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Original Research Article

Cytotoxic and Molecular Docking Potential of *β*-Sitosterol Isolated from *Lantana camara* Leaves against Breast (T47D) and Cervical Cancer (HeLa) Cell Lines

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ABSTRACT

Lantana camara is a plant used in traditional medicine to treat various diseases. The cytotoxic potential of *Lantana camara l*eaves has been reported for extracts and isolated pure compounds. So this study aims to test the cytotoxic potential of *β*-sitosterol isolated from the leaves of *Lantana camara* using *in silico* (Molecular docking) as well as *in-vitro* MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against T47D and HeLa cancer cells. In this study, the isolation of *β*-sitosterol from the ethyl acetate extract of *Lantana camara* leaves was carried out by gravity column chromatography and purification by recrystallisation. The isolated compound was obtained as a white amorphous solid with a melting point of 136-137 °C. The structure of the isolated compound was determined by extensive IR, ^{1}H-NMR, and ^{13}C-NMR spectra data analysis and with comparison to literature data. The molecular docking results for *β*-sitosterol showed good docking score values of -8.11 kcal/mol against Bcl- 2 and -7.276 kcal/mol against HPV16 E7, as well as a Root Mean Score Deviation (RMSD) value ≤ 2 compared to the standard anticancer drug doxorubicin, -9.230 kcal/mol against Bcl-2 and -8.288 kcal/mol (HPV16 E7), respectively. The results of the potential cytotoxic test of *β*-sitosterol using the MTT method showed its high cytotoxic properties against T47D (breast cancer cell line) and HeLa (cervical cancer cell line) with IC_{50} values of 9.98 µg/mL and 10.31 µg/mL**,** respectively.

***Keywords*:** *Lantana camara*, T47D cell, HeLa cell, molecular docking, and Microculture tetrazolium assay

Introduction

Lantana camara is a plant used in traditional medicine to treat various diseases, including cancer, tumours, and fever. The essential oil of this plant has also been used to treat wounds and as antiseptics.[1] The cytotoxic potential of *Lantana camara* leaves has been reported for extracts and isolated pure compounds. Ediruslan *et al*. reported strong cytotoxic potential of hexane, ethyl acetate, and methanol leaf extracts of this plant with the Brine Shrimp Lethality Test (BSLT) method against *Artemia salina* L shrimp larvae with LC_{50} values of 34.2972, 27.4254 and 133.1930 µL/mL, respectively, as well as lantanilic acid compound isolated from ethyl acetate extracts with LC_{50} values of 27.9903 µL/mL.[2] Suryati *et al*. reported a very strong cytotoxic potential of lantadene A isolated from the hexane extract of the leaves of this plant against *Artemia salina* L shrimp larvae with an LC_{50} value of 48.97 µL/mL,[3]. Also, they reported a very strong cytotoxic potential of lantadene B isolated from the hexane extract of the leaves of this plant using the Microculture tetrazolium test (MTT) method against MCF-7 breast cancer cells with an IC_{50} value of 1.1336 µM.[4] The cytotoxic potential of *Lantana camara* leaves essential oil has also been tested against T-47D breast cancer cells and HeLa cervical cancer cells. The results showed potent activity against both cell lines with IC_{50} values of 10.67 µM and 44.86 µg/mL, respectively.[5,6]

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In other studies, it has also been reported that the plant's essential oil exhibited potent cytotoxicity against *Artemia salina* L shrimp larvae from various regions with LC_{50} values of 36.04, 51.55, 28.34, and 50.35 µg/mL.[7] Other bioactivities of the leaves of this plant have also been reported. The ethyl acetate extract showed strong antioxidant activity with an IC_{50} value of 36.18 mg/mL.[8] In addition, the essential oil of the leaves of this plant was also reported to have antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* with inhibition zones of 12.9 mm and 7.8 mm, respectively.[7]

This current study aims to test the cytotoxic potential of a compound isolated from the leaves of *Lantana camara* using both *in silico* (Molecular docking) and *in-vitro* MTT assay against T47D and HeLa cancer cell lines. So far this assay has not been reported from β-Sitosterol compounds isolated from *Lantana camara* plants. The isolation compound from of *Lantana camara* leaves was carried out by gravity column chromatography and purification by recrystallization. The structure of the isolated compound was determined using IR (infrared) and Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) spectrum data. At the same time, preliminary cytotoxic potential tests were carried out through molecular docking analysis of the interaction of the isolation compound (β-sitosterol) with cancer cell protein receptors breast T47D (Bcl-2) and cervical cancer cell protein (HPV16 E7) in silico. Furthermore, the isolated compound was tested for cytotoxic potential *in vitro* using the MTT method against T47D breast and HeLa cervical cancer cells by determining the Inhibition Concentration (IC_{50}).

