353, attractive charge/salt bridge with GLU323, Pi-alkyl with MET357 and PRO324, and Carbon Hydrogen Bond with PRO325 and GLY442 (Fig. 6).

**3.3.4.4 Flavonoids.** The control compound, 5757 showed better binding ability than all other compounds studied at  $-10.9$  kcal mol<sup>-1</sup>. The next compounds with a low binding affinity were 439246 and 5281607, both at  $-8.2$  kcal.

**3.3.4.5 Hexane extract.** The control compound exhibited the lowest binding affinity at  $-8.8$  kcal mol<sup>-1</sup>. The next hit with a low binding affinity was compound 610065 at  $-7.5$  kcal mol<sup>-1</sup>.

**3.3.4.6 Methanolic extract.** None of the compounds under study exhibited a lower binding affinity than 5757, the control at  $-8.6$  kcal mol<sup>-1</sup>.

**3.3.4.7 Phenols.** The control, 5757 exhibited the lowest binding affinity when compared to the phenols studied. It

exhibited a low binding affinity of  $-10.9$  kcal mol<sup>-1</sup>. Compound 5281855 exhibited the next low binding affinity at  $-9.1$  kcal mol<sup>-1</sup>.

### 3.3.5 Screening of compounds against EGFR

**3.3.5.1 Aqueous extract.** Compound 10531 exhibited the lowest binding affinity of  $-9.7$  kcal mol<sup>-1</sup> which was considerably lower than that of the control, 123631 at  $-7.9$  kcal mol<sup>-1</sup>. The interactions formed are Conventional Hydrogen Bonds with LEU694 and ASP831, Pi-sigma with VAL702 and LEU694, Pi-alkyl with LYS704 and LEU820, and Carbon Hydrogen Bonds with PHE771 and GLY772 (Fig. 7).

**3.3.5.2 Chloroform extract.** Compound 93344 exhibited a binding affinity of  $-8.2$  kcal mol<sup>-1</sup> which is considerably lower than the binding affinity of the control at  $-8$  kcal mol<sup>-1</sup>. The interactions formed by compound 93344 are Pi-anion with



Table 6 HPLC quantification of the flavonoids, phenols and saponin content in *P. ostreatus* extracts

S/N	Compounds (mg kg <sup>-1</sup> )	PO-A	PO-M	PO-E	PO-C	PO-H
<b>A</b>	<b>FLAVONOIDS</b>					
1	2,5-Dihydroxybenzoic acid	0.0014	—	0.0107*	—	0.0009
2	Caffeic acid	0.0013	—	0.0329*	—	0.0165
3	Phenylacetic acid	—	—	—	8.4189*	0.0022
4	<i>Trans</i> -cinnamic acid	66.6939*	—	—	27.7456	0.0018
5	Quercetin	—	—	—	35.3818	95.5490*
6	Naringenin	—	—	—	—	4.4120
7	Chrysin	—	—	—	—	0.0161
8	3,4-Dimethoxybenzoic acid	—	79.6578*	—	—	—
9	Gallic acid	—	0.0944	0.0066	—	—
10	4-Hydroxybenzoic acid	—	0.2174	—	—	—
11	Salicylic acid	—	0.1216	—	—	—
12	<i>p</i> -Coumaric acid	—	19.9073	—	—	—
13	Rutin hydrate	0.0009	—	0.0308	—	—
14	<i>O</i> -Coumaric acid	0.0011	—	0.0231	—	—
15	Benzoic acid	—	—	76.1210	—	—
16	Chlorogenic acid	33.2979	—	23.6395	—	—
17	Luteolin	—	—	0.0594	—	—
18	4-Methoxycinnamic acid	0.0012	—	—	—	—
19	Unidentified	0.0012	—	—	—	—
20	Unidentified	0.0011	—	—	—	—
21	Unidentified	—	—	—	0.0185	—
22	Unidentified	—	—	—	—	0.0015
23	Unidentified	—	—	0.0291	—	—
24	Unidentified	—	—	0.0280	—	—
<b>B</b>	<b>PHENOLS</b>					
1	Gallic acid	—	0.0932	0.0010	—	0.0128
2	Syringic acid	59.1016	76.2015	12.0117	36.6614	49.0412
3	Ferulic acid	—	—	—	24.8694	21.2843
4	Quercetin	40.8968	22.7721	—	38.4585	17.9785
5	Apigenin	—	0.9332	—	—	11.3508
6	Caffeic acid	0.0016	—	—	—	—
7	Vanillic acid	—	—	69.9143	—	—
8	Ellagic acid	—	—	18.0513	—	—
9	Gallic acid	—	0.0932	0.0010	—	0.0128
<b>C</b>	<b>SAPONINS</b>					
1	Saponin	168.9560*	59.0371	17.5690	34.3375	16.5521
2	Unidentified	30.8881	0.0257	0.0019	1.0807	0.0073
3	Unidentified	0.1474	18.1823	0.0285	1.0833	0.2756
4	Unidentified	0.0009	22.7383	44.9442	13.4500	4.1655
5	Unidentified	0.0003	—	17.6892	0.0130	8.8328
6	Unidentified	0.0012	—	1.1126	0.0032	0.0092
7	Unidentified	0.0003	—	18.6404	0.0023	0.0180
8	Unidentified	0.0003	—	—	0.0020	0.0339
9	Unidentified	0.0007	—	—	0.0018	0.0198
10	Unidentified	0.0005	—	—	0.0040	0.0152
11	Unidentified	0.0017	—	—	0.0020	0.0207
12	Unidentified	—	—	—	0.0008	0.0256a
13	Unidentified	—	—	—	0.0034	0.0067
14	Unidentified	—	—	—	0.0016	0.0064
15	Unidentified	—	—	—	0.0028	0.0034

Table 7 The proteins studied and their description

Protein	Substrate/inhibitor	PDB ID
Progesterone receptor (PR)	Progesterone; 5994	1A28
Estrogen receptor (ER)	Estradiol; 5757	1ERE
Epidermal growth factor receptor (EGFR)	Geftinib; 123631	1M17

ASP831, Pi-sigma with LEU820, Pi-Pi stacked with PHE699, and alkyl/Pi-alkyl with LEU694 and VAL702 (Fig. 8).

**3.3.5.3 Ethanol extract.** No compound exhibited a lower binding affinity than that of the control, 123631 at  $-7.9$  kcal mol<sup>-1</sup>.

