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Two N-Phenyl-Carbamic Acid Methyl Esters from *Spatholobus littoralis* and their Cytotoxic Effect Against Breast Cancer Using *In Vitro* and *In Silico* Methods

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ABSTRACT

Spatholobus littoralis is an herbal plant widely used in traditional medicine in Kalimantan, Indonesia. This study aimed to isolate and characterize phytoconstituents from the wood of *Spatholobus littoralis*, and evaluate their cytotoxic activity against breast cancer cells *in vitro* and *in silico*. The ethanol extract was fractionated by solvent-solvent partitioning using *n*-hexane, chloroform, and ethyl acetate sequentially. The chloroform fraction was subjected to gravity silica gel column chromatography using various solvent system to isolate the phytoconstituents. The structures of the isolated compounds were determined using a combination of spectroscopic techniques, including ultra-violet (UV), infra-red (IR), 1D and 2D nuclear magnetic resonance (NMR) spectroscopy, and liquid chromatography-high resolution mass spectrometry (LC-HRMS). The cytotoxic activity of the isolated compounds was tested against T47D and 4T1 breast cancer cells, and their potential activity against breast cancer receptor (2IOG) was also studied *in silico* using molecular docking simulations. The chromatographic separation of the chloroform fraction of *Spatholobus littoralis* wood led to the isolation of two carbamic acid esters characterized as dimethyl (methylene bis(4,1-phenylene)) dicarbamate (**1**) and asperteramide A (**2**). Compound **1** was cytotoxic to 4T1 cancer cells with an IC_{50} of 84.82 µg/mL, while compound **2** was cytotoxic to T47D with an IC_{50} of 134.71 µg/mL. Compounds **1** and **2** showed high affinity with the 2IOG receptor with binding affinity of -6.8 and -8.1 kcal/mol, respectively. Therefore, *S. littoralis* wood extract has the potential to be developed as a natural source of anticancer agents against breast cancer.

Keywords: Breast cancer, Cytotoxic, *Spatholobus littoralis*, N-phenylcarbamic acid methyl ester.

Introduction

Spatholobus is a genus of plants that climb woody trees from the Phaseoleae tribe. The genus was first classified in 1842 by a German botanist.[1] Several species of the genus *Spatholobus* are considered efficacious plants for treating various diseases.[2] One of the species that is abundant on the island of Kalimantan, Indonesia, is *Spatholobus littoralis*, which is known locally as "Bajakah tempala" and is usually used as an herbal medicine.[3-4] Findings from a previous study showed that the total ethanol extract of *S. littoralis* exhibited no cytotoxic effects on T47D cells; however, it demonstrated weak toxicity against 4T1 cells. In contrast, the *n*-hexane, chloroform, and ethyl acetate fractions exhibited strong to moderate cytotoxic effects on both T47D and 4T1 cells.[5]

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There has been limited reports on the structure characterization of chemical compounds in *S. littoralis.* Several studies still report results of phytochemical tests such as the presence of terpenoids, steroids, and flavonoids.Some researchers predicted structures of some phytoconstituents in *S. littoralis* using LC-MS data.[6-9] Predicting molecular structures using LC-MS data alone is insufficient to determine the accurate chemical structure, as it relies solely on matching molecular mass and fragmentation with information in the library database. Reported structural predictions from different researchers often lack consistency due to reliance on limited spectral data. Therefore, it is necessary to carry out more comprehensive and continuous research to determine the structures of the bioactive compounds found in *S. littoralis*. Understanding the structures of bioactive compounds in plants will enhance the utilization and advancement of herbal plants as herbal medicines in the form of phytopharmaceuticals. It will also aid in drug development through the synthesis of more promising derivative compounds. In the present study, the chloroform fraction of *S. littoralis* was subjected to chromatographic separation to isolate its phytoconstituents. The structures of the compounds were elucidated using various spectroscopic methods, including ultra violet (UV), infra-red (IR), and nuclear magnetic resonance (NMR) spectroscopy (both 1D and 2D), as well as liquid chromatography-mass spectrometry (LC-MS) analysis. The cytotoxic activity of the isolated compounds was assessed against T47D and 4T1 cancer cells using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. The 3D structures of the isolated compounds were docked with breast cancer receptors to

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investigate their binding interactions against the selected protein target *in silico*.