Materials and Methods

Materials

The solvents include hexane (Technical), chloroform (Merck Germany), and ethyl acetate (technical). Silica gel 60 (0.063-0.200 mm, Merck Germany). The materials for cytotoxic tests such as T47D breast cancer cell and HeLa cervix (Cell Culture Laboratory, Andalas University), Roswell Park Memorial Institute (RPMI) 1640 medium

(Capricorn Scientific, Germany), Fetal Serum Bovine (RM10432, HiMedia), antibiotic (1% penicillin-streptomycin) Gibco, US, Trypsin-EDTA (Gibco, US), Phosphate Buffer Saline (Gibco, US), and MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) Invitrogen, US.
Others include a Chromatography column (Pyrex, USA), melting point apparatus (SMP10 Stuart, USA), FT-IR spectrophotometer (Thermo Scientific, Nicolet iS10, Amarican), NMR spectrometer (JOEL JNM ECA-500), USA) at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR, T-75 flask (Falcon^{TM}), conical tubes, Eppendorf tubes, serology pipettes, 96-well plates, automated cell counter hemocytometer TC-10 (Bio-Rad, US), 37 °C incubator with 5% CO_2 (Thermo Fisher Scientific, US), inverted microscope (Nikon Ts2R, Thermo Fisher Scientific, US) centrifuge (Thermo Fisher Scientific, US), laminar air flow refrigerator (Lebtech), and Elisa reader (x-mark microplate spectrophotometer Bio-Rad, US). Graph Pad Prism 9.0 software for IC_{50} value analysis. While the tools used for molecular docking are MOE 2022.02 software as well as the Worldwide Protein Data Bank (PDB) online database (https://www.rcsb.org/) and the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Plant collection and preparation
Fresh leaves of the *Lantana camara* were obtained from the Andalas University campus area, Padang City, West Sumatra Province (0° 54' 52.2648" S and 100° 27' 34.2936" E) on September 15, 2022. The leaf sample was identified in the Andalas University Herbarium Laboratory (ANDA) with specimen code 381/K-ID/ANDA/VII/2022.

Sample Preparation and Extraction
Fresh leaves (± 13 kg) of *Lantana camara* were air-dried until brittle, then crushed and weighed. Powder samples (± 3.5 kg) were macerated with hexane and then filtered. The filtrate obtained was evaporated with a rotary evaporator at 40°C. The crude extract was stored in a refrigerator until further use study. The marc from the hexane extract was re-extracted with ethyl acetate, filtered, and evaporated to dryness under vacuum. The ethyl acetate extract obtained was used for compound isolation.[9]

Isolation, Purification of Compounds, and Elucidation of Structures
The ethyl acetate extract (60 g) was fractionated in a gravity column chromatography using hexane: ethyl acetate as eluents (10 : 0 - 0: 10) with increasing polarity. Five fractions (F1-F5) were collected based on differences in their Rf values. Fraction 4 (13.0213 g) was further separated using hexane: ethyl acetate eluent (10 : 0 - 0: 10) using column chromatography, and nine subfractions (F4.1-F4.9) were obtained. Subfraction F.4.3 (0.3645 g) was further separated using hexane: ethyl acetate (9: 1) as eluent in a gravity column, and three subfractions (F4.3.1-F4.3.3) were obtained. Subfraction F4.3.2 (105 mg) was further purified by recrystallisation using hexane and chloroform to get a pure compound. The melting point of the isolated was determined. The was characterised by FTIR, NMR (H-NMR, and C-NMR), and by comparison with the literature. The compound was tested for cytotoxic potential against T47D breast and HeLa cervix cancer cell lines.

Molecular Docking Proteins of T47D Breast and HeLa Cervical Cancer Cells
The isolated compound, *β*-sitosterol, and doxorubicin, a standard anticancer agent (positive control), were docked against the protein targets of the two cancer cell lines. The docking was done using the MOE 2022.02 software, the Protein Data Bank (PDB), and PubChem databases. The ligand structure (*β*-sitosterol) obtained from the PubChem database was imported into MOE 2022.02 software, and energy was minimised using MMFF94X parameters. The structure of the T47D breast cancer protein in the form of Bcl-2 protein (PDB: 6YBL) and the cervical cancer cell protein in the form of HPV16 E7 protein (PDB: 7sqp) were also imported into MOE software. The active sites in the protein structures were identified via the Site Finder menu. Then, the docking process was carried out using Triangle Matcher placement with the London dG score function and 30 poses. Refinement was done using the Rigid Receptor method with the GBVI/WSA dG score function for 5 poses. The docking result data was displayed in the Database Viewer window in a tabular form containing Docking Score (S) and Root Mean Square Deviation (RMSD) values. The results of ligand interactions with amino acid residues in 2D and 3D were displayed via the Ligand Interactions Menu. The shape of the complex molecular surface resulting from docking was shown via the Surfaces and Maps Menu. The process was repeated for the positive control agent.[10]