**3.3.5.4 Flavonoids.** Four compounds showed a better binding affinity than that of the control at  $-7.8$  kcal mol<sup>-1</sup>. These compounds include 5280343, 5280445, 5280805, and



Table 8 The binding affinity of top ligands targeting PR

	Compound ID	Compound name	Binding affinity (kcal mol <sup>-1</sup> )
Aqueous extract	10531	Ergotaman-3',6',18-trione	-8.1
	565346	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine	-7.8
	Control: 5994	Progesterone	-7.7
Chloroform extract	Control: 5994	Progesterone	-11.4
Ethanol extract	6876597	<i>o</i> -Tolylamino-acetic acid (4-nitro-benzylidene)-hydrazide	-8.4
	Control: 5994	Progesterone	-6.9
Flavonoids	5280445	Luteolin	-9.6
	5280343	Apigenin	-9.5
	439246	Naringenin	-9.2
	Control: 5994	Progesterone	-7.7
Hexane extract	Control: 5994	Progesterone	-7.7
Methanolic extract	Control: 5994	Progesterone	-11.4
Phenols	5280343	Apigenin	-9.5
	5280443	Quercetin	-8.1
	5281855	Ellagic acid	-7.2
	Control: 5994	Progesterone	-7.2

Table 9 The binding affinity of top ligands targeting ER

	Compound ID	Compound name	Binding affinity (kcal mol <sup>-1</sup> )
Aqueous extract	Control: 5757	Estradiol	-10.9
Chloroform extract	Control: 5757	Estradiol	-10.9
Ethanol extract	6876597	<i>o</i> -Tolylamino-acetic acid (4-nitro-benzylidene)-hydrazide	-7.5
	Control: 5757	Estradiol	-7
Flavonoids	Control: 5757	Estradiol	-10.9
Hexane extract	Control: 5757	Estradiol	-8.8
Methanolic extract	Control: 5757	Estradiol	-8.6
Phenols	Control: 5757	Estradiol	-10.9

Table 10 The binding affinity of top ligands targeting EGFR

	Compound ID	Compound name	Binding affinity (kcal mol <sup>-1</sup> )
Aqueous extract	10531	Ergotaman-3',6',18-trione	-9.7
	Control: 123631	Gefitinib	-7.9
Chloroform extract	93344	Phenol, 2,4-bis(1,1-dimethyl ethyl)	-8.2
	Control: 123631	Gefitinib	-8
Ethanol extract	Control: 123631	Gefitinib	-7.9
Flavonoids	5280343	Quercetin	-8.5
	5280445	Luteolin	-8.4
	5280805	Rutin	-8.4
	5281607	Chrysin	-7.8
	Control: 123631	Gefitinib	-7.8
	Control: 123631	Gefitinib	-7.7
Hexane extract	Control: 123631	Gefitinib	-7.7
Methanolic extract	Control: 123631	Gefitinib	-7.7
Phenols	5281855	Ellagic acid	-8.8
	5280343	Apigenin	-8.5
	5280443	Quercetin	-7.8
	Control: 123631	Gefitinib	-7.8

5281607 at best binding affinities of -8.5, -8.4, -8.4, and -7.8 kcal mol<sup>-1</sup> respectively. Compound 5280343 formed interactions which include Conventional Hydrogen Bond with MET769, GLN767, GLU738, MET742 and ASP831, Pi-cation with LYS721, Pi-sigma with LEU820 and LEU694, sulfur-X with

MET742 and Pi-alkyl with VAL702, LEU820 and ALA719 (Fig. 9A). Compound 5280445 formed interactions which include MET742 and MET769, Pi-sigma with VAL702, LEU820 and LEU694, and Pi-alkyl with LYS721, ALA719, and VAL702 (Fig. 9B). Compound 5280805 formed interactions which



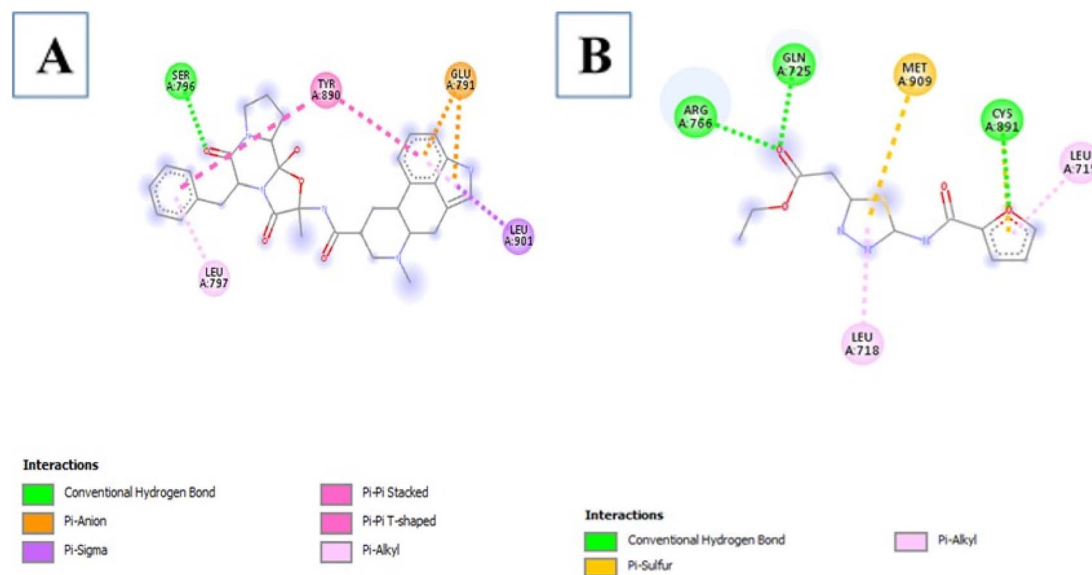


Fig. 2 The intermolecular interactions between 10531 (A); 565346 (B) and PR.

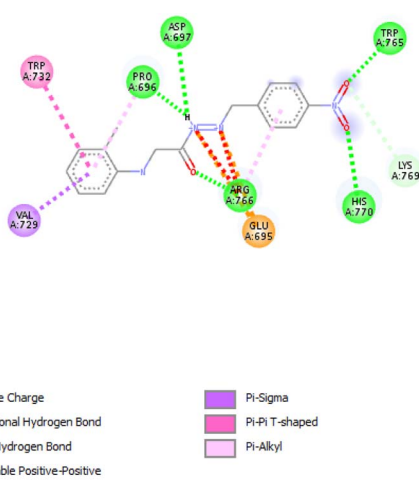


Fig. 3 The intermolecular interactions between 6876597 and PR.

include Conventional Hydrogen Bonds with CYS773, LYS721, and LEU764, Pi-Pi stacked with PHE699, Pi-anion with ASP831 and Pi-alkyl with VAL702 and ALA719 (Fig. 9C). Compound 5281607 formed interactions which include Pi-cation with LYS721, Pi-sulfur with MET742, Pi-sigma with VAL702 and LEU820, and Pi-alkyl with LEU694, ALA719 and VAL702 (Fig. 9D).

**3.3.5.5 Hexane extract.** Among the 22 compounds, none exhibited a lower binding affinity than that of the control, 123631 at  $-7.7$  kcal mol $^{-1}$ .

**3.3.5.6 Methanolic extract.** Among the 36 Compounds, none exhibited a lower binding affinity than that of the control, 123631 at  $-7.7$  kcal mol $^{-1}$ .