Materials and Methods

Solvents and Equipment

Solvents used for extraction and isolation were *n*-hexane (Merck, Germany), ethyl acetate (Merck, Germany), chloroform (Merck, Germany), acetone (Merck, Germany), ethanol (Teknis, UK), and methanol (Merck, Germany). Equipment employed included a rotary evaporator (Buchi Rotavapor R-114), autoclave, (GEA Vertical Pressure Steam Sterilizer HD), an inverted microscope (ThermoFischer), a binocular microscope (LED XSZ BN-107, Oregon), water bath (Thermo Fischer) micropipette (Socorex, Swiss), microplate reader (BioRad, Hercules, CA, USA). All a glassware were product of pyrex.

UV and IR spectra were recorded on Varian Cary 100 Conc and Shimadzu 8300 FTIR instruments. ^{1}H- and ^{13}C-NMR spectra were recorded on a Jeol JNM A-5000 spectrometer, which operates at frequencies of 500 MHz for ^{1}H and 125 MHz for ^{13}C. Mass spectral data were acquired using Thermo Scientific™ Orbitrap™ Exploris 240 High Resolution Mass Spectrometry (HRMS, Bremen, Germany) with acquisition mode Full MS/dd-MS2 and positive and negative polarity (polarity switching). Residual and deuterated solvent peaks served as internal standards for these measurements. Vacuum liquid chromatography (VLC) was conducted using silica gel Merck 60 GF_{254} (230-400 mesh), while gravity column chromatography utilized silica gel Merck 60 (200-400 mesh). Thin-layer chromatography (TLC) analysis was performed on pre-coated silica gel plates, specifically Merck Kieselgel 60 F254, measuring 0.25 mm in thickness and 20 x 20 cm in size. A high-performance computer (Intel Xeon CPU, 10 cores, 32 GB RAM, 500 GB SSD) was used for performing the *in-silico* study. The software used was AutoDock Tools 1.5.7, PyMOL, Avogadro 2.0, LigPlot+2.2.8, and GIMP 2.0.

Cell lines and culture medium

T47D and 4T1 cell cultures were obtained from the Parasitology Laboratory, Universitas Gadjah Mada, Indonesia, and grown in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin-streptomycin (Gibco), and incubated at 37°C with 5% CO_2, 5% (Heraeus), MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), 87.5% RPMI (Roswell Park Memorial Institute (RPMI) 1640 Medium (Merck, Germany), FBS (Fetal Bouvine Serum) (Merck, Germany), and DMSO (Dimethyl Sulfoxide) (Merck, Germany).

Plant collection and identification

Wood of *S. littoralis* was obtained from a traditional market in Pontianak (X9JJ+9F) Kalimantan, Indonesia, in July 2024. The plant was identified at the Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia, and voucher specimen with voucher number HERB-SL-2023 was deposited in the Herbarium.

Isolation and identification of compounds

About 3 kg of *S. littoralis* wood was macerated in 96% ethanol (5 L) at room temperature for 24 h. The extract was filtered, and the marc was remacerated twice. The combined extract was concentrated in a vacuum evaporator at 70ºC resulting in a thick extract (420 g). The ethanol extract was fractionated by solvent-solvent partitioning using *n*-hexane (3 x 500 mL), chloroform (3 x 500 mL), and ethyl acetate (3 x 500 mL) sequentially. Each fraction was then concentrated to obtain fractions of *n*-hexane (13.63 g), chloroform (104.26 g), and ethyl acetate (153.57 g), respectively. A 100 g sample of the chloroform fraction of *S. littoralis* was separated using vacuum liquid chromatography (VLC) with a mixture of *n*-hexane and ethyl acetate in different ratios (9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8), along with ethyl acetate, acetone, and methanol, to produce twelve different sub-fractions (1 – 12). This process allowed for the effective separation of the compounds present in the chloroform fraction, facilitating further analysis of their properties and potential applications. These fractions were combined based on their TLC profiles. Fractions 4 and 5 were concentrated, and a shiny white precipitate appeared, which was then purified by recrystallization in methanol to obtain a shiny white crystal of compound **1** (0.13 g). Fractions 6 and 7 were combined and separated using gravity chromatography with a mixture of *n*-hexane and ethyl acetate (9:1 and 8:2) as the eluting solvents, which resulted in two compounds: compound **1** (200 mg) and compound **2** (1.1 g) that appeared as white solids. Furthermore, compounds **1** and **2** were tested for purity using various eluent mixtures, showing a single spot, with compound **2** being relatively more polar than compound **1**. The structures of the compounds were elucidated by combination of spectroscopic data.

