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Original article

Bioactivity and molecular docking of lactones isolated from *Centaurea pseudosinaica* Czerep

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ABSTRACT

Two cytotoxic sesquiterpene lactones, 17-epichlorohyssopifolin A (**1**) and chlorjanerin (**2**), and a monoterpene lactone, loliolide (**3**) were isolated from *Centaurea pseudosinaica*. The cytotoxicity of the total extract and terpenoids **1**–**3** were evaluated against three human cancer cells (HepG2, PC-3, and HT-29), along with the human normal primary epidermal keratinocytes (HEKa) cells. With IC_{50} values ranging between 0.6 ± 0.04 and 5.0 ± 0.61 μg/mL against HepG2; 0.2 ± 0.01 and 11.9 ± 1.31 μg/mL against PC-3, and 0.04 ± 0.013 and 8.9 ± 0.97 μg/mL against HT-29, the total extract, and lactones **1**–**3** demonstrated cytotoxic effects. Compound **1** displayed the strongest impact on all cancer cells and a slightly safe effect on the normal cells HEKa. Compound **1** caused accumulation of HepG2 and HT-29 cells in G1 phase as displayed cell cycle analysis. On the other hand, the cell distributions were increased in the S phase in PC-3 cells. Furthermore, **1** caused apoptosis in PC-3 and HePG2 cells with 91.50%, and 79.72 %, respectively. A higher fraction of necrotic cells was observed in HT-29 cells amounting to 23.60%. These results suggested that the promising cytotoxicity exhibited by **1** is brought by the apoptosis induction in the cancer cells, which were evaluated. As the compounds showed antiproliferative effect against the HT-29 cells, the docking simulation was performed aiming at determining how they would interact with the EGFR enzyme, whose PDB: 4I23 is considered one of the two distinct wild types of EGFR enzymes. The antibacterial activity results revealed that **3** showed the most remarkable antibacterial effects, especially against the examined Gram-positive bacteria. The total extract exhibited potent activity against all examined bacteria. The total extract showed a potent antifungal effect against two *Candida* and two *Aspergillus* pathogens. The antioxidant activity revealed the potency of the total extract and **3** as antioxidant candidates. The obtained results refer to the importance of *Centaurea pseudosinaica* as a source of potent antiproliferative agents and the whole plant as an antipathogenic and antioxidant agent.

Abbreviations: IC_{50}, The half-maximal inhibitory concentration; EGFR, Epidermal Growth Factor Receptor; CC, Column Chromatography; TLC, Thin Layer Chromatography; PTLC, Preparative Thin Layer Chromatography; NMR, Nuclear Magnetic Resonance; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

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1. Introduction

Cancer is an uncontrollable cell growth that can spread to various tissues. It raises the fatality rate and is one of the main global health issues (International Agency for Research on Cancer, 2020). It is also listed as the second leading cause of mortality, right after cardiovascular disease, with about 10 million people perished of cancer in 2018 (De Martel et al., 2020). With a range between 1.09 and 2.26 million cases, the most prevalent cancers were those of the breast, respiratory, intestines and rectum, prostate, epidermis (non-melanoma), and gut. According to GCO (Global Cancer Observatory), lung, intestines, liver, rectum, stomach, and breast cancer accounted for most of the cancer-related death in 2020, with 1.80, 0.935, 0.830, 0.769, and 0.685 million cases, respectively. The research, particularly in natural products, has increased, aiming at finding new or bioactive anticancer metabolites.

Asteraceae is a prominent flowering plant family, comprising 1,911 genera, 32,913 species (Al-Wahaibi et al., 2020) and was featured by many aromatic and medicinal plants. *Centaurea* is one of the largest genera of over 700 species of herbaceous thistle-like flowering plants in Asteraceae. Members of this genus are widely distributed in the Mediterranean and Western Asia regions (Albayrak et al., 2017; Carev et al., 2017; Ifantis et al., 2013; Sharonova et al., 2021). There are diversities of folk medicinal applications of plants belonging to the genus *Centaurea* as diuretic, antifebrile, antimalarial, mildly astringent, digestive, cytotoxic, phytotoxic, antineoplastic, and emmenagogue (Albayrak et al., 2017; Dereli et al., 2020; Fattaheian-Dehkordi et al., 2021; Karamenderes et al., 2007; 2008; Politeo et al., 2019; Shoeb et al., 2005). Sesquiterpene lactones found in *Centaurea* are believed to possess a broad variety of biological features, including anticancer, anti-inflammatory, and antimicrobial (Kebbi et al., 2021).

