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Metabolic Profiling, *in vitro* Cytotoxicity and *in silico* Investigation of *Lycium shawii* Roem. Extract

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ABSTRACT

Background: Breast cancer is a prevalent global health concern. Traditional medicine often incorporates the use of medicinal plants to address various diseases.

Methods: The cytotoxicity, oxidative stress and cell migration effects of saponin and phenol extracts were evaluated through MTT assay, ROS analysis and wound-healing assay. Following the identification of the active extract, it underwent GC-MS analysis and in silico investigations.

Result: Our results revealed significant inhibition of cell proliferation in MDA-MB-231 (IC_{50}:407.3 µg/mL) and HUVECs (IC_{50}:500 µg/mL), which was achieved only with the ethyl acetate extract (Fraction 2). Fraction 2 extract induced notable morphological changes and significantly inhibited time-dependent migration in MDA-MB-231 cells. Additionally, it elevated cellular ROS levels compared to the control cells. In molecular docking analysis, out of the 51 chosen secondary metabolites from *L. shawii*, stigmast-5-en-3-ol, (3α,24S) (-10.0 kcal/mol) and lup-20(29)-ene-3,28-diol (-9.5 kcal/mol) were found to be the best docked to their respective targets-6CHZ and 4MAN, respectively. Therefore, this plant holds promise as a potential therapeutic agent for breast cancer treatment.

Key words: 6CHZ, Cell migration, Cytotoxicity, MDA-MB-231, Molecular docking.

INTRODUCTION

Significant progress has been made in cancer therapy, leading to a remarkable improvement in the rates of survival of breast cancer (BC) patients. However, even with these progressions, BC still stands as the primary contributor to cancer-related fatalities in women across the globe (Hortobagyi *et al.*, 2005). In 2016, it accounted for 535,000 deaths in 195 countries, presenting considerable clinical challenges (Fitzmaurice *et al.*, 2018), (Xiao *et al.*, 2019), (Carpenter *et al.*, 2019). BC can be classified into four primary molecular subtypes based on the expression of estrogen receptor (ER), epidermal growth factor receptor 2 (HER2) and progesterone receptor (PR). Among these subtypes, triple-negative BC (TNBC) stands out as the most aggressive and fast-growing form of BC, characterized by the absence of HER2, ER, PR and receptors (Burguin *et al.*, 2021). Consequently, standard treatments like hormone therapy and targeted drugs are ineffective, leaving limited options for TNBC treatment. Cytotoxic chemotherapy is the primary approach in this context, showing initial efficacy in earlier stages but a higher recurrence rate than other BC types (Wang *et al.*, 2019). Managing TNBC, especially its highly metastatic variant, remains a considerable challenge due to the absence of targeted therapies. Therefore, there is an urgent need for innovative treatment modalities to save lives (Mehanna *et al.*, 2019).

Between 1981 and 2014, over 150 drugs derived from natural products were introduced to the pharmaceutical market (Baskar *et al.*, 2012). The extensive biodiversity of plant species offers a vast resource for the discovery of new compounds with anticancer potential and ongoing investigations continue to unveil the healing potentials of these natural extracts in the fight against cancer (Cragg *et al.*, 2009) (Feng *et al.*, 2023) (Manosroi *et al.*, 2017). *Lycium shawii* Roem. and Schult, an indigenous plant found in the Arabian Peninsula (Ali *et al.*, 2020). It is a thorny shrub belonging to the Solanaceae family. Locally referred to as “Awsaj,” it has a history of use in traditional medicine for treating conditions like jaundice, stomach ailments, mouth sores and coughs (Rehman *et al.*, 2016). Notably, *L. shawii* has demonstrated a wide range of beneficial properties, including anti-inflammatory, antimicrobial, antioxidant, anti-diabetic and anticancer properties (Albarrak, 2021; Lee *et al.*, 2012; Ali *et al.*, 2020; Usha *et al.*, 2016; Tahraoui *et al.*, 2007).

This study aimed to explore the cytotoxic, apoptotic and anti-migration properties of *L. shawii* extracts. In addition, in silico molecular docking identified potential bioactive anticancer compounds, offering valuable insights for the development of novel drugs.

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MATERIALS AND METHODS

Study area

This experiment was conducted at King Saud University, Diriyah, Kingdom of Saudi Arabia, from October 2022 to December 2023.

Plant material

The plant used in this study was collected from Irqah, Riyadh province (Saudi Arabia). A specimen of *Lycium shawii* Roem was deposited in the herbarium collection under the acquisition number BRC-IRQA7-23. The plant was dried using a hot air oven at 50°C for 48 h. The aerial parts were ground in a commercial mill and used for extraction.