**3.3.5.7 Phenols.** The control compound, 123631 exhibited a low binding affinity at  $-7.8$  kcal mol $^{-1}$ . Three compounds

showed a better binding affinity than that of the control. These compounds are 5281855, 5280343, and 5280443 at binding affinity of  $-8.8$ ,  $-8.5$ , and  $-7.8$  kcal mol $^{-1}$ . Compound 5281855 formed interactions which include Conventional Hydrogen Bonds with ASP831, LYS721, GLU738, THR766 and MET769, Pi-sigma with VAL702 and LEU820, and Pi-alkyl with LEU694, ALA719, VAL702, LEU820 and LYS721 (Fig. 10A). Compound 5280343 formed interactions which include Conventional Hydrogen Bond GLN767 and MET769, sulfur-X with MET7422, Pi-cation with LYS721, Pi-sigma with LEU694 and LEU820 and Pi-alkyl with VAL702, LEU820 and ALA719 (Fig. 10B). Compound 5280443 formed interactions which include Conventional Hydrogen Bond with MET769, Pi-sigma with VAL702, LEU820, and LEU694, and Pi-alkyl with LYS721, VAL702 and ALA719 (Fig. 10C).

**3.3.6 Pharmacological properties of compounds.** The pharmacological properties of the selected hit compounds are shown in Table 11. Positive results were observed for blood-brain barrier (BBB) penetrability for all compounds except 5281607 of the Flavonoids group. A high GI absorption was predicted for all compounds except 5280805 which reported a low GI absorption. 10531, 93344, 439246 and 5280805 were the only P-glycoprotein substrates among these hits and no inhibitor was recorded among these non-substrates (Table 11).

**3.3.7 Drug likeness of compounds.** Eight (8) of the selected hits fully adhered to the drug-likeness rule according to Lipinski, Veber, Ghose, Egan and Muegge. All other compounds had at least one violation (Table 12).

**3.3.8 Physiochemical properties of compounds.** The physiochemical properties of the selected hits shown in Table 13 impact the drug-likeness of each compound.

**3.3.9 Cytochrome P450 (CYP) potentials of compounds.** The cytochrome P450 isoforms inhibitory properties of the selected hits are contained in Table 14. The results showed that with the exception of 5280805 and 565346, all other compounds



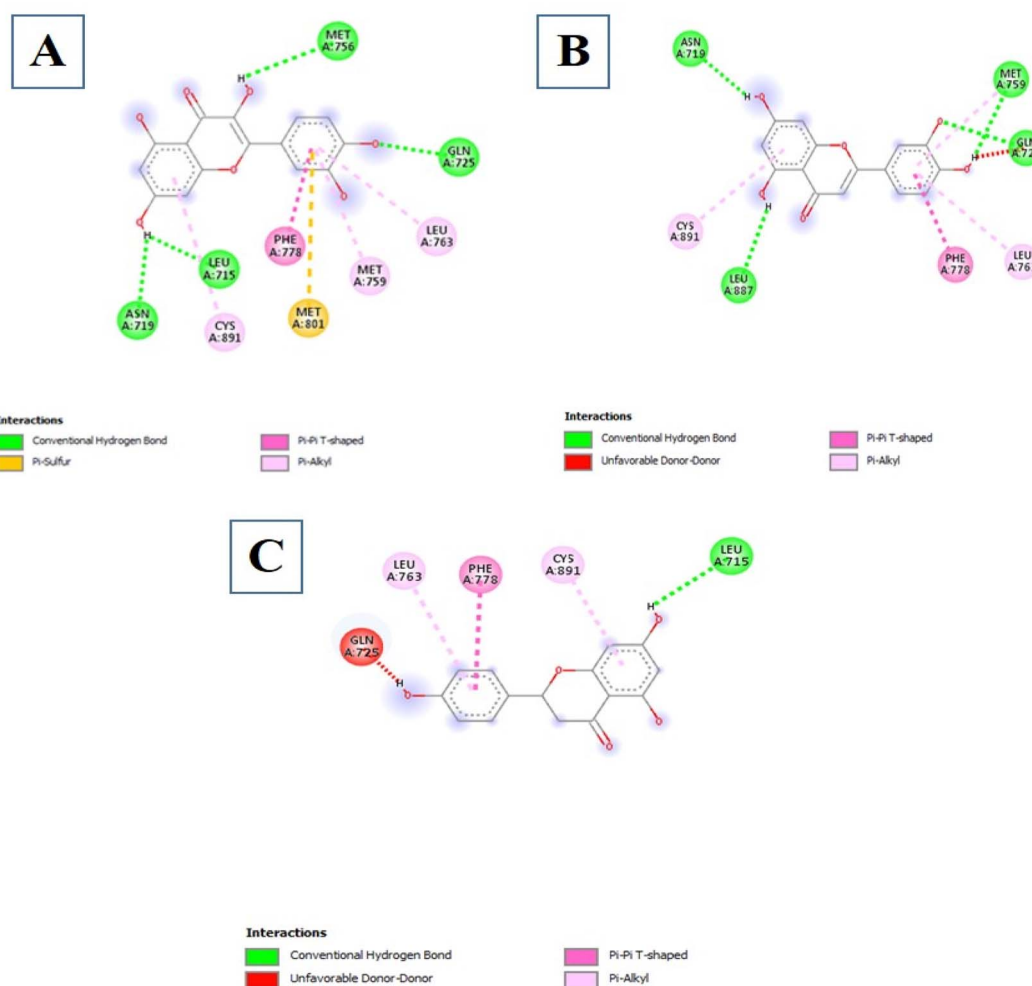


Fig. 4 The intermolecular interactions between 5280343 (A); 5280445 (B); 439246 (C) and PR.

exhibited inhibitory properties of the isoforms of cytochrome P450.

## 4 Discussion

*P. ostreatus* is highly nutritious and medicinal mushroom that contains a wide variety of bioactive compounds which may possess anti-cancer properties.<sup>28</sup> The isolation and identification of these bioactive compounds using a combination of GC-MS and HPLC provides an opportunity to explore their anti-cancer potential and mechanisms of actions. As a result, this study was carried out to identify the bioactive compounds in *P. ostreatus* using GC-MS and HPLC and evaluate their inhibition potential on breast cancer receptors *in silico*.

### 4.1 GC-MS profile of *P. ostreatus* bioactive compounds and their biological activities

Gas chromatography-mass spectrometry (GC-MS) is a valuable analytical tool for profiling and studying secondary metabolites in a variety of biological samples.<sup>41</sup> The GC-MS profile of the phytocompounds from the five extracts of *P. ostreatus* revealed that chloroform solvent extracted more versatile compounds

compared to other solvents. This is similar to the reports by Effiong *et al.*<sup>42</sup> Overall the GC-MS identified bioactive compounds in *P. ostreatus* have been found to possess numerous medicinal and biological significance ranging from anti-tumor, antioxidant, anti-cancer, anti-diabetic, anti-microbial, anti-inflammatory, immunomodulatory, anti-malarial, probiotic, neuroprotective, anti-tuberculosis, *etc.*