Cytotoxic Potential Test of Isolated Compound Against T47D Breast Cancer Cells and HeLa Cervix
The isolated compound was dissolved with DMSO to obtain a 1000 µg/ml solution. Different concentrations of the test compound (100 µg/mL, 10 µg/mL, 1 µg/mL, and 0.1 µg/mL) were prepared using RPMI medium. T47D breast cancer cells and HeLa cervix were collected from storage and grown on RPMI medium (10% fetal serum bovine (FBS) and antibiotic (1% penicillin-streptomycin), incubated for 24 hours in an incubator at 37 °C, humidity 95% and 5% CO_2. Cell growth was observed daily using an inverted microscope. Cells that were confluent ≥ 80% were harvested and suspended. The cells were grown on 96-well plates and incubated for 24 hours at 37 °C, humidity 95 %, and 5% CO_2. Then, 20 µL of each test solution was added to individual wells. The cells were incubated again in the incubator for 48 hours at 37 °C, 95% humidity, and 5% CO_2. Next, the cell medium was removed and washed with 100 µL FBS. MTT reagent (0.5 mg/mL) was added to each well and incubated for 4 hours. The MTT solution was discarded, the formazan salt crystals formed were dissolved with 100 µL DMSO, and the absorbance was measured at a wavelength of 550 nm using an ELISA plate reader. The absorbance data obtained was converted into percentage cell viability, and the IC_{50} value was determined using the Graph Pad Prism 9.0 software.[7,11]

Statistical analysis
Data were analysed using Graph Pad Prism 9.0 software. The IC_{50} value was obtained from the result of the curve fit from the plot of the log of dose-response (inhibitor) vs. normalised response.[12]

Results and Discussion

The extraction yields from ± 3.5 kg of powder leaves sample was 102.598 g (hexane) and 128.609 g (ethyl acetate). Further purification of 60 g of ethyl acetate extract using a gravity chromatographic column gave a pure compound (22 mg), a white (amorphous) solid with a melting point of 136-137 °C. The structure of the compound was determined using FTIR and NMR spectra analysis.
Infrared spectrum data shows the presence of vibrations at several wave numbers: 3343 cm^{-1} (OH stretching), 2935 cm^{-1} and 2867 cm^{-1} (CH_3 stretching), 1725 cm^{-1} (C=C), 1464 cm^{-1} indicates the presence of CH bending from CH_2, 1377 cm^{-1} and 1332 cm^{-1} indicate the presence of a CH group from gem-dimethyl, 1243 cm^{-1} (C-C stretching) and 1107 cm^{-1} (C-O). The results of the infrared spectra of the isolated compounds agree with those reported by Adeyemi *et al.*[12] The structure of the isolated compound was determined by ^{1}H-NMR, ^{13}C-NMR, DEPT 135, and DEPT 90 data analysis. The spectra analysis showed a similarity in chemical shifts between the isolated compound and the chemical shifts of *β*-sitosterol compounds reported by Chaturvedula & Prakash.[13] The ^{1}H-NMR and ^{13}C-NMR spectral data of the isolated compound and *β*-sitosterol from the literature are shown in Table 1.
Table 1 shows 13 proton signals in the chemical shift range δH 0.664 - 5.342 ppm. The number of chemical shifts in this compound is generally below two ppm, which is characteristic of aliphatic compounds. The ^{1}H-NMR spectrum showed 6 methyl proton (3H) signals that appeared at δH ppm: 0.664 (H-28); 0.803 (H-27); 0.813(H-26); 0.830 (H-24); 0.992 (H-19); and 0.995 (H-29). One olefinic proton signal at δH 5.342 ppm (1H, H-6) and one multiplet signal at 3.500 (1H, H-3) indicated the presence of methoxy protons.
The ^{13}C-NMR chemical shift data showed 29 carbon signals at δC 11.951-140.823 ppm. Based on DEPT data analysis, the 29 carbon signals consist of six primary carbons (CH_3), eleven secondary carbons ($-CH_2$), nine tertiary carbons (CH), and three quaternary carbons (C). At δC 72.0 ppm, a hydroxyl carbon (C-OH) is seen at the C-3 position.