Extraction of plant material

Extraction of saponin

The dry aerial parts (25 grams) of *L. shawii* were refluxed in an EtOH-H_2O mixture (2:8, v/v, 0.3 L × 2) for 4 hours and subsequently sonicated for 30 minutes. The resulting extract was filtered using cheesecloth and centrifugation at 4350 × g for 3 minutes. The volume was then reduced to 200 mL using a rotavapor at 45°C. The extract was defatted with hexane (3 × 200 mL) and then extracted with n-BuOH (3 × 200 mL). The butanol extract was subsequently evaporated using a rotavapor at 50°C, isolating an n-BuOH-soluble fraction (520 mg).

Extraction of phenol

For 5 days, the powdered material (34 g) was allowed to macerate in 400 mL of 80% methanol while occasionally shaken. It was then filtered and concentrated under reduced pressure using a rotary evaporator at 45°C.

Fractionation

The methanol extract was suspended in distilled water (500 ml) and transferred into a separatory funnel followed by the addition of 200 mL of *n*-hexane (× 2) and shaking vigorously and then left until two layers were formed. The hexane layer was separated and kept for evaporation. The exact process was repeated using solvents of increasing polarity, namely ethyl acetate (EtOAc) and butanol (n-BuOH). Each separated fraction was concentrated under reduced pressure using a rotary evaporator at 45°C.

Extraction of bound phenolic compounds

Alkaline hydrolysis was used to extract the bound phenolic compounds of *L. shawii* according to the described method (Irakli *et al.*, 2018) with some modifications. Briefly, the 23-gram residues obtained after free phenolic compounds extraction were washed with water and dried in an oven. They were then treated with 2 N NaOH (200 mL) for 15 min using a sonicator at 60°C. The pH was adjusted to 7 using 2 N HCl and was extracted 5 times with EtOAc (3 × 300 mL). The EtOAc fraction was concentrated individually under reduced pressure at 45°C.

Cell culture

MDA-MB-231 (metastatic breast cancer), HepG2, Huh-7 (human hepatoma cell lines) and normal HUVECs (Human umbilical vein endothelial) cell lines were sourced from the DSMZ Cell Bank (Germany). These cell lines were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with fetal bovine serum (FBS, 10%). The cultures were kept at 37°C in a 5% CO_2 humidified atmosphere.

MTT cytotoxicity assay

The cells were seeded in 24-well culture plates (1000 µL of medium/well) at a 5 × 10^4 cells/well density. After 24 hours, the test substances were added to the wells in triplicate at different concentrations (50 to 500 µg/mL) and 0.01% methanol as the control. Proliferation activity was assessed by quantifying mitochondrial activity through the previously reported MTT reduction method (Al-Zharani *et al.*, 2019).

Reactive oxygen species (ROS)

Fraction 2 (IC_{50} concentration) was added to MDA-MB-231 cells (5 × 10^4 cells/well) and then incubated for 24 h. Subsequently, 25 µM of DCFH-DA was added and incubated for 30 minutes. As a control, methanol (0.01%) was maintained. Images were captured using a fluorescent microscope (EVOS, USA).

Wound-healing assay

The impact of Fraction 2 on cellular migration was qualitatively assessed using a wound-healing method (Al-Zharani *et al.*, 2019). In brief, 5×10^4 MDA-MB-231 cells/well were seeded in 6-well plates, forming a confluent monolayer. Scratching was done with a sterile pipet tip and fresh FBS-deprived medium was added. Plates were incubated for 24 hours with 200 µg/mL Fraction 2. Scratch areas were imaged at 0, 24 and 48 hours and quantified using Image J.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using the method of Abd El-Kareem *et al.* (2016). The chemical composition fraction 1 was carried out using GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) using TG–5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The compounds were identified using NIST14 and WILEY 09 mass spectral databases. di-o-glucoside).

Molecular docking

Based on the literature, estrogen receptor alpha Y537S (ER-ALPHA (PDB ID-6CHZ), apoptosis regulator Bcl-2 (PDB:4MAN), Myeloid leukemia 1 (MCL-1) (PDB ID: 5FDO) and BCL-W (PDB ID: 2Y6W) were selected as a drug target for breast cancer (Abdulrahman *et al.*, 2023; Kaur *et al.*, 2022). The X-ray crystal structure of protein targets and its co-crystallized ligands were obtained from the RCSB Protein Data Bank. The protein and the chemical structures of compounds (ligands) sourced from the PubChem database

were prepared using AutoDock Tools (version 1.5.7) (Trott and Olson, 2010). After calculating the docking scores for various protein-ligand pairs, we selected the one with the most negative energy for further investigation. This chosen protein-ligand complex was subjected to more detailed scrutiny using a Protein-Ligand Interaction Profiler (PLIP) (Adasme *et al.*, 2021) and PyMol software.