Antioxidant compounds perform the role of removing free radicals that cause oxidative damage to cells and tissues. They can combat oxidative stress, protect cells from injury, maintain cellular integrity, regulate growth, and aid in immune function which plays a significant role in preventing and managing breast cancer development.<sup>43</sup> The identified antioxidant compounds in *Pleurotus ostreatus* extracts were 4*H*-pyran-4-one, furanones,<sup>44,45</sup> cyclohexanecarboxylic acid, pentadecanoic acid, hexadecanoic acid, dodecanoic acid, heptacosanoic acid,<sup>46,47</sup> 2-pyrrolidinone;<sup>48</sup> niacinamide; niacin;<sup>49</sup> 1,2,4-triazol-3-amine;<sup>50</sup> acetamide;<sup>51</sup> propanamide;<sup>52</sup> pyrrolizine derivatives,<sup>53</sup> piracetam;<sup>54</sup> D-limonene<sup>55</sup> and eicosane.<sup>56</sup>

Anti-inflammatory compounds were also identified in the extracts of *P. ostreatus*. These compounds includes 4*H*-pyran-4-one, furanones,<sup>44</sup> pyrazine, thiadiazole;<sup>57,58</sup> acetamide;<sup>59</sup>



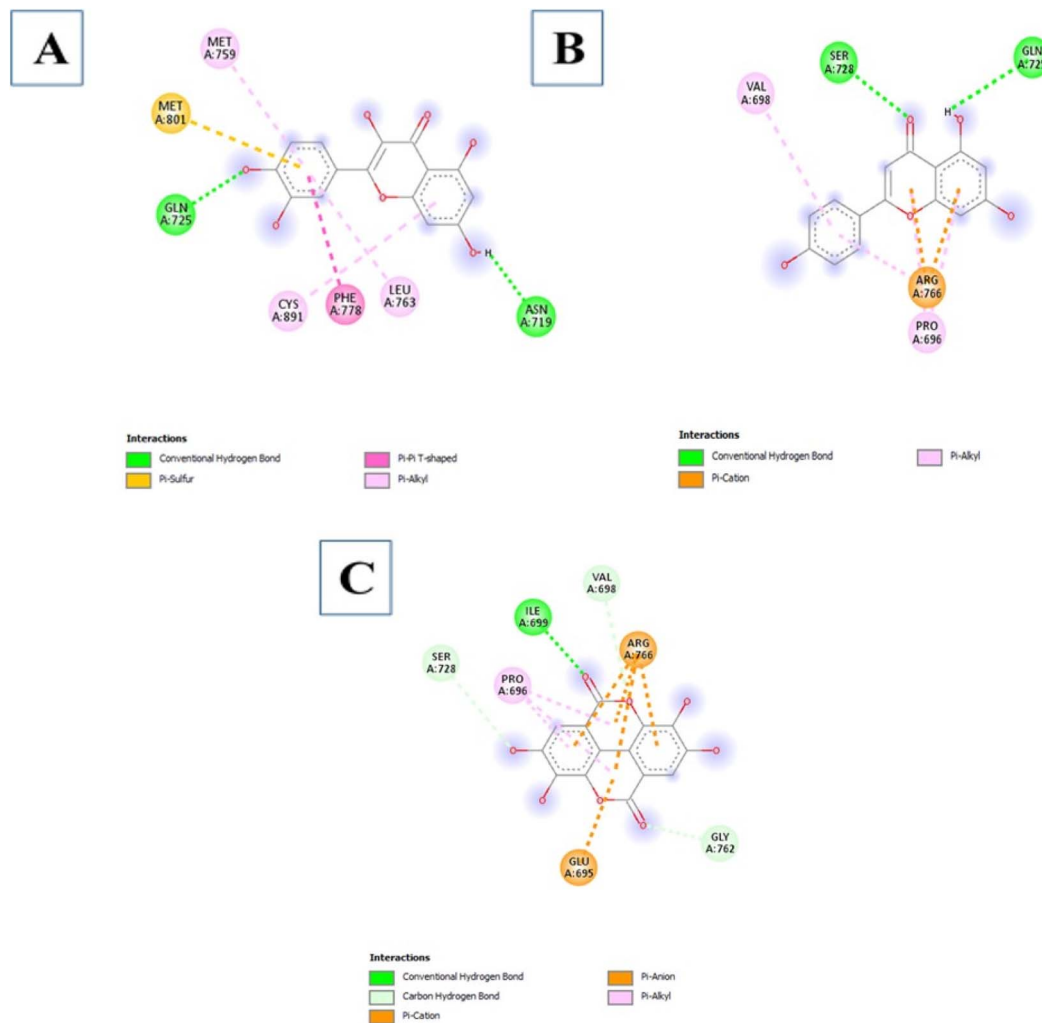


Fig. 5 The intermolecular interactions between 5280343 (A); 5280443 (B); 5281855 (C) and PR.

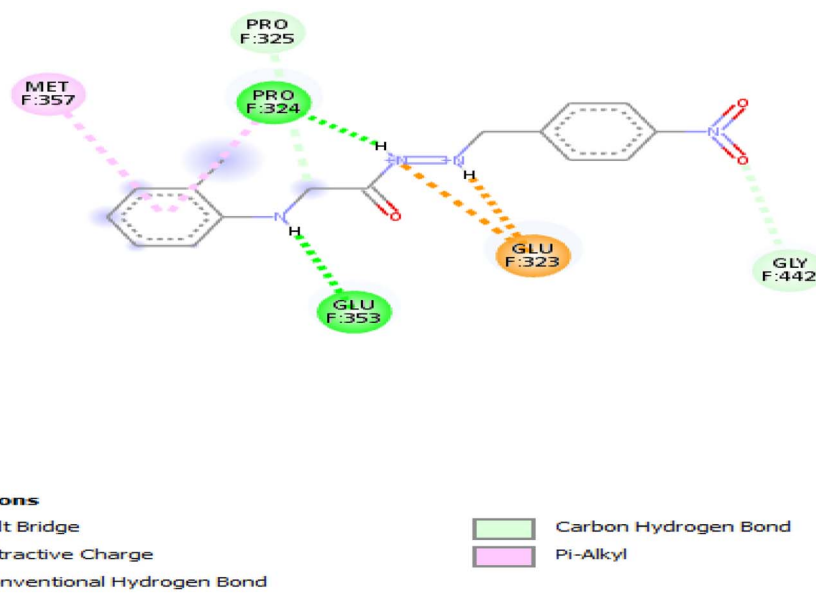


Fig. 6 The intermolecular interactions between 6876597 and ER.



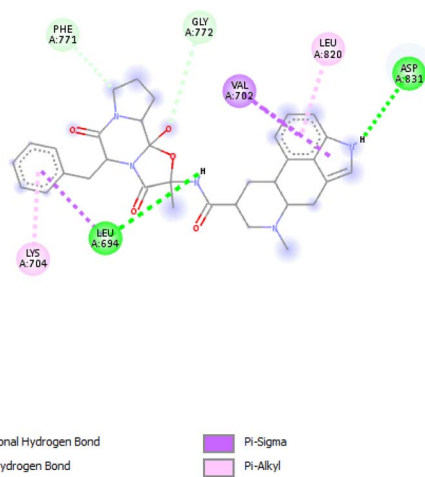


Fig. 7 The intermolecular interactions between 10531 and EGFR.