Determination of cytotoxic activity

The isolated compounds were tested for their cytotoxicity against T47D and 4T1 breast cancer cells. The cytotoxicity test was performed in a 96-well plate. A total of 100 µL of cells at a density of 2 x 10^4 cells/mL in culture medium (87.5% RPMI 10.4 g/L, 2% penicillin-streptomycin, and 10% FBS) were added to each well. A total of 100 µL of sample (at concentrations of 10 - 500 µg/mL in 0.05% DMSO) was added to each well. The plate containing the mixture was incubated in a CO_2 incubator at 37°C for 24 hours. After 24 hours, 10 µL of MTT solution was added to each well, and the plate was kept in a CO_2 incubator at 37°C for 4 hours. Living cells will react with MTT to form purple colour formazan crystals. The reaction was stopped by adding 100 µL of 10% SDS in 0.01 N HCl, then incubated at room temperature overnight, then, the absorbance was read at 595 nm using a microplate reader (BioRad, USA). The percentage cell viability was calculated using the formula below (Equation 1).

$$\% \text{ Cell viability} = \frac{\text{Average absorbances of extract treated cells}}{\text{Average absorbances of control cells}} \; x \; 100 \quad \text{(Eq.1)}$$

The linear regression equation of the graph of the relationship between the average percentage (%) of cell viability and log concentration was used to calculate the IC_{50} for each sample.[10] The cytotoxic effect was categorized as follows: IC_{50} <20 µg/mL (high cytotoxic activity), IC_{50}: 20-200 µg/mL (moderate cytotoxic activity), IC_{50}: 201-500 µg/mL (weak cytotoxic activity), IC_{50}> 500 µg/mL (no cytotoxic activity).[11]

In silico study

The prediction of the cytotoxic activity of isolated compounds against breast cancer receptor (PDB 2IOG) was carried out *in silico* using AutoDock Tools 1.5.7, PyMOL, Avogadro 2.0, LigPlot+2.2.8, and GIMP 2.0 software.

Breast cancer receptor (PDB 2IOG) was obtained from the Protein Data Bank (https://www.rcsb.org/structure/2IOG). The 3D crystal structure of the breast cancer protein 2IOG was co-crystallized with N-[(1R)-3-(4-hydroxyphenyl)-1-methylpropyl]-2-[2-phenyl-6-(2-piperidine-1-ylethoxy)-1H-ind. The protein was separated from solvents, ligands, and residues using PyMOL software and saved in pdb format.[12] The docking procedure was conducted using AutoDockTools-1.5.7 software. To validate the docking protocol, re-docking was performed using the 11F ligand, which is the native ligand of the 2IOG receptor. The re-docking process was carried within a designated grid box, resulting in a root mean square deviation (RMSD) value of less than 2 Å.[13] Compounds **1** and **2** were constructed as 3D structures, stabilized using Avogadro software, and saved in pdb.ent format. The test compound was subsequently attached to the receptor binding site following grid box validation. The findings from this docking procedure were presented in terms of the binding affinity of the compound or ligand. To further elucidate the interaction between the ligand and the receptor's active site, LigPlot+ 2.2.8 software was utilized for visualization.

Statistical analysis

Data for the in vitro cytotoxic activity was presented as the mean ± standard deviation (SD) of triplicate determination (n =3).

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Results and Discussion

Characterization of compounds 1 and 2

Compound **I** was obtained as a white powder (330 mg), LC-HRMS/MS m/z 314 [M+] (C_{28} $H_{22}O_7$), UV (MeOH) λmax: 209; 245; 278 nm, IR (KBr) υmax: 3500 cm^{-1} medium absorption indicates the presence of NH; 1705 cm^{-1} indicates the C=O ester group; 1600-1400 cm^{-1} (C=C stretch) indicates the presence of aromatic rings; and absorption at 1250 cm^{-1} indicates the presence of a single C-O bond. The ^{1}H- and ^{13}C-NMR data (Acetone-d_6, 500.0 and 150 MHz) are presented in Table 1.