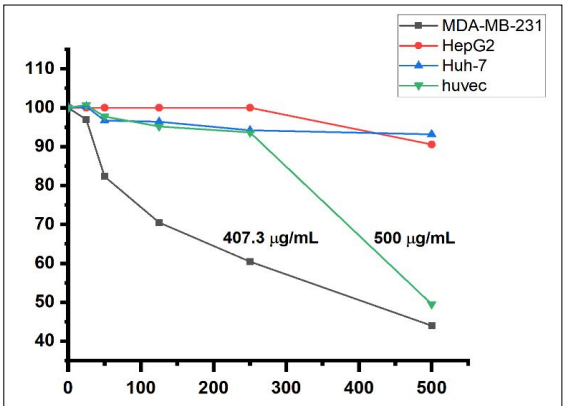
Statistical analysis

All experiments were conducted in triplicate and the significance of the findings was determined using a t-test. The results are presented as mean ± SD, with a p-value less than 0.05 considered significant.

RESULTS AND DISCUSSION

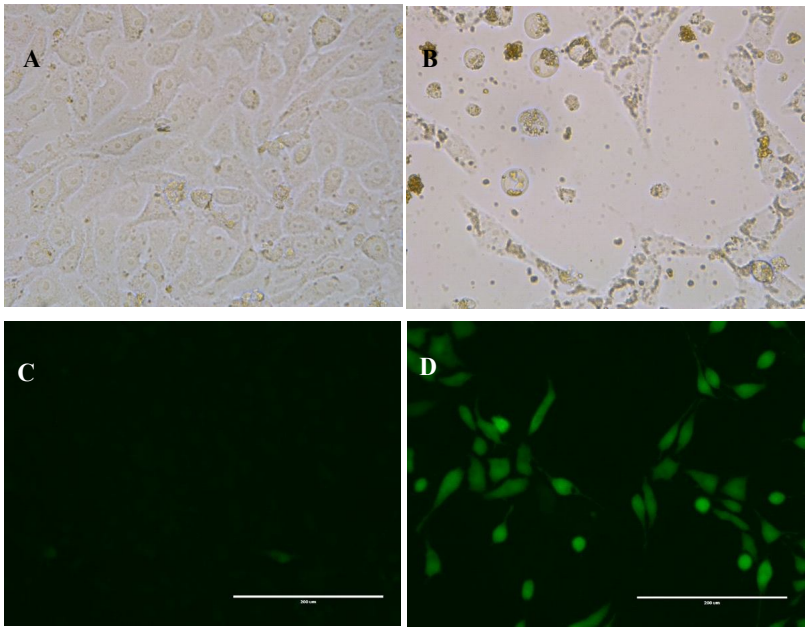
Upon extracting 34 grams of *L. shawii* powder with 85% ethanol, the fractions obtained were as follows: 370 mg from Hex (Fraction 1), 205 mg from EtOAc (Fraction 2) and 490 mg from n-BuOH (Fraction 3), while 23 grams of bound phenol extraction yielded 220 mg for EtOAc (Fraction 4) and 940 mg for n-BuOH (Fraction 5), with 520 mg for saponin (Fraction 6).

Our findings indicated that MDA-MB-231 (IC_{50}: 407.3 µg/mL) and HUVECs (IC_{50}: 500 µg/mL) cell proliferation was inhibited only with Fraction 2. However, the extract did not exhibit activity against HepG2 and Huh-7 liver cancer cell lines. Fig 1 illustrates the cytotoxic properties of *L. shawii* against these cell lines. The most potent anticancer effect of the extract was observed against MDA-MB-231 cancer cells. Fraction 2 induced notable morphological alterations in MDA-MB-231 cells, characterized by cytoplasmic shrinkage, contraction and detachment, leading to the complete loss of cellular integrity. In contrast, untreated cells displayed normal cellular morphologies (Fig 2 A and b). Similar results were observed in a previous study documenting that costunolide (IC_{50}: 32) and aloe emodin



The graph displays dose-response curves, illustrating the impact of various concentrations of the ethyl acetate extract from *Lycium shawii* on the tested cells after a 24-hour treatment.

Fig 1: The assessment of cytotoxicity caused by the ethyl acetate extract on MDA-MB-231, HUVEC, Huh-7 and HepG2 cells through MTT assays.