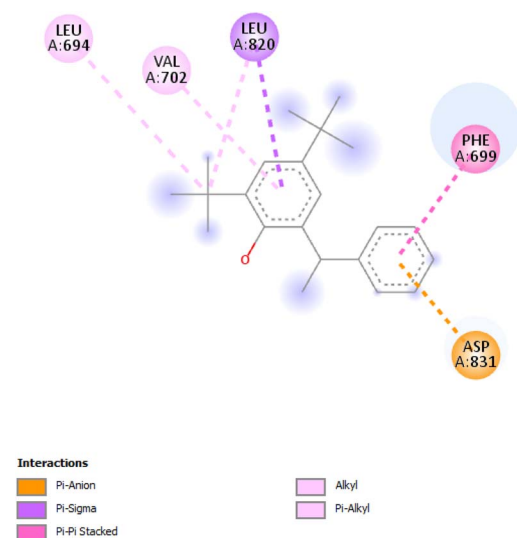


Fig. 8 The intermolecular interactions between 93344 and EGFR.

succinamide<sup>60</sup> and pyrrolizine,<sup>61</sup> D-limonene<sup>62</sup> and eicosane.<sup>63</sup> These compounds functioning as anti-inflammatory chemicals help to prevent breast cancer by modulating inflammatory pathways. They reduce inflammation, prevent pro-inflammatory cytokine production, and scavenge free radicals. They also influence the hormonal pathways and cellular mechanisms that lead to the progression of breast cancer.<sup>64</sup>

Compounds with anti-proliferative, anti-cancer and anti-tumor properties were identified among the GC-MS profile of *P. ostreatus*. These compounds include 2,3-butanediol,<sup>65</sup> 1-dodecanol,<sup>66</sup> 4H-pyran-4-one, furanones, succinimide,<sup>67</sup> propanamide,<sup>68</sup> pyrrolizine,<sup>69</sup> acetamide,<sup>51</sup> butyrolactone,<sup>70</sup> piperazine,<sup>71</sup> heptane,<sup>72</sup> pyrazine,<sup>73</sup> pyrrole,<sup>74</sup> thiadiazole,<sup>75</sup> fumaric acid,<sup>76</sup> linoleic acid ethyl ester,<sup>77</sup> methoxyacetic acid,<sup>78</sup> phenol;<sup>79</sup> D-limonene;<sup>80</sup> eicosane<sup>81</sup> and 1,2,4-triazol-3-amine.<sup>82</sup> These compounds can combat cancer initiation and progression by preventing cancer cells from proliferating, dividing, and spreading uncontrolled. They can induce apoptosis and inhibit

angiogenesis, preventing breast cancer initiation, progression, and metastasis.<sup>83</sup>

Immunomodulatory compounds were identified among the GC-MS profile of *P. ostreatus* extracts. These compounds includes succinimide,<sup>84</sup> propanamide,<sup>85</sup> D-limonene,<sup>86,87</sup> fumaric acid and its derivatives.<sup>87</sup> These compounds enhance the immune system's response to cancer cells, aid in tumor detection and eradication, hence lowering breast cancer growth and progression. Compounds with anti-diabetic properties were also identified. These compounds includes pyrazine,<sup>88</sup> pyrrolizine;<sup>53</sup> pyrrole;<sup>74</sup> phenol;<sup>79</sup> dodecanoic acid;<sup>89</sup> D-limonene,<sup>90</sup> acetamide<sup>91</sup> and eicosane.<sup>56</sup> These compounds functions in breast cancer prevention and management by addressing diabetes and obesity, which are risk factors of breast cancer. They target insulin resistance, hyperglycemia, and inflammation leading to a lower risk of breast cancer.<sup>92</sup>

Antimicrobial compounds have been demonstrated to lower breast cancer risk by altering the microbiome and immune response.<sup>93</sup> The GC-MS profile of *P. ostreatus* revealed the presence of various compounds with antimicrobial properties. These compounds includes fumaric,<sup>94</sup> dodecanoic acid, nonadecanoic acid, ethyl ester,<sup>95</sup> heptacosanoic acid, methyl ester,<sup>96</sup> acetic acid,<sup>97</sup> heptane,<sup>98</sup> nonane,<sup>99</sup> hexanal,<sup>100</sup> propanol,<sup>101</sup> furanone,<sup>102</sup> phenol,<sup>79</sup> 2-pyrrolidinone,<sup>69</sup> furanone,<sup>103</sup> propanamide,<sup>104</sup> pyrazine,<sup>73</sup> piperazine,<sup>105</sup> pyrrolizine,<sup>106</sup> pyrrole,<sup>74</sup> D-limonene<sup>107</sup> and eicosane.<sup>108</sup> These compounds indirectly aid in breast cancer prevention by lowering chronic inflammation, promoting hormonal balance, and strengthening the body's natural defenses against malignant growth.<sup>83</sup>

#### 4.2 HPLC profile of *P. ostreatus*

HPLC has high sensitivity, resolution, and reproducibility, making it possible to identify and quantify various classes of bioactive compounds such as phenols, flavonoids, saponins, among others.<sup>109</sup> The HPLC profile of *P. ostreatus* revealed the presence of numerous unidentified saponins. The aqueous extract had the highest saponin content, followed by the methanol, chloroform, ethanol and hexane extracts. The presence of saponins suggests the ability of these extracts to prevent cancer cell proliferation by interrupting the cell cycle and triggering apoptosis. Although the specific mechanisms of action is not clearly understood with little research into their effects on breast cancer.

Phenols are a class of bioactive molecules that have the potential to prevent and treat breast cancer. These bioactive compounds contain antioxidant and anti-inflammatory properties, which reduce oxidative stress, protect DNA from damage, and prevent cancer cell proliferation.<sup>83</sup> They also influence hormone signaling pathways, potentially lowering the risk of hormone receptor-positive breast cancer.<sup>110,111</sup> The HPLC profile of *P. ostreatus* extracts revealed the presence of the following phenolic compounds: gallic acid, syringic acid, ferulic acid, quercetin, apigenin, caffeic acid, vanillic acid and ellagic acid. Syringic acid was found to be the most abundant phenolic compound present in all the extracts of *P. ostreatus*. It targets distinct pathways in cancer cells, increasing DNA repair,