At δC 140.823 and δC 121.827, it shows the presence of olefinic carbon (C=CH_2) at positions C-5 and C-6 characteristic for the β-sitosterol structure (Figure 1).

The molecular docking test was carried out as a preliminary study to determine the cytotoxic potential of the *β*-sitosterol compound against T47D breast and cervical cancer cells. This cytotoxic ability can be seen from the interaction between the receptor in the Bcl-2 protein for T47D breast cancer cells and the HPV16 E7 protein for HeLa cervical cancer cells with the ligand *β*-sitosterol. The results of this interaction are known through the docking score, Root Mean Score Deviation (RMSD), and bond length between the cancer cell protein and the inhibitor. The results of this docking are shown in Figure 2 and Table 2.

The data results from Table 2 and Figure 2 show an interaction between the *β*-sitosterol compound and the Bcl-2 protein with a docking score value of -8.11 kcal/mol. This interaction occurs between the ligand (β-sitosterol) and the amino acid tryptophan 144 (Trp 144), with a bond distance of 4.08 Å. The H-π bond was formed due to the interaction between the hydrogen atom in the ligand and the aromatic group of the amino acid tryptophan 144 (Trp 144). Meanwhile, there was also an interaction in the HPV16 E7 protein with a docking score value of -7.276 kcal/mol. This interaction occurs between *β*-sitosterol and the amino acid tyrosine 87 (Tyr 87) with a bond distance of 4.11 Å. The H-π bond was formed due to the interaction between the hydrogen atoms in the ligand and the aromatic group originating from the amino acid tyrosine 87 (Tyr 87). The formation of hydrogen bonds between ligands and amino acids indicates a specific interaction in a molecule.[13]

The molecular docking results of the positive control, doxorubicin, showed interaction with the Bcl-2 and the HPV16 E7 proteins with docking score values of -9.230 kcal/mol and -8.288 kcal/mol, respectively. In the Bcl-2 protein, there was an interaction between the ligand and four amino acids: glutamic acid -85, lysine -181, arginine -130, and tyrosine -41 (Glu -85, Lyn -181, Arg -130, and Try -41) with a bond distance of 1.91 Å – 4.62 Å. Bonding interactions between ligands and proteins are formed from several interactions, namely H-donor, which occurs between the H atom in the hydroxyl group of the doxorubicin and the acceptor of the side chain of the amino acid glutamic acid (Glu-85), H-acceptor which occurs between the H atom in the hydroxyl group.

Table 1: Data ^{1}H-NMR (500 MHz in $CDCl_3$) and ^{13}C-NMR (125 MHz in $CDCl_3$) isolated compound and comparative β-sitosterol[12]

Position (C)	Isolated compound			Comparative β-sitosterol (Chaturvedula & Prakash [12])	
	δC (ppm)	DEPT (135 and DEPT 90)	δH (ppm)	δC (ppm)	δH (ppm)
1	37.321	CH_2	1.819 (t, 2H)	37.5	
2	31.993	CH_2		31.9	
3	71.895	CH	3.500 (m, 1H)	72.0	3.53 (tdd, 1H, J = 4.5, 4.2, 3.8 Hz)
4	42.389	CH_2	2.265 (d, 2H)	42.5	
5	140.823	C		140.9	
6	121.827	CH	5.342 (t, 1H)	121.9	5.36 (t, 1H, J = 6.4 Hz
7	31.724	CH_2		32.1	
8	31.964	CH		32.1	
9	50.183	CH		50.3	
10	36.581	C		36.7	
11	21.156	CH_2	1.47 (m,2H)	21.3	
12	39.835	CH_2		39.9	
13	42.360	C		42.6	
14	56.835	CH		56.9	
15	26.080	CH_2		26.3	
16	28.346	CH_2		28.5	
17	56.105	CH		56.3	
18	36.236	CH		36.3	
19	19.102	CH_3	0.992 (d, 3H)	19.2	0.92 (d, 3H, J = 6.5 Hz)
20	33.999	CH_2		34.2	
21	24.391	CH_2	1.15 (m, 2H)	26.3	
22	36.236	CH		46.1	
23	23.124	CH_2		23.3	
24	12.066	CH_3	0.830 (t, 3H)	12.2	0.84 (t, 3H J = 7.2)
25	29.181	CH	1.64 (m, 1H)	29.4	
26	19.928	CH_3	0.813 (d, 3H)	20.1	0.83 (d, 3H, J= 6.4)
27	19.496	CH_3	0.803 (d, 3H)	19.6	0.81 (d, 3H, J= 6.4)
28	18.862	CH_3	0.664 (s, 3H)	19.0	0.68 (s, 3H)
29	11.951	CH_3	0.995 (s, 3H)	12.0	1.01 (s, 3H)