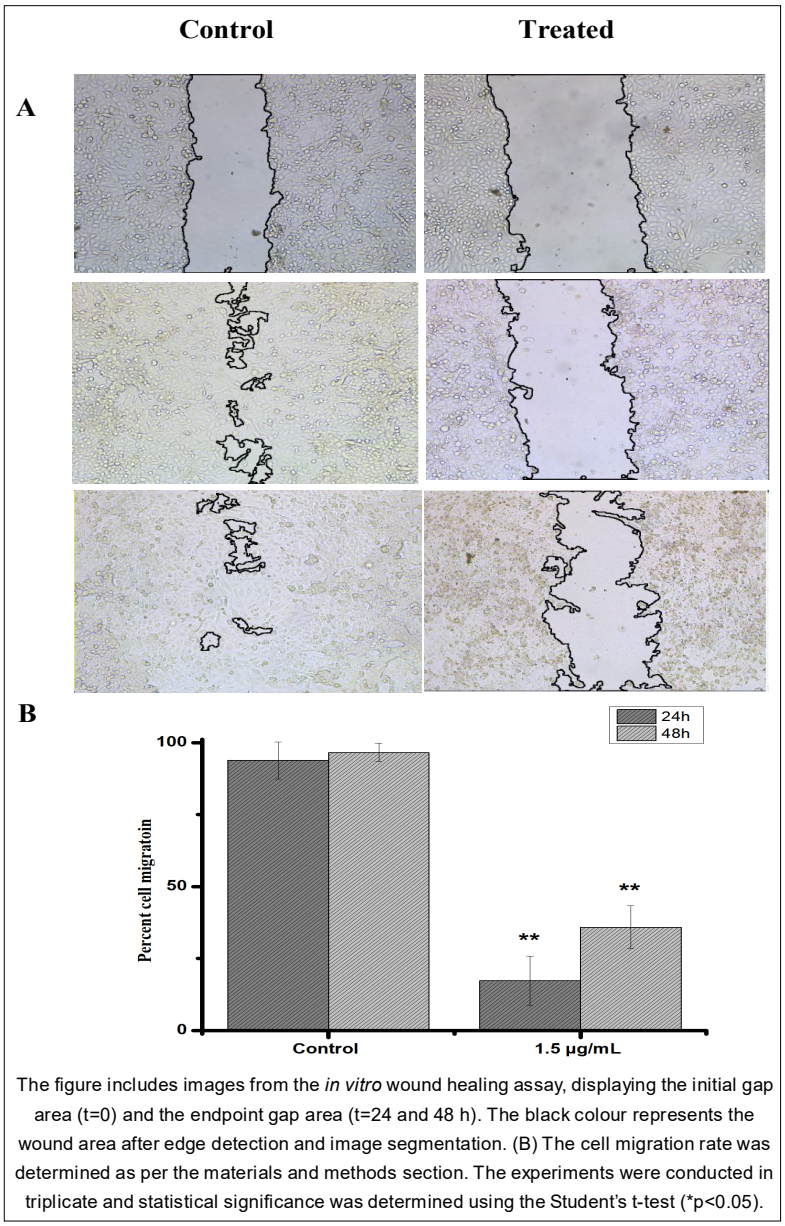
The analysis of ROS formation was conducted by applying DCFH-DA staining to MDA-MB231 cells incubated with the ethyl acetate extract. Images were captured after 30 min using an EVOS microscope at a 20× magnification. The images include (A) the control group and (B) the ethyl acetate extract-treated group.

Fig 2: Morphological assessment of MDA-MB231 cells incubated with EtOAc extract using a phase-contrast microscope (A: control B: treated with cells).

(IC_{50} 38 µM) isolated from *L. shawii* stem extract exhibited substantial apoptotic potential against oral squamous cell carcinoma OSCC cells. Notable cellular morphological alterations and gene and protein expression (BAK, caspase 3, 6 and 9) indicated the presence of apoptosis in treated cells.

In cancer, the invasion and migration of cells are pivotal factors contributing to recurrence and metastasis. Effectively inhibiting cell migration is essential for successful cancer therapy, as metastasis significantly impacts survival rates, reducing them to approximately 50% (Irani, 2016). As illustrated in Fig 3A and 3B, fraction 2 significantly hindered the time-dependent migration of MDA-MB-231 cells. In the absence of treatment, the cells exhibited wound closure, reaching up to 93.8% and 96.6% at 24 and 48 hours, respectively. The scratch closures at IC_{50} doses were smaller, measuring up to 17.2% at 24 hours and 35.8% at 48 h, reflecting the potential of the extract in reducing cell migration. Several in vitro studies have provided evidence that phytochemical compounds derived from *L. shawii* can impede the invasion and migration of various cell lines (Choi *et al.*, 2013; Xiao and Guo, 2009; Shams *et al.*, 2023).