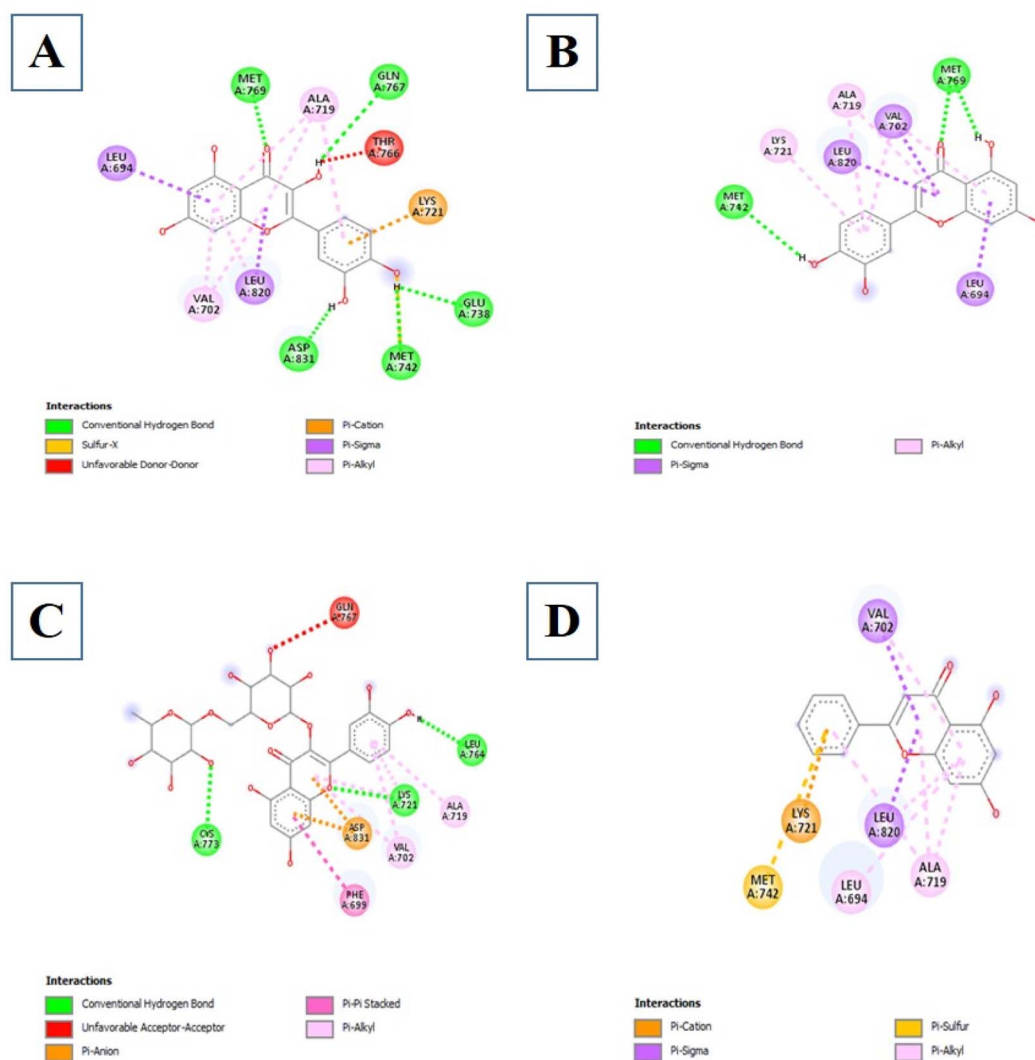


Fig. 9 The intermolecular interactions between 5280343 (A); 5280445 (B); 5280805 (C); 5281607 (D) and EGFR.

apoptosis and reducing angiogenesis.<sup>112</sup> It was highest in the methanolic extract, followed by the aqueous, *n*-hexane, chloroform and ethanolic extract. Ferulic acid was also identified in the non-polar extracts of *P. ostreatus*, with the chloroform extract having higher ferulic acid content compared to the *n*-hexane extract. The presence of ferulic acid shows the ability of these extracts to inhibit enzymes involved in proliferation and reduce NF- $\kappa$ B activity.<sup>113</sup> Caffeic acid interferes with estrogen signaling, potentially impacting hormone-receptor positive breast cancers.<sup>114</sup> It was only found to be present in the aqueous extract of *P. ostreatus*. Ellagic acid is an ellagitannin that inhibits various signaling pathways involved in cancer cell proliferation and survival. It also improves DNA repair and inhibits enzymes that enable cancer cells to evade cell death.<sup>115</sup> Ellagic and vanillic acids was found to be present only in the ethanol extract. Quercetin and apigenin functions as antioxidants, protecting cells from free radical damage, which can result in cancer growth. They also inhibit enzymes that promote cell proliferation and induce apoptosis.<sup>116</sup> Angenin was found to

be present in the hexane and methanol extracts, with higher amounts in the hexane extract compared to the methanol extract. However, quercetin was present in all extracts except the ethanol extract, following the trend of aqueous > chloroform > methanol and hexane extracts.

Flavonoids have antioxidant qualities that neutralize free radicals, which can damage cells and promote cancer growth.<sup>117</sup> They regulate inflammation, cell cycle progression, inhibit cell division enzymes, and modulate estrogen signaling. They also have anti-angiogenic properties, which prevent the formation of new blood vessels necessary for tumor growth.<sup>83</sup> The HPLC profile of *P. ostreatus* extracts revealed the presence of 2,5-dihydroxybenzoic acid, caffeic acid, phenylacetic acid, *trans*-cinnamic acid, quercetin, naringenin, chrysin, 3,4-dimethoxybenzoic acid, gallic acid, 4-hydroxybenzoic acid, salicylic acid, *p*-coumaric acid, rutin hydrate, *o*-coumaric acid, benzoic acid, chlorogenic acid, luteolin, 4-methoxycinnamic acid and other six unidentified flavonoid compounds. Quercetin was found to be the most abundant flavonoid in the *n*-hexane extract,