A ligand with a side chain donor of the amino acid lysine -181 (Lyn -181), an H-acceptor that occurs between the H atom in the amine and hydroxyl groups of the ligand with a side chain donor of the amino acid arginine -130 (Arg -130). In the HPV16 E7 protein, there is also an interaction between the ligand and several amino acids, namely aspartic acid 173, glutamine 175, and tyrosine 170 (Asp 173, Gln 175, Try 170), with a bond distance of 3.16 Å - 4.57 Å. Interactions between ligands and proteins are also formed from several interactions, including H-donor, H-acceptor, and H-π interactions.

The docking results between *β*-sitosterol and the Bcl-2 protein (-8.11 kcal/mol) and the HPV16 E7 protein (-7.276 kcal/mol) were comparable to the docking score of doxorubicin (positive control) against the Bcl-2 protein (- 9.230 kcal/mol) and HPV16 E7 protein (-8.288 kcal/mol). The docking score value shows the strength of the interaction between the ligand-receptor (protein-compound); the more negative the docking score value, the stronger the bond and interaction between the protein-compound.[14,15] Apart from that, the results showed good RMSD values. RMSD is the deviation or error values from docking scores. The smaller the RMSD value, the smaller the error from docking. Ramirez & Caballero explain several divisions of RMSD values. They posited that docking results were good if the RMSD value was ≤ 2 Å, acceptable if the RMSD value is between 2 Å – 3 Å, and not good if ≥ 3.[16] The RMSD value obtained from interaction between the ligand and receptor was ≤ 2. Hence, it could be predicted that *β*-sitosterol isolated from *Lantana camara* have potential as an anticancer agent, and could be investigated *in vitro* to validate the docking study results.

The cytotoxicity potential of the isolated compound (test sample) was evaluated using the MTT assay. This was done to determine the ability of the isolated compound to inhibit cancer cell growth by determining the percentage of cell viability.[17] The percentage of cell viability was determined by measuring the absorbance of the test sample at various concentrations against the two cancer cell lines (T47D breast cancer cells and HeLa cells) evaluated. The results are shown in Figures 3a and 3b.

The results (Figures 3a and 3b) of the *in vitro* assay showed that the higher the concentration, the smaller the absorbance produced. In other words, the cytotoxic activity of the test compound is concentration-dependent. This indicates that low levels of dehydrogenated succinate enzyme were produced as well as the formazan crystals formed, resulting in low absorbance values. Figures 3a and 3b also show that the positive control has a higher absorbance value, indicating that it has many living cells. This is because the positive control was untreated, hence no cell death. Thus, a lot of formazan crystals will be produced, leading to higher absorbance values. However, the negative control shows that the absorbance produced is minimal. This is because there are no live cells in the negative control. The shape of cell morphology before and after treatment with the test sample is shown in Figure 4. The figure shows that the higher the test sample concentration, the smaller the number of cancer cells that survive, characterised by a round shape, shrinking, and increase in size. In control (Figures 4a & 4f), it can be seen that the cells have an oval shape with an extensive distribution, which indicates that in the positive control, all cells were alive. Meanwhile, at a sample concentration of 100 µg/mL (Figure 4e and 4j), it can be seen that almost all cells give a round, wrinkled, and small shape, which indicates that at this concentration, there was complete death of the treated cells.[18]

Figure 5 shows the percentage viability of T47D breast cancer cells (Figure 5a) after treatment with the test sample at 100 µg/mL, resulting in cell viability of 8%, indicating about 92% cell death. That is, there was almost complete inhibition of cell growth at this concentration. However, at 0.1 µg/mL of the test sample, there was 96% cell viability, indicating that only 4% of the cells were inhibited. The same scenario was observed in HeLa cervical cancer cells (Figure 5b). At the highest concentration, the isolated compound provided complete inhibition of cell growth with a viability percent of 1%. In comparison, *β*-sitosterol induces a substantial inhibition of cell growth of T47D breast cancer than in HeLa cells.