To assess the impact of oxidative stress on the cytotoxicity of fraction 2, cell lines were exposed to its IC_{50} concentration determined through cytotoxicity assays. Fraction 2 significantly elevated cellular ROS levels in MDA-MB-231 cells compared to the 0.01% MeOH-treated cells (control) (Fig 2 C and D).



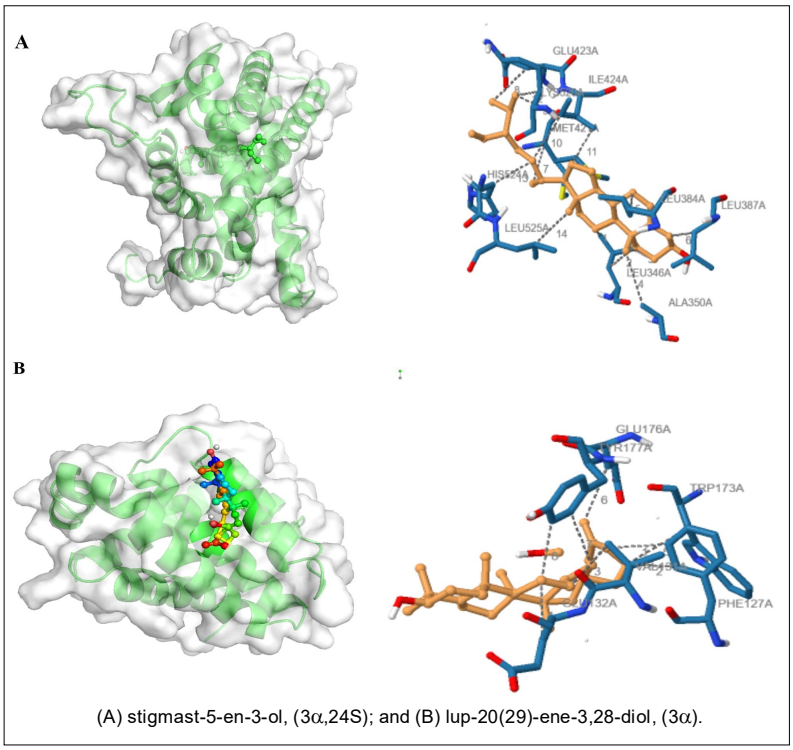
The figure includes images from the *in vitro* wound healing assay, displaying the initial gap area (t=0) and the endpoint gap area (t=24 and 48 h). The black colour represents the wound area after edge detection and image segmentation. (B) The cell migration rate was determined as per the materials and methods section. The experiments were conducted in triplicate and statistical significance was determined using the Student's t-test (*p<0.05).

Fig 3: The impact of the EtOAc extract on MDA-MB-231 cell migration.

Docking is essential for systematically exploring large chemical libraries and it remains a key tool in rational drug design and drug repurposing strategies (Friesner *et al.*, 2004) (Huang and Zou, 2007). The GC–MS analysis revealed the presence of fifty-one compounds within fraction 2 of *L. shawii* (Table 1). Out of fifty-one phytoconstituents, it was observed that stigmast-5-en-3-ol, (3α,24S) and lup-20(29)-ene-3,28-diol, (3α), exhibited the most favourable interactions with 6CHZ, featuring binding energies of -10.0 and -9.9 kcal/mol, respectively (Table 1). Stigmast-5-en-3-ol, (3α,24S)-showed 15 hydrophobic interactions with Leu 346 (3x), Ala 350, Leu 384, Leu 387, Met 421, GLU 423, 424 ILE (3x), LYS 520, HIS 524 and LEU 525 (Table 1). The 2D and 3D interactions are shown in fig 4 A and B. Similarly, the best-docked secondary metabolite with BCL-2 was LUP-20(29)-ENE-3,28-DIOL (-9.5 kcal/mol). In the binding site of BCL-2 lup-20(29)-ene-3,28-diol, (3α), interacted through 8 hydrophobic interactions with the following residues: 127A PHE (2x), 131A VAL, 132A GLU, 173A TRP, 176A GLU and 177A TYR (2x) The 2D and 3D interactions are shown in Fig 4 B.