The pharmacological and economic values of Asteraceae, particularly genus *Centaurea*, stimulate the researchers to spend much effort and time aiming at discovering new and/or bioactive constituents. Accordingly, certain studies have been published investigating different members of the genus *Centaurea* (Khammar and Djeddi, 2012). This led to the publication of acetylenes, flavonoids, and terpenoids like the sesquiterpene lactones of the guaianolide and germacranolide types. Recently, some elemanolides and eudesmanolides have been reported. Finally, Sesquiterpene lactones seem responsible for the genus *Centaurea*'s pharmacological effects. In our continuous endeavor to investigate the secondary metabolites and/or new biological activities. A specimen of the Saudi Asteraceae plant identified as *C. pseudosinaica,* was collected, extracted, and explored for its secondary metabolites. Two sesquiterpenes were isolated and identified as 17-epichlorohyssopifolin A (**1**), chlorjanerin (**2**), and a monoterpene lactone known as loliolide (**3**) (Fig. 1). The antiproliferative, antipathogenic, and antioxidant properties of the isolated metabolites and the total extract were assessed.

2. Materials and methods

2.1. General

Equipment qualification, chemical manufacturer and chromatographic separation have been previously reported (Abdul-Hameed et al., 2021).

2.2. Plant material

During its blooming stage, *Centaurea pseudosinaica* Czerep's semi-dried root and aerial segments originated from the Al-Swdah, Abha, the southern part of Saudi Arabia (18°14′33.7″N; 42°24′07.8″E) were gathered on September 4, 2021. The plant was authenticated by Prof. Ibrahim Mashaly (Department of Botany, Faculty of Science, Mansoura University, Egypt). A plant sample was stored at the Chemistry Department, King Abdulaziz University with a voucher specimen no of CP-F-100.

2.3. Extraction and isolation

The semi-dried aerial parts and roots (1.24 Kg) were extracted until exhaustion using a mixture of equal volumes of dichloromethane, *n*-hexane, and methanol at room temperature for one day. A rotary evaporator was employed to evaporate the mixed extract (The evaporation temperature was 36 °C), which yielded a greenish residue (42.76 g). The residue (20.0 g) was dissolved in methanol and kept overnight in a refrigerator; the residue was filtered to remove fatty materials. The methanol-soluble material was dried and homogenized with the lowest amount of column chromatography silica gel. The silica gel-supported *C. pseudosinaica* extract was fractionated on a silica gel open column. Gradient technique was employed from *n*-hexane to diethyl ether mixtures to yield 186 fractions (1.FC–186.FC), followed by mixtures of *n* hexane: ethyl acetate to yield 97 fractions (187.FC–284.FC), followed by dichloromethane: methanol to yield 115 fractions (285.FC–400.FC). TLC was used to observe the fractionation sequence, with the assistance of ultraviolet light and sulfuric acid reagent. Similar spots were combined according to their TLC pattern. The fractions from 20% ethyl acetate in *n*-hexane were processed with PTLC (25% EtOAc in *n*-hexane). The spot with R_f 0.48 was collected to yield **3** (19.7 mg). The fractions from 30% ethyl acetate in *n*-hexane were further purified with PTLC (35% EtOAc in *n*-hexane). The spots with R_f values 0.55 and 0.25 were collected to yield **1** (10.4 mg) and **2** (12.1 mg), respectively.