Table 1: ^{1}H- and ^{13}C-NMR spectrum data for compounds **1** and **2** isolated from ethanol extract of *S. littoralis* wood

Compound 1			Compound 2	
No	**δ ^{13}C ppm**	**δ ^{1}H (∑H, m, J Hz) ppm**	**δ ^{13}C ppm**	**δ ^{1}H (∑H, m, J Hz) ppm**
1	136.90		135.25	-
2	129.99	7.15 (1H, d, 8.5)	130.01	7.13 (1H, d, 8,5)
3	119.41	7.46 (1H, d, 8.5)	119.30	7.46 (1H, d, 8.5)
4	138.32	-	135.10	-
4-NH	-	8.56 (1H, br s)	-	8.54 (1H, br s)
5	119.41	7.46 (1H, d, 8.5)	119.30	7.46 (1H, d, 8.5)
6	129.99	7.15 (1H, d, 8.5)	130.01	7.13 (1H, d, 8,5)
7 (C=O)	155.07	-	155.02	-
8 (OCH3)	52.12	3.67 (3H, s)	52.12	3.65 (3H. s)
1’	136.90		138.34	-
2’	129.99	7.15 (1H, d, 8.5)	131.71	7.01 (1H, d, 8.5)
3’	119.41	7.46 (1H, d, 8.5)	138.41	-
4’	138.32	-	135.34	-
4’-NH	-	8.56 (1H, br s)	-	8.54 (1H, brs)
5’	119.41	7.46 (1H, d, 8.5)	128.09	7.05 (1H, d, 8.5)
6’	129.99	7.15 (1H, d, 8.5)	131.71	7.07 (1H, m)
7’ (C=O)	155.07		155.02	-
8 (OCH_3)	52.12	3.67 (3H, s)	52.11	3.67 (3H, s)
1’’			136.66	-
2’’			129.95	7.07 (1H, d, 8.5)
3’’			119.34	7.45 (1H, d, 8.5)
4’’			138.45	-
4’’-NH			-	8.54 (1H, br s)
5’’			119.34	7.46 (1H, d, 8.5)
6’’			129.95	7.07 (1H, d, 8.5)
7’’ (C=O)			155.02	
8’ (OCH_3)			52.12	3.67 (3H, s)
1’’’	41.15	3.87 (2H, s)	37.39	3.97 (2H, s)
2’’’			41.13	3.87 (2H, s)

The analysis of ^{1}H-NMR spectral data of compound-**1** showed signals corresponding to disubstituted 1,4-benzene rings at δ 7.15 (2H, d, *J* = 8.5 Hz, H2; H6) and δ 7.46 (2H, d, *J* = 8.5 Hz, H3; H5) ppm. The methoxyl proton at 3.67 (3H, s) and amide proton at 8.56 (1H, br s). The presence of amide protons was confirmed by IR data showing absorption at 3500 cm^{-1} and carbonyl groups at 1715 cm^{-1}. These data indicate the presence of aryl units substituted with methoxycarbonyl amino groups.

The aryl units substituted with methoxycarbonyl are bound to methylene carbon, with methylene protons at 3.87 (2H, s). The presence of this methylene group is also confirmed by DEPT 135 carbon spectrum data showing a negative signal at 41.15 ppm. Furthermore, the ^{13}C-NMR data showed the presence of a carbamate group carbonyl carbon of 155.07 ppm, a methoxyl group carbon of 52.12 ppm, and substituted aromatic carbon at the para position with a carbon chemical shift of 136.90 (C-1), 129.99 (C-2 and C-6), 119.41 (C-3 and C-5), and 138.32 (C-4) ppm. This compound is a symmetric compound with a methylene group as a bridge that connects two aryl units substituted with a methoxycarbonyl amino group. Further confirmation using two-dimensional NMR (HMQC, HMBC, and COSY spectra) and LC-HRMS/MS showing molecular mass data at m/z 314. Compound **1** has been reported to be isolated from the *Magnolia kachirachirai* plant, with the systematic name dimethyl [methylene bis(4,1-phenylene)] dicarbamate (Figure 1).[14]