Stigmast-5-en-3-ol, (3α,24S)- and Lup-20(29)-ene-3,28-diol, (3α)- interact with 6CHZ, presenting a binding energy of -10.0 and -9.9 kcal/mol respectively. Notably, their binding affinity was equal to or greater than the control compounds (-9.9 kcal/mol). ER-α is associated with both hormone-dependent and hormone-independent tumours. This dual role of ER-α is notable, as it has been reported to play a part in both cancer suppression and cancer progression (Liu *et al.*, 2020). Given that 60–70% of breast cancers in women are ER-α positive, the current therapy primarily relies on tamoxifen, which helps control ER-α-induced breast cancer progression. However, the prolonged use of tamoxifen may lead to resistance in breast cancer patients. Consequently, there is a pressing need to explore novel natural drugs and understand ER-α signalling to enhance breast cancer therapy (Xue *et al.*, 2019).

The capacity to avoid apoptosis is a critical characteristic of cancer cells. Therefore, they frequently disrupt the apoptotic pathway to ensure the tumour cells survival by upregulating the expression of anti-apoptotic proteins within the Bcl-2 family. These proteins include Bcl2-A1, Mcl-1, Bcl-2, Bcl-w and Bcl-xL (Williams *et al.*, 2019). In various cancer types, the elevated levels of anti-apoptotic proteins, such as Mcl-1, Bcl-2 and Bcl-xL, have been established to not only confer resistance to chemotherapy but also promote tumour initiation and progression through the microRNAs regulation and transcription factors (Valentini *et al.*, 2022; Choi *et al.*, 2016). The VINA scoring function predicted the ability of lup-20(29)-ene-3,28-diol, (3α) and stigmasta-5,22-dien-3-ol, acetate, (3α), to bind to Bcl-2 and Mcl-1 with similar affinities and more effectively than BCL-W.



(A) stigmast-5-en-3-ol, (3α,24S); and (B) lup-20(29)-ene-3,28-diol, (3α).

Fig 4: Binding poses of two top-ranked ligands at the estrogen receptor alpha (ERα) (PDB ID-6CHZ) (A) and apoptosis regulator Bcl-2 (PDB:4MAN) binding sites and 3D and 2D interaction diagrams.

Table 1: The outcomes obtained from AutoDock Vina show the binding energies of secondary metabolites from *L. shawii* with different protein targets associated with breast cancer.