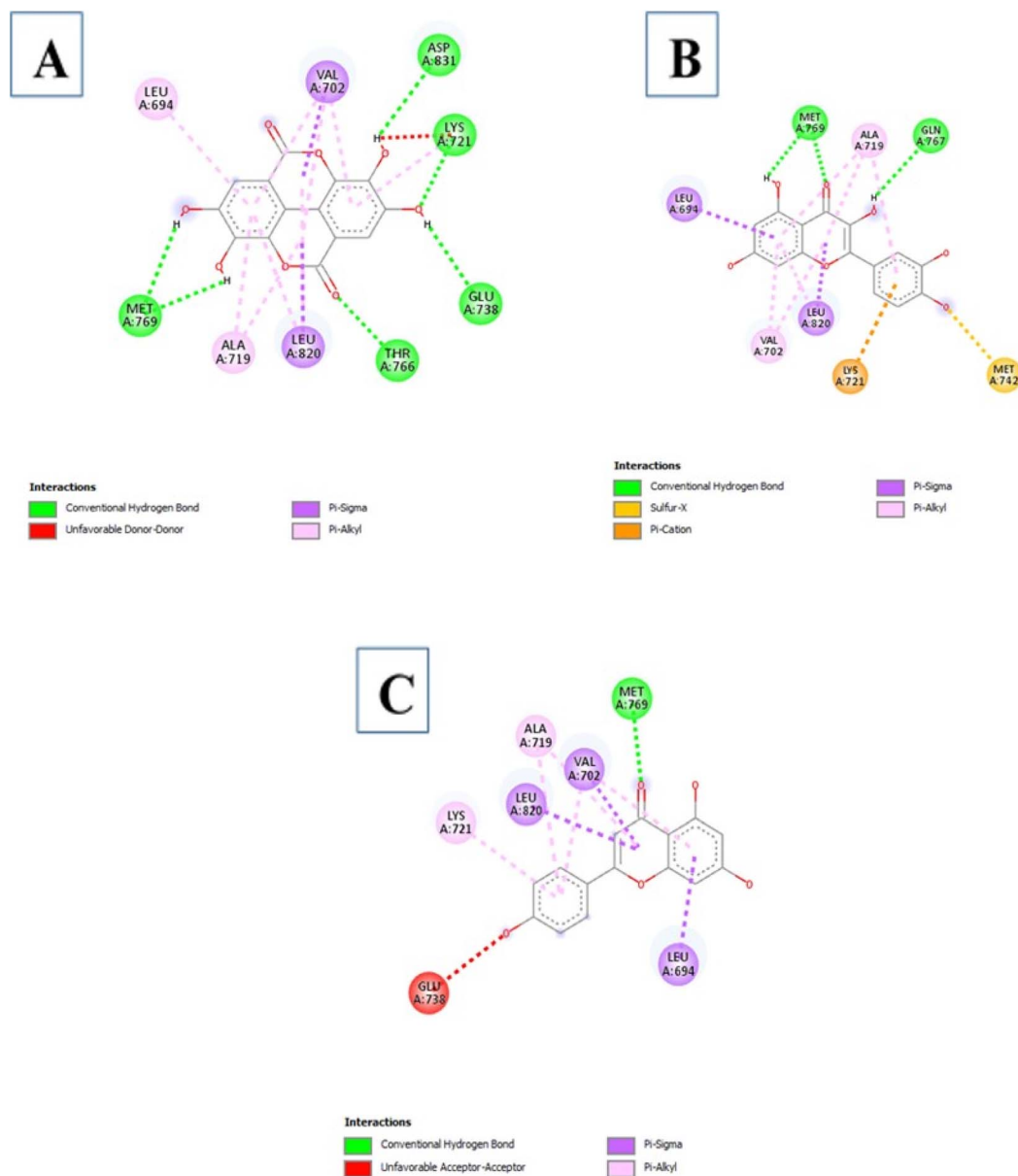


Fig. 10 The intermolecular interactions between 5281855 (A); 5280343 (B); 5280443 (C) and EGFR.

Table 11 Pharmacokinetic properties of selected hits

	Compounds	Water solubility (ESOL)	Lipophilicity (iLOGP)	GI absorption	BBB permeation	P-glycoprotein substrate
Aqueous extract	10531	−4.88 (moderately soluble)	3.31	High	No	Yes
	565346	−2.22 (soluble)	1.62	High	No	No
Chloroform extract	93344	−6.40 (poorly soluble)	4.01	High	No	Yes
Ethanol extract	6876597	−3.64 (soluble)	2.03	High	No	No
Flavonoids	5280445	−3.71 (soluble)	1.86	High	No	No
	5280343	−3.16 (soluble)	1.63	High	No	No
	439246	−3.49 (soluble)	1.75	High	No	Yes
	5280805	−3.30 (soluble)	1.58	Low	No	Yes
	5281607	−4.19 (moderately soluble)	2.27	High	Yes	No
	5280343	−3.16 (soluble)	1.63	High	No	No
Phenols	5280443	−3.94 (soluble)	1.89	High	No	No
	5281855	−2.94 (soluble)	0.79	High	No	No



Table 12 Drug likeness of Selected Hits<sup>a</sup>

	Compounds	Lipinski	Ghose	Veber	Egan	Muegge
Aqueous extract	10531	No; 1 violation	No; 3 violation	Yes	Yes	No; 1 violation
	565346*	Yes	Yes	Yes	Yes	Yes
Chloroform extract	93344	Yes	No; 1 violation	Yes	No; 1 violation	No; 2 violations
Ethanol extract	6876597*	Yes	Yes	Yes	Yes	Yes
Flavonoids	5280445*	Yes	Yes	Yes	Yes	Yes
	5280343*	Yes	Yes	Yes	Yes	Yes
	439246*	Yes	Yes	Yes	Yes	Yes
	5280805	No; 3 violations	No; 4 violations	No; 1 violation	No; 1 violation	No; 4 violations
	5281607*	Yes	Yes	Yes	Yes	Yes
	5280343*	Yes	Yes	Yes	Yes	Yes
Phenols	5280443*	Yes	Yes	Yes	Yes	Yes
	5280443*	Yes	Yes	Yes	Yes	Yes
	5281855	Yes	Yes	No; 1 violation	No; 1 violation	Yes

<sup>a</sup> Compound\* – compounds with no violation of the drug likeness rules.

followed by naringenin and other flavonoids in minute quantities. *Trans*-cinnamic and chlorogenic acid were the most abundant flavonoids in the aqueous extract. 3,4-Dimethoxybenzoic and *p*-coumaric acid were the most abundant flavonoids in the methanolic extract, whereas, benzoic and chlorogenic acid were the most abundant flavonoid in the ethanolic acid. In the chloroform extract, quercetin and *trans*-cinnamic acid were the most abundant, phenylacetic acid was also found to be present in small quantities.

### 4.3 *In silico* evaluation of the anti-cancer effects of *P. ostreatus* on breast cancer receptors

Breast cancer receptors play a crucial role in breast cancer development and progression. They aid in identifying the molecular subtype of breast cancer, tumor behavior and therapy response.<sup>118</sup> Hormone receptor-positive breast cancers, ER+ and PR+, respond well to hormonal therapy, while HER2-positive breast cancers benefit from targeted therapies combined with chemotherapy. HER2-positive breast cancers necessitate HER2-targeted therapies, whereas triple-negative breast cancers lack these receptors.<sup>119</sup> Understanding the roles of these receptors has led to the development of personalized therapies and exploration of natural phytochemicals that have improved survival rates and quality of life for many patients.<sup>120</sup>

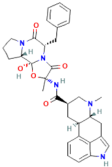
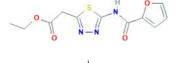
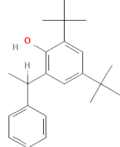
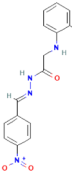
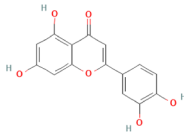
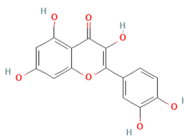
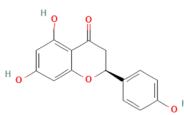
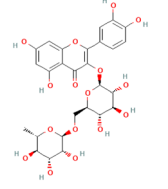
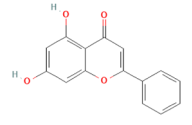
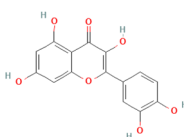
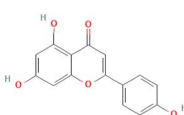
**4.3.1 Effects of *P. ostreatus* on ER+ breast cancer.** ER+ breast cancer is identified by the presence of estrogen receptors on the cells, which activate downstream signaling pathways that promote cell growth, proliferation, and survival. This estrogen-dependent signaling system is essential to tumor growth and progression.<sup>119</sup> It may interact with other biological systems, hence boosting tumor growth and metastasis. Targeting the estrogen receptor system with inhibitors is an important part of treating ER-positive breast cancer. The *in silico* evaluation of bioactive compounds in *P. ostreatus* revealed that only one bioactive compound in the ethanolic extract among the 188 compounds analyzed had a better binding affinity to estrogen receptor when compared to the control (Estradiol). *o*-Tolylamino-acetic acid (4-nitro-benzylidene)-hydrazide (compound 6876597) in the ethanolic extract exhibited a better binding affinity to estrogen receptor compared to

estradiol as the control. The compound was found to have the ability to inhibit CYP1A1 and CYP2C9 but not CYP19, CYP2D6 and CYP3A4. The ability of the compound to inhibit CYP1A1 and CYP2C9 may have benefits on estrogen metabolism and beyond, such as reducing the activation of harmful environmental chemicals that might cause cancer.<sup>121,122</sup> The compound was also found to possess 7 rotatable bonds, 7 hydrogen acceptors and 2 H-bond donors, making it highly water soluble. Its pharmacokinetic properties showed that it has a high gastrointestinal tract absorption with no blood-brain barrier penetration and glycoprotein binding which implies that is a promising therapeutic candidate. These characteristics can result in greater bioavailability, fewer side effects, lower drug interaction risk, and more predictable pharmacokinetics, all of which contribute to safer and more effective medications.<sup>123</sup>