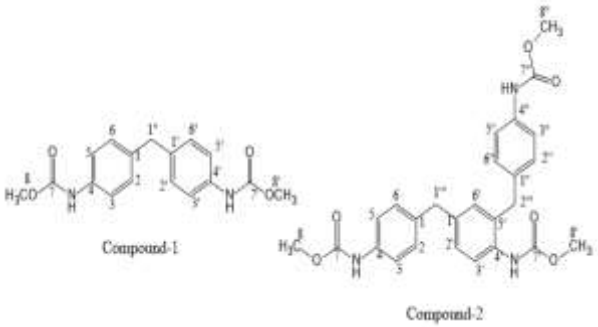

Figure 1: Molecular structures of Compounds **1** and **2**

Compound **2** was isolated as a yellowish white solid (1.1 g), LC-HRMS/MS m/z 477 [M $^+$] ($C_{26}H_{27}N_3O_6$), UV (MeOH) λ max: 210; 242; 277 nm, IR (KBr) υ max: 3450 cm^{-1} medium absorption indicates the presence of NH; 1705 cm^{-1} indicates the C=O ester group; 1600-1400 cm^{-1} (C=C) indicates the presence of aromatic rings; and absorption at 1250 cm^{-1} indicates the presence of a single C-O bond. The UV and IR spectral data of compound 2 are very similar to compound 1. ^{1}H- and ^{13}C-NMR (Acetone-d_6, 500.0 and 150 MHz) are presented in Table 1.

The ^{1}H-NMR spectrum showed the presence of three protons bound to the NH heteroatom at a chemical shift of 8.54 (3H, br s) ppm. This is in accordance with the FTIR spectral data which shows a medium peak at 3450 cm^{-1} which is characteristic of NH absorption. The NMR spectral data (measured in Acetone-d_6) showed the presence of two substituted benzene rings at the 1,4 position, indicated by δ 7.13 (H-2 and H-6, d, *J* = 8.5 Hz) and 7.46 (H-3 and H-5, d, *J* = 8.5 Hz), and 7.07 (H-2’’ and H-6’’, d, *J* = 8.0 Hz) and 7.45 (H-3’’ and H-5’’, d, *J* = 8.5 Hz) ppm, respectively. The proton NMR spectrum also showed one substituted aromatic ring at the 1,4,5 position, thus having three protons at δ 7.01 (H-2’, d, *J* = 8.5 Hz); 7.05 (H-3’, d, *J* = 8.5 Hz); and 7.07 (H-6’, s). Furthermore, the proton NMR spectrum also shows the presence of 9 methoxyl protons in the δ 3.65 (3H, s), and 3.67 (6H, s) ppm. Interestingly, there were methylene protons at 3.87 (2H, s) and 3.97 (2H, s) ppm. The presence of methylene protons is also confirmed by the DEPT 135 carbon spectral data which showed the presence of two methylene carbons in the δ 37.39 (C-1’’’) and δ 41.13 (C-2’’’) ppm.

The ^{13}C-NMR spectrum also shows the presence of three carbonyl carbons of the carbamate group in the region δ 155.2 (C-7; C-7’; C-7’’) ppm, three carbons of the methoxyl group δ 52.12 (3C) ppm, and 11 aromatic methine carbons at δ 130.01 (C-2 & C-4); 119.30 (C-3 & C-5); 131.71 (C-2’); 128.09 (C-5’); 131.71 (C-6’); 129.95 (C-2’’ and C-6’’); and 119.34 (C-3’’ and C-5’’) ppm. Furthermore, there were seven quaternary carbons at δ 135.25 (C-1); 135.10 (C-4); 138.34 (C-1’); 138.41 (C-3’); 135.34 (C-4’); 136.66 (C-1’’); and 138.45 (C-4’’) ppm. Further structure confirmation using two-dimensional NMR (HMQC, HMBC, and COSY NMR), LC-HRMS data showing molecular ions at m/z 477 [M $^+$] ($C_{26}H_{27}N_3O_6$), and by comparison of the chemical shift data with that of asperteramide A, proved that compound **2** is

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asperteramide A (Figure 1).[15] The two compounds that have been isolated and identified from the ethanol extract of *S. littoralis* are of the N-phenyl-carbamic acid methyl ester group which show a very interesting biogenesis relationship.