Phytoconstituents	RT	Molecular weight	Molecular formula	Area %	Energy (kcal/mol)			
					4MAN	2Y6W	5FDO	6CHZ
6-Dodecanone	7.00	178	$C_{12}H_{18}O$	0.31	-3.6	-3.6	-5.7	-5.3
3,5-Heptadienal, 2-ethylidene-6-methyl-	11.67	150	$C_{10}H_{14}O$	0.57	-5.4	-4.4	-6.1	-5.5
PHENOL, 2-METHOXY-5-(1-PROPENYL)-,(E)-	14.91	164	$C_{10}H_{12}O_2$	0.59	-5.9	-4.7	-6.5	-6.2
Phenol,2,6-dimethoxy-4-(2-propenyl)-	20.50	194	$C_{11}H_{14}O_3$	0.54	-6.0	-5.0	-6.3	-5.7
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	21.33	196	$C_{12}H_{20}O_2$	0.94	-6.3	-5.1	-6.4	-6.8
Hexadecanoic acid, 2,3-dihydroxypropyl ester	22.44	330	$C_{19}H_{38}O_4$	0.87	-5.7	-4.0	-6.1	-5.5
Pentadecanoic acid, 14-methyl-, methyl ester	25.64	270	$C_{17}H_{34}O_2$	0.83	-5.6	-3.9	-6.0	-5.6
6,9,12,15-Docosatetraenoic acid, methyl ester	25.95	346	$C_{23}H_{38}O_2$	0.54	-6.3	-4.9	-6.7	-6.6
Hexadecadienoic acid, methyl ester	26.10	266	$C_{17}H_{30}O_2$	0.31	-5.4	-4.7	-6.5	-5.9
Hexadecanoic acid	26.46	256	$C_{16}H_{32}O_2$	15.95	-5.1	-4.1	-6.0	-5.7
7,10-octadecadienoic acid, methyl ester	28.65	294	$C_{19}H_{34}O_2$	3.20	-5.9	-4.1	-6.7	-5.9
Cis-13-Octadecenoic acid, methyl ester	28.83	296	$C_{19}H_{36}O_2$	3.62	-5.6	-3.9	-6.4	-6.2
10-octadecenoic acid, methyl ester	28.93	296	$C_{19}H_{36}O_2$	0.67	-5.3	-3.4	-6.7	-5.4
1-heptatriacotanol	29.15	536	$C37H_{76}O$	0.81	-6.0	-3.4	-5.9	-6.9
9,12,15-Octadecatrienoic acid,(Z,Z,Z)-	29.55	278	$C_{18}H_{30}O_2$	25.59	-6.2	-4.5	-6.5	-6.1
9,12-Octadecadienoyl chloride,(Z,Z)-	29.64	298	$C_{18}H_{31}C_1O$	17.12	-5.6	-4.0	-7.0	-6.3
9-octadecenoic acid (z)-	30.11	282	$C_{18}H_{34}O_2$	2.47	-5.9	-4.9	-6.7	-6.0
ANDROSTAN-17-ONE, 3-ETHYL-3-HYDROXY-, (5α)-	30.25	318	$C_{21}H_{34}O_2$	0.79	-7.4	-6.2	-8.7	-9.1
9,12,15-Octadecatrienoic acid	31	352	$C_{21}H_{36}O_4$	1.02	-5.4	-3.5	-7.0	-6.5
9-(3-hexenylidenecyclopro pylidene)-, 2-hydroxy-1-(hydroxymet hyl)ethyl ester, (z,z,z)-	31.34	352	$C_{21}H_{36}O_4$	0.48	-5.4	-5.1	-5.4	-5.7
7,10,13-Eicosatrienoic acid, methylEster	32.05	352	$C_{21}H_{36}O_4$	0.48	-6.6	-4.7	-6.8	-6.6
6,9,12-octadecatrienoic acid, methyl ester	32.24	292	$C_{19}H_{32}O_2$	0.20	-6.3	-4.2	-6.5	-6.2
Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)-	32.31	352	$C_{21}H_{36}O_4$	0.42	-6.2	-4.6	-6.3	-6.6
5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	32.48	318	$C_{21}H_{34}O_2$	0.35	-6.4	-3.9	-7.0	-6.6
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethylEster	33.95	354	$C_{21}H_{38}O_4$	0.36	-5.6	-4.4	-6.7	-6.3
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	34.02	352	$C_{21}H_{36}O_4$	0.48	-5.8	-3.7	-6.5	-6.6
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethylEster	34.09	356	$C_{21}H_{40}O_4$	0.21	-6.0	-5.4	-6.6	-5.6
9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl Ester, (z,z,z)-	34.71	352	$C_{21}H_{36}O_4$	0.41	-5.5	-4.5	-6.4	-6.2

Table 1: Continue...

Table 1: Continue...