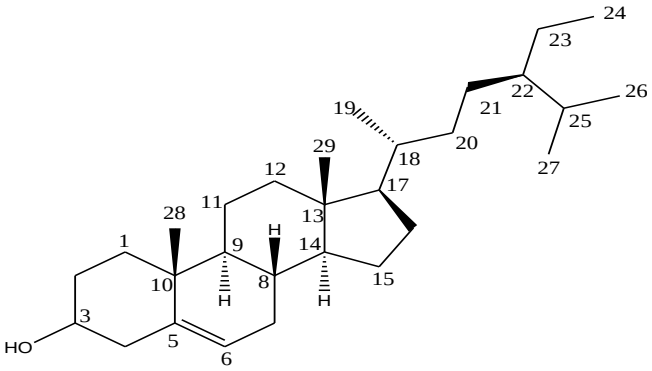

Figure 1: Structure of β-sitosterol isolated

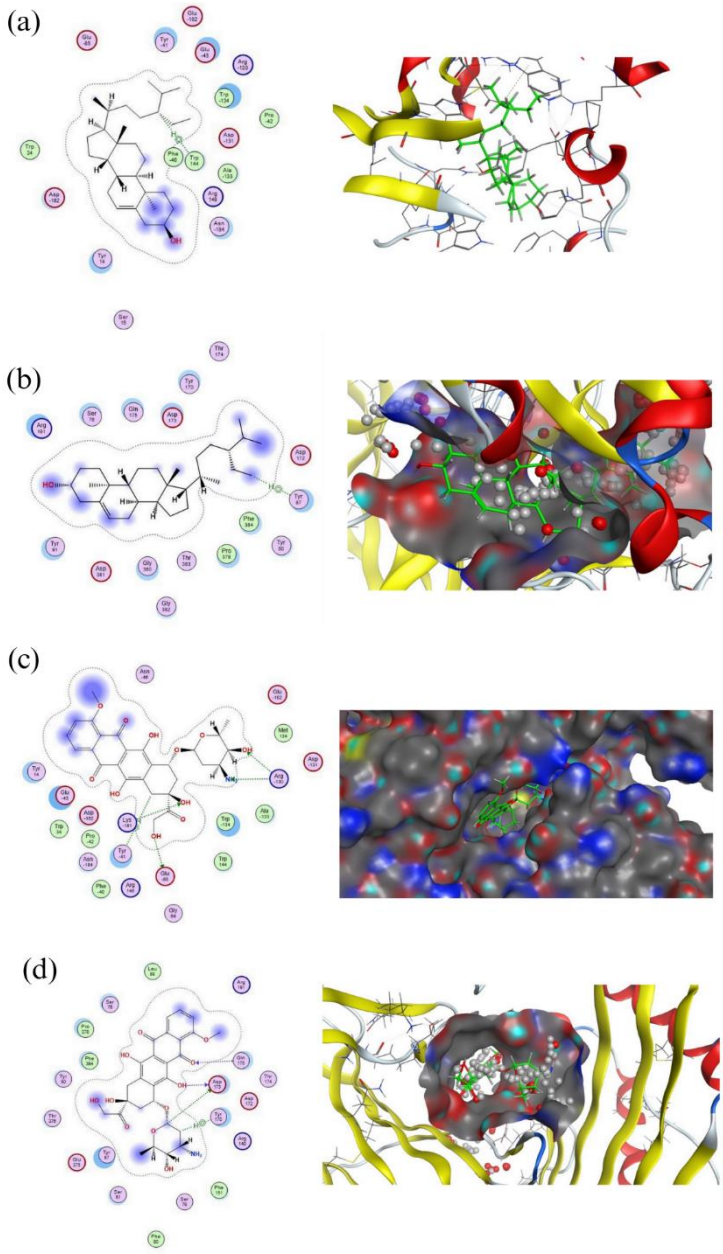

Figure 2: 2D and 3D interactions (b) β-sitosterol with Bcl-2 protein (b) β-sitosterol with HPV16 E7 protein (c) Doxorubicin with Bcl-2 protein (d) Doxorubicin with HPV16 E7 protein

Table 2: Results of Docking Score, RMSD, and bond length of β-sitosterol compounds and positive control for Bcl-2 and HPV16 E7 proteins

Compounds	Proteins	Docking Score (kcal/mol)	RMSD	Bond length (Å)	Bond energy (kcal/mol)	Bond type	Amino acid
β-sitosterol	Bcl-2	-8.11	1.859	4.08	-0.5	H-π	Trp 144
	HPV16 E7	-7.276	1.962	4.11	-0.7	H-π	Tyr 87
	Bcl-2	-9.230	1.5067	2.91	-3.6	H-donor	Glu -85
				2.80	-1.6	H-acceptor	Lyn-181
				2.96	-4.5	H-acceptor	Arg-130
				3.21	-1.2	H-acceptor	Arg-130
				4.62	-0.5	H-π	Try -41
Doxorubicin (Positive Control)	HPV16 E7	-8.288	1.059	3.16	-0.5	H-donor	Asp 173
				2.69	-0.5	H-donor	Asp 173
				3.43	-0.5	H-acceptor	Gln 175
				4.57	-0.5	H-π	Try 170