This is the first report of the discovery of two N-phenyl-carbamic acid methyl esters from *S. littoralis* wood. Both compounds are rarely found in plants. Compound **1** (Dimethyl [methylene bis(4,1-phenylene)] dicarbamate) was previously found in *Magnolia kachirachirai*,[14] while compound **2** (Asperteramide A) was found in the coral-derived fungus *Aspergillus terreus*.[15]

*Cytotoxic effect of compounds **1** and **2** in vitro*

Cytotoxic data as presented in Table 2 showed that compound **1** had moderate cytotoxic activity against 4TI cancer cells with IC_{50} value of 84.82 ± 0.40 µg/mL, and was not cytotoxic to T47D cells, while compound 2 had moderate cytotoxic activity against T47D cancer cells with IC_{50} value of 134.71 ± 1.60 µg/mL, and showed no cytotoxic effect against 4T1 cells. These results are supported by results from previous studies, which have shown the cytotoxic and anticancer activities of several plants from the genus *Spatholobus*.[5,16-18]

Table 2: Cytotoxic activity of compounds **1** and **2** isolated from *S. littoralis* against T47D and 4T1 cancer cells

No	Sample	T47D IC_{50} (µg/mL)	cytotoxic activity category	4T1 IC_{50} µg/mL	Cyto-toxic activity category
1	Compound-**1** (Dimethyl [methylene bis(4,1-phenylene)] dicarbamate)	509.09 ± 2.50	no cytotoxic activity	84.82 ± 0.40	Mode-rate cyto-toxic activity
2	Compound-**2** (Aspertera-mide A)	134.71 ± 1.60	moderate cytotoxic activity	2578.39 ± 11.20	no cyto-toxic activity

*Cytotoxic effect of compounds **1** and **2** in silico*

The cytotoxic activity of two N-phenyl-carbamic acid methyl esters (compounds **1** and **2**) isolated from the wood of *S. littoralis* was predicted *in silico* through molecular docking interaction with the human breast cancer receptor (2IOG). The redocking results produced a grid box with a center x: 31.976; y: -0.732; z: 24.202, with a box size of x = y = z = 30 Å, and had a root-mean-square deviation (RMSD) value of 0.343 Å. The results of method validation through redocking of natural ligands with their respective receptor proteins obtained an RMSD value of <2 Å, so it can be stated that the method used is valid.[13] For clarity of validation results, the receptor was excluded and only the ligand 11F structures before and after redocking are shown in Figure 2. The grid box configuration derived from the validation results was subsequently employed in the docking of the ligands and tamoxifen (as positive control) with the breast cancer receptor. Tamoxifen is a pharmaceutical agent with anti-oestrogenic activity that is frequently used in breast cancer chemotherapy.[19] Tamoxifen is one of the most widely used anticancer drugs in the world. This drug is safe with generally well-tolerated side effects, and has been prescribed for the treatment of early and advanced breast cancer or metastatic estrogen receptor α (ERα/ESR1) positive breast cancer. Tamoxifen therapy has been shown to provide a 38% reduction in the risk of breast cancer in high-risk women.[20]

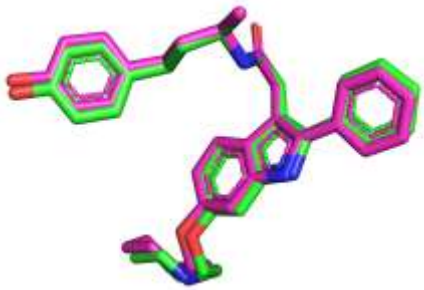

Figure 2: Superimposed ligand 11F before (green color) and after (pink color) redocking against receptor 2IOG

The docking protocol facilitated the assessment of binding energy, hydrogen bonds, and hydrophobic interactions between ligands and amino acid residues of the 2IOG receptor. The binding energy data for the compounds is expressed in Gibbs free energy (ΔG kcal/mol). In this expression, a reduced binding energy is associated with enhanced stability of the bond between the ligand and the receptor.[21] The results of the docking analysis are presented in Table 3. The docking analysis of the isolated compounds and tamoxifen revealed that they exhibited a greater binding energy than the native ligand (11F) associated with the 2IOG receptor. This finding suggests that the native ligand demonstrates greater stability in its binding to the 2IOG receptor. Compound **2** had a binding energy of -8.1 kcal/mol, which signified a better activity than compound **1** with a binding energy of -6.8 kcal/mol. Compound **2** showed hydrogen bond interaction with amino acid residue Arg352, while both compounds **1** and **2** exhibited hydrophobic bond interaction at the amino acid residue Asp351.