Nonanoic acid, 9-(3-hexenylidenecycloproPylidene)-, 2-hydroxy-1-(hydroxymetHyl)ethyl ester, (z,z,z)-	34.78	352	$C_{21}H_{36}O_4$	7.65	-6.5	-5.8	-7.8	-7.7
Arachidonoyl ethanolamide	35.12	699	$C_{22}H_{37}NO_2$	0.23	-6.6	-5.4	-6.8	-6.7
9,12,15-octadecatrienoicAcid,	35.35	496	$C_{27}H_{52}O_4Si_2$	1.02	-5.9	-4.0	-6.8	-6.7
4h-1-benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-6, 8-Di-α-d-glucopyranosyl-5,7- dihydroxy-	35.82	300	$C_{16}H_{12}O_6$	0.57	-7.6	-7.1	-7.6	-7.2
Stigmast-5-en-3-ol	37.51	414	$C_{29}H_{50}O$	0.27	-7.5	-6.2	-8.6	-7.9
9 cis-2-phenyl-1, 3-dioxolane-4-methylOctadec-9, 12, 15-trienoate	37.59	440	$C_{28}H_{40}O_4$	0.71	-6.3	-4.6	-7.5	-8.3
Butyl 9,12,15-octadecatrienoate	37.93	334	$C_{22}H_{38}O_2$	7.65	-6.3	-4.6	-7.0	-6.5
3-dioxolane-4-methyl octadec-9, 12, 15-trienoate	39.02	440	$C_{28}H_{40}O_4$	0.92	-6.3	-4.8	-6.2	-6.2
9,12,15-octadecatrienoicAcid,2-phenyl-1,3-dioxan-5-yl ester	40.38	352	$C_{21}H_{36}O_4$	0.21	-7.2	-4.9	-6.6	-7.9
4h-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-Dihydroxy-7-methoxy-	41.02	610	$C_{27}H_{30}O_{16}$	0.59	-6.9	-6.0	-7.4	-7.6
Trilinolein	41.58	878	$C_{57}H_{98}O_6$	0.30	-5.9	-4.4	-5.4	-6.7
LUP-20(29)-ENE-3,28-DIOL, (3α)-	41.76	442	$C_{30}H_{50}O_2$	0.38	-9.5	-7.7	-9.1	-9.9
STIGMAST-5-EN-3-OL, (3α,24S)-	41.93	414	$C_{29}H_{50}O$	0.48	-8.1	-6.3	-9.0	-10.0
Betulin	42.19	442	$C_{30}H_{50}O_2$	0.83	-7.5	-6.2	-8.1	-8.6
Stigmasta-5,22-dien-3-ol, acetate,(3α)-	42.32	454	$C_{31}H_{50}O_2$	0.79	-8.1	-6.5	-9.3	-9.4
Ergosta-5,22-dien-3-ol, acetate,(3α,22E)-	42.55	440	$C_{30}H_{48}O_2$	1.17	-7.5	-6.5	-8.4	-7.8
Loperamide	42.94	476	$C_{29}H_{33}ClN_2O_2$	0.22	-8.4	-6.7	-8.2	-6.9
Arabinitol, pentaacetate	42.99	362	$C_{15}H_{22}O_{10}$	0.32	-5.1	-4.6	-5.2	-5.6
Rhodopin	43.93	554	$C_{40}H_{58}O$	0.24	-7.4	-5.9	-6.5	-7.2
Glycidyl oleate	44.23	338	$C_{21}H_{38}O_3$	0.76	-5.2	-4.2	-6.3	-6.2
Flavone 4'-oh,5-oh,7-di-o-glucoside	44.75	594	$C_{27}H_{30}O_{15}$	1.88	-8.5	-7.7	-7.4	-7.7
15,17,19,21-hexatriacontatetrayne	44.87	490	$C_{36}H_{58}$	0.55	-5.8	-4.3	-4.7	-6.8
Methyl 7-ethyl-10-hydroxy-11-hydroxy(18O)-3, 11-dimethyl-2,6-tridecadienoate	45.17	312	$C_{18}H_{32}O_4$	0.13	-7.6	-5.6	-6.8	-8.7
Positive control								
4-[4-({4'-chloro-3-[2-(dimethylamino)ethoxy]biphenyl-2-yl}methyl)piperazin-1-yl]-2-(1H-indol-5-yloxy)-N-({3-nitro-4-[(tetrahydro-2H-pyran-4-ylmethyl)amino] phenyl}sulfonyl)benzamide	-	-	-	-	-9.0	-	-	-
Di(hydroxyethyl)ether	-	-	-	-	-	-3.5	-	-
3-[3-(4-chloranyl-3,5-dimethyl-phenoxy)propyl]-~{N}-(phenylsulfonyl)-1~{H}-indole-2-carboxamide	-	-	-	-	-	-	-9.5	-
4-[(2-{4-[(1E)-1-(1H-indazol-5-yl)-2-phenylbut-1-en-1-yl]phenoxy}ethyl)amino]-N,N-dimethylbutanamide	-	-	-	-	-	-	-	-9.9
Negative control (glycerol)	-	-	-	-	-3.4	-3.4	-3.4	-3.8

Various studies have proved that Stigmast-5-en-3-ol can induce apoptotic cancer cell death, including MCF-7 (BC), U937 and HL-60 leukemia cell lines with IC_{50} values of 45.17, 37.82 and 8.294 µg/ml, respectively (Fernando *et al*., 2018; Moon *et al*., 2008). Similarly, it has been documented that the apoptotic effects induced by Stigmast-5-en-3-ol are linked to an elevation in Bax, Caspase-9, PARP cleavage and p53, while concurrently reducing Bcl-xl levels (Fernando *et al*., 2018). Likewise, Lup-20(29)-ene-3β,11β-diol displayed cytotoxic potential against HeLa cell lines (IC_{50}: 28.5 µM) (Nguyen *et al*., 2017).

CONCLUSION

The study examined the Fraction 2 of *L. shawii* for its impact on breast cancer cell lines and results revealed significant inhibition of cell proliferation in MDA-MB-231(IC_{50}:407.3 µg/mL) and HUVECs (IC_{50}:500 µg/mL). Molecular docking highlighted the favourable binding of specific compounds to targets associated with breast cancer. While promising, further *in vivo* investigations are crucial for validating these findings and exploring potential drug discovery and nutraceutical development applications.

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Authors' contribution

N. Abutaha designed the study, R. Alghamdi and N. Abutaha conducted experiments. F.A. Almekhlafi, M.A. Wadaan helped in writing the manuscript and conducted data analyses.

Data availability statement

All the data is available within the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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