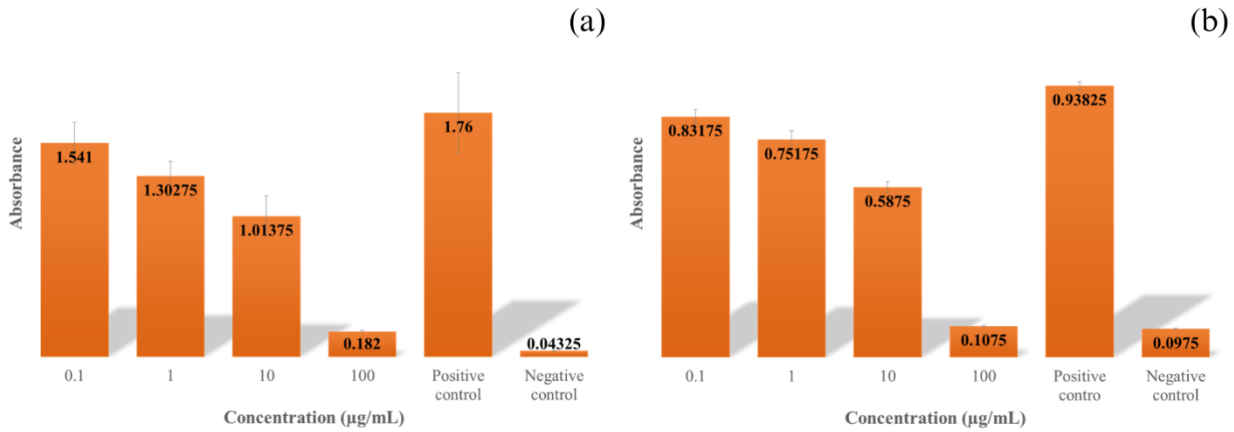

Figure 3: MTT test absorbance value of isolated compounds against T47D breast cancer cells (a) cervical HeLa (b) Positive control: cell + medium; Negative control: medium

The cytotoxic activity of the isolated compounds was determined by determining the IC_{50} value. This IC_{50} value is the concentration needed to inhibit cell proliferation by 50%. The calculations show that the isolated compound has an IC_{50} value of 9.98 µg/mL for T47D breast cancer cells and 10.31 µg/mL for HeLa cervical cancer cells. These two IC_{50} values are categorised as highly cytotoxic based on the National Cancer Institute (NCI) cytotoxic categorisation. A compound is said to be very cytotoxic if $IC_{50} \leq 20$ µg/mL, moderate cytotoxic IC_{50} 21-200 µg/mL, weak cytotoxic IC_{50} 201-500 µg/mL, and not cytotoxic $IC_{50} >$ 501µg/mL. A compound has the potential as an anticancer drug if the compound has an IC_{50} value of <100 µg/mL.[19] Thus, the isolated compound has the potential to be an anticancer.

Previous studies have also reported that *β*-sitosterol has various cytotoxic activities against several cancer cells. Anwar *et al*. reported that *β*-sitosterol isolated from the *Berberis lyceum* had cytotoxic activity against Hep-1 and HepG2 cancer cells with IC_{50} values of 123.12 ± 3.51 µM and 140 ± 4.21 µM.[20] Katja *et al*. reported that *β*-sitosterol isolated from the *Chisocheton celebicus* plant had cytotoxic activity against P-388 murine leukaemia cancer cells with an IC_{50} value of 12.45 ± 0.050 µg/mL.[21] Da Silva *et al*. also reported that *β*-sitosterol isolated from the *Passiflora mucronata* plant showed cytotoxic activity against K562 cancer cells with an IC_{50} value of 8.13 µg/mL.[22] Another report showed that *β*-sitosterol isolated from the *Chisocheton celebicus* plant had cytotoxic activity against MCF-7 and PC3 cancer cells with IC_{50} values of 57 ± 3.8 µg/mL and 90 ± 8.9 µg/mL, respectively.[23] Malek et al. reported that *β*-sitosterol isolated from the *Pereskia bleo* (Kunth) DC plant. (Cactaceae) had cytotoxic activity against CasKi and A549 cancer cells with IC_{50} values of 62 µM and 78 µM.[24]