Table 3: Binding energy, hydrogen bonds, and hydrophobic interactions of native ligand (11F), N-phenyl-carbamic acid methyl esters (compound **1** and **2**), and tamoxifen against the 2IOG receptor

No	Compound/ Ligand	ΔG (Kcal/mol)	Hydrogen bond	Hydrophobic interactions (Amino acid residue)
1	Native ligan (11F)	-18.8	Arg394	Phe425; Leu391; Glu353; Leu387; Leu384; Thr347; Trp383; Leu354; Asp351; Lys531; Ala330; Cys550; Leu525; Gly521; Met421; His524; Val418; Ile424; Met528; Glu419; Phe404; Met388
2	Compound **1**	-6.8	-	Pro535; Leu536; Lys531; Leu354; Asp351; Ala350; Trp383; Asn532; Tyr526
3	Compound **2**	-8.1	Arg352	Leu327; His356; Val355; Asp351; Thr347; Asn348
4	Tamoxifen	-8.1	-	Lys531; Trp383; Asp351; Ala350; Leu525; Thr347; Met343; Cys530; Met528; Leu346; Ile424; Leu428; Phe404; Met388; Met421

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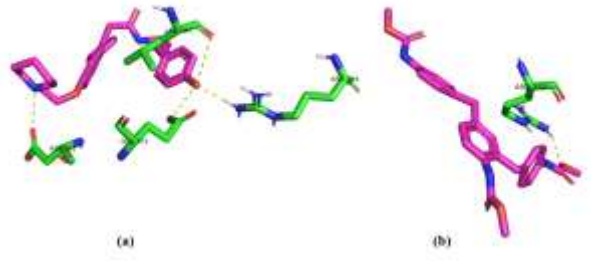

Figure 3: Binding site of native ligand (11F) **(a)** and compound **2 (b)** on 2IOG receptor

The analysis of the binding site for each ligand on the 2IOG receptor was conducted using PyMOL software. The binding site is defined as a hollow region or pocket within the protein target, where small molecules or ligands interact with specific amino acid residues corresponding to each ligand type.[22,23] The results of the analysis indicated that compound **1** and tamoxifen did not exhibit any interaction with specific amino acid residues at the protein (receptor) binding sites. In contrast, compound **2** and the native ligand displayed interaction with specific amino acids within the receptor binding sites (Figure 3). The binding site for the native ligand is located at the amino acid residues Asp 351, Glu 353, Arg 394, and Leu 387, while compound **2** interacted with the amino acid residue Arg 352. The binding site analysis for the ligand-protein complex of compound **1** and tamoxifen against the 2IOG receptor docking results using PyMOL software revealed no specific interactions between the ligands and the amino acid residues on the 2IOG receptor. However, this does not imply that both compounds lack potential for breast cancer treatment, as various other factors influence the activity of a ligand on the receptor. Other factors beyond direct amino acid interactions, such as receptor-mediated signaling pathways and overall biological activity, may determine its efficacy.[24,25]

Conclusion

The isolation and characterization of phytoconstituents from the ethanol extract of *S. littoralis* wood yielded two compounds of N-phenyl-carbamic acid methyl ester: dimethyl (methylenebis(4,1-phenylene)) dicarbamate (Compound **1**) and asperteramide A (Compound **2**). These compounds are reported for the first time in *S. littoralis* and are seldom documented in other plant species. Compound **1** exhibits cytotoxicity against 4T1 cancer cells, with an IC_{50} of 84.82 µg/mL, while compound **2** demonstrates cytotoxicity against T47D cells, with an IC_{50} of 134.71 µg/mL. The binding affinities of compounds **1** and **2** to the 2IOG receptor are -6.8 and -8.1 kcal/mol, respectively. These findings highlight the need for further investigations to assess the potential of *S. littoralis* as a source of natural therapeutic agents against breast cancer.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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