**4.3.2 Effects of *P. ostreatus* on PR+ breast cancer.** PR+ breast cancer is characterized by the presence of progesterone receptors on the surface of cancer cells. These receptors initiate downstream signaling pathways that promote cell growth, proliferation, and survival.<sup>124</sup> This activation leads PR+ tumors to develop and spread in the same way that ER+ breast cancer does. The signaling system may interact with other biological pathways, encouraging tumor growth and metastasis.<sup>119</sup> Targeting the progesterone receptor system with hormone therapy, which are usually combined with estrogen-targeted drugs, is an essential technique for treating PR+ breast cancer. Identifying inhibitors of PR is crucial for effective treatments. The *in silico* evaluation of the bioactive compounds in the various extracts in *P. ostreatus* showed total of nine compounds were better inhibitors of PR. These compounds consists of three flavonoid compounds (luteolin, apigenin, naringenin), three phenol compounds (apigenin, quercetin and ellagic acid), two aqueous extract compounds (10531 and 565346) and one compound in the ethanolic extract (6876597) could bind better to the progesterone receptor compared to the control (progesterone). Of the two compounds in the aqueous extract, compound 10531 showed the ability to inhibit CYP2D6 and CYP3A4, while compound 565346 did not show any inhibition on the enzymes. Compound 10531 was found to be moderately soluble with a high gastrointestinal tract and glycoprotein binding but no



Table 13 Physicochemical properties of selected hits

	Compounds	2D structure	Molecular weight	Num. Rotatable bonds	Num. H-bond acceptors	Num. H-bond donors
Aqueous extract	10531		583.68	5	6	3
	565346		281.29	7	6	1
Chloroform extract	93344		310.47	4	1	1
Ethanol extract	6876597		312.32	7	4	2
Flavonoids	5280445		286.24	1	6	4
	5280343		302.24	1	7	5
	439246		272.25	1	5	3
	5280805		610.52	6	16	10
	5281607		254.24	1	4	2
	5280343		302.24	1	7	5
Phenols	5280443		270.24	1	5	3

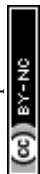


Table 13 (Contd.)

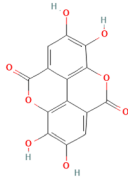
Compounds	2D structure	Molecular weight	Num. Rotatable bonds	Num. H-bond acceptors	Num. H-bond donors
5281855		302.19	0	8	4

Table 14 CYP potentials of the selected hits<sup>a</sup>

	Compounds	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Aqueous extract	10531	No	No	No	Yes	Yes
	<b>565346*</b>	<b>No</b>	<b>No</b>	<b>No</b>	<b>No</b>	<b>No</b>
Chloroform extract	93344	No	Yes	No	Yes	No
Ethanol extract	6876597	Yes	No	Yes	No	No
Flavonoids	5280445	Yes	No	No	Yes	Yes
	5280343	Yes	No	No	Yes	Yes
	439246	Yes	No	No	No	Yes
	<b>5280805*</b>	<b>No</b>	<b>No</b>	<b>No</b>	<b>No</b>	<b>No</b>
	5281607	Yes	No	No	Yes	Yes
Phenols	5280343	Yes	No	No	Yes	Yes
	5280443	Yes	No	No	Yes	Yes
	5281855	Yes	No	No	No	No

<sup>a</sup> Compound\* – compounds with no inhibition on all the CYP isoforms.

blood–brain barrier crossing. However, compound 565346 possessed only a high gastrointestinal tract binding but no blood–brain barrier crossing and glycoprotein binding. Compound 565346 looks to be a more promising treatment candidate than compound 10531 due to its lack of glycoprotein binding, which may result in fewer side effects and more predictable pharmacological action.<sup>123</sup> However, Compound 565346's intermediate solubility may provide formulation issues, especially at large doses.

**4.3.3 Effects of *P. ostreatus* on HER2+ breast cancer.** HER2+ breast cancer is characterized by the overexpression or amplification of HER2 protein, resulting in uncontrolled cell proliferation and tumor progression. This hyperactivity encourages aggressive tumor behavior, metastatic potential, and resistance to standard treatments.<sup>125</sup> HER2+ breast cancer is treated by targeting the receptor with inhibitors. The *in silico* evaluation of the bioactive compounds in the various extracts in *P. ostreatus* showed that a total of nine compounds inhibits EGFR. These consists of four flavonoid compounds (quercetin, luteolin, rutin and chrysin), three phenol compounds (apigenin, quercetin and ellagic acid), one aqueous extract compounds (10531) and one compound in the chloroform extract (93344) could bind better to the epidermal growth factor receptor compared to the control (gefitinib). Of the two compounds in the aqueous and chloroform extract, compound 10531 showed the ability to inhibit CYP2D6 and CYP3A4, while compound 93344 inhibits CYP2D6 and CYP2C19. Compound

10531 was found to be moderately soluble while compound 93344 is poorly soluble. They both possessed high gastrointestinal tract and glycoprotein binding but no blood–brain barrier crossing. In comparison, compound 10531 is a better drug candidate than compound 93344 due to its better solubility.<sup>123</sup>

## 5 Conclusion

This study focused on the discovery of potent inhibitors of EGFR, ER and PR from *Pleurotus ostreatus* extracts as a potential therapy for breast cancer using *in silico* methods. When compared with the already established drugs as well as their natural substrates, the binding energy of some of the compounds was better. With ER, only one compound out of all compounds studied exhibited a better binding affinity than estradiol, its substrate. PR and EGFR on the other hand recorded many compounds with better binding affinity than that of the control. Most of these compounds were in the class of phenols and flavonoids demonstrating the proven anticancer potential of compounds in this class. None of the hexane and methanolic extracts performed better than the control for each of the proteins. These compounds possess good ADMET properties indicating that these compounds can be considered safe for further development to anticancer drugs. Further *in vitro* and *in vivo* studies should be undertaken to validate these docking results.



## Data availability

Data is available upon request from the corresponding author.

## Author contributions

All authors conceptualized this research. Experiments, data analysis and writing of original draft was carried out by Effiong Magdalene Eno and Mercy Bella-Omunagbe. Methodology validation and writing (review and editing) was carried out by Israel Sunmola Afolabi and Shalom Nwodo Chinedu.

## Conflicts of interest

The authors declare no conflict of interest.

## Acknowledgements

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