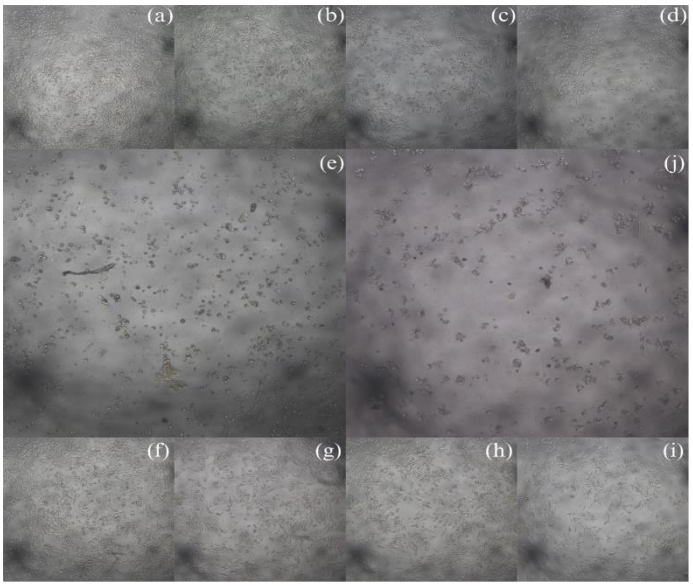

Figure 4: Morphology of control T47D cancer cells (a) and various concentrations of the test solution 0.1 µg/mL T47D (b) 1 µg/mL T47D (c) 10 µg/mL T47D (d) 100 µg/mL T47D (e) control T47D cancer cells (f) and various concentrations of the test solution 0.1 µg/mL HeLa (g) 1 µg/mL HeLa (h) 10 µg/mL HeLa (i) 100 µg/mL HeLa (j)

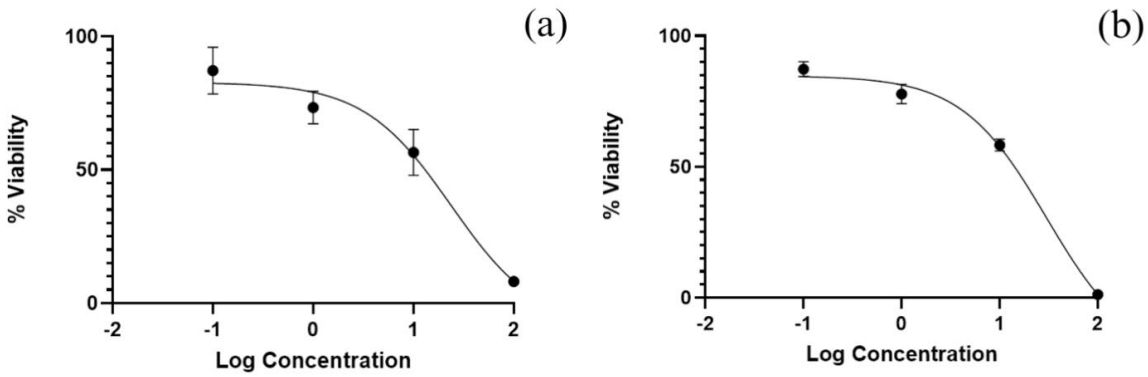

Figure 5: Relationship between log concentrations of isolated compounds and % viability of T47D cancer cells (a) Relationship between log concentrations of isolated compounds and % viability of HeLa cancer cells (b)

The mechanism of the isolated compound (β-sitosterol) in inhibiting cancer cell growth could be due to the induction apoptosis, as well as inhibiting the PI3K/Akt/mTOR signalling pathway. In addition, *β*-sitosterol also inhibits the growth of cancer cells in the G0/G1 phase by increasing the proportion of cells in the G0/G1 phase and reducing the proportion in the S phase, resulting in suppression of cell proliferation.[25] *β*-sitosterol can also suppress cell proliferation by stopping the cell cycle in the S and mitotic phases.[26]

The results of molecular docking and the MTT test showed that *β*-sitosterol isolated from the ethyl acetate extract of the leaves of *Lantana camara* has good potential as an anticancer drug. This potential was determined *in silico* using the molecular docking method and *in vitro* using the MTT method. These two methods also show that *β*-sitosterol has the best cytotoxic ability for breast cancer cells.

Conclusion

This study reports the isolation of *β*-sitosterol from the ethyl acetate extract of the leaves of the *Lantana camara*, characterised by different spectroscopic (FTIR, 1D & 2D NMR) data analysis. The results of the cytotoxic potential of the *β*-sitosterol compound showed good docking scores for both cancer cells (breast and cervical cancer cells). The MTT test results also show that the compound was cytotoxic to T47D breast cancer cells and HeLa cervical cancer cells with IC_{50} values of 9.98 µg/mL and 10.31 µg/mL, respectively. Further cytotoxic studies on other cancer cells, such as HepG2 liver cancer and A375 skin cancer, may be necessary for the isolated compound.

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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