2.4. Characterization of the isolated compounds

All isolates were identified by comparing each isolate's spectral data to the information previously published in publications. Those

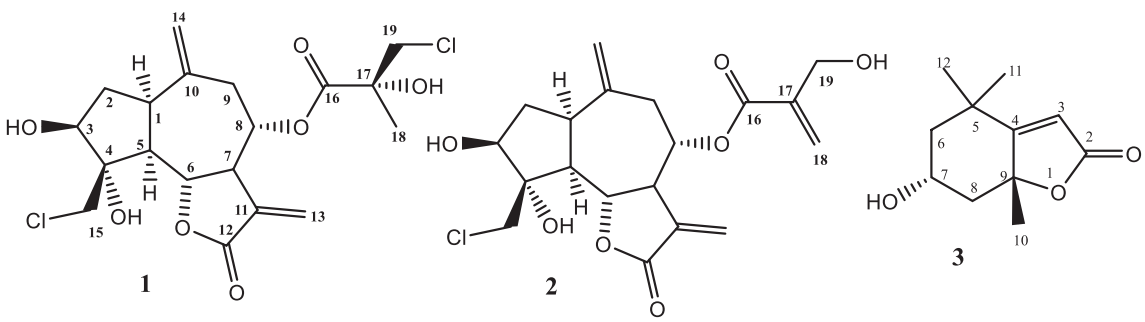

Fig. 1. Chemical structures of compounds **1**–**3** isolated from *Centaurea pseudosinaica.*

terpenoids were identified as 17-epichlorohyssopifolin A (**1**), chlorjanerin (**2**), and loliolide (**3**).

2.5. Biological activities

2.5.1. Cell culture

Human hepatoma (HepG2), human prostate cancer (PC-3), human colon cancer (HT-29), and HEKa cells were grown in RPMI-1640 medium. The cell lines and the culture medium were purchased from the American Type Culture Collection (ATCC) (Gibco, Thermo Fisher Scientific, USA). Fetal bovine serum (FBS) at 10% and penicillin/streptomycin (PS) at 100 units/mL were added to the growth media. A humid environment, 37 °C, and 5% CO_2 were the conditions for cells growth.

2.5.2. Cell viability assay

Roughly 2000 cells/well of HepG2, PC-3, HT-29, and HEKa were inoculated in 96-well plates. The cells were rinsed once using PBS and five times using distilled water. The full procedure was conducted as described by Elbehairi and his coworkers (Elbehairi et al., 2020).

2.5.3. Cell cycle analysis

The pre-calculated IC_{50} values of **1** were administered to HepG2, PC-3, and HT-29 cells for 48 h. Cells were then collected using trypsinization, cleaned twice with PBS (phosphate-buffered saline), preserved at 40 °C in ice-cold 60% ethanol, and then rinsed once more in PBS. Following that, cells were resuspended in 500 L of propidium iodide (PI) with RNase staining solution and cultured for 30 min. Lastly, the ACEA Novocyte™ flow cytometer, ACEA Biosciences Inc., USA, were applied to carry out the FACS investigation. Data from 12,000 cells were gathered for every sample, and cell cycle phase distribution was examined using ACEA Novo Express™ software, ACEA Biosciences Inc., USA (Alam et al., 2021).

2.5.4. Apoptosis analysis

Sesquiterpenoid **1** was applied to HepG2, PC-3, and HT-29 cells for 48 h before they were trypsinized and given two PBS washes. The Annexin V- FITC/PI Apoptosis Detection Kit, BD Biosciences, USA, was used to inspect apoptosis (Abdel-Lateff et al., 2015).

2.5.5. Antimicrobial assay

All isolates were screened for the presence of antibacterial and antifungal activities by the methods of Tajbakhsh et al. (2011) and Yen and Duh (1994), respectively.

2.5.6. Antioxidant assay

The formula referred to Yen and Duh (1994) was used to determine the DPPH radical's percentage inhibition (PI), as the following:

$$PI = \frac{Abs_0 - Abs_T}{Abs_0} \times 100$$

Abs_0 : absorbance of the control at t = 0; Abs_T: absorbance of the sample with the addition of DPPH at t = 16 min, respectively.

2.6. Molecular docking study

Using the molecular docking procedure, molecular docking was appraised through MOE "2015.10". The EGFR enzyme PDB was acquired from (https://www.rcsb.org/PDB codes: 4I23). The created protein was arranged according to the guidelines below: i) The enzyme's structure has been cleared of objective heteroatoms and water molecules. ii) The MOE technique had RMS values lower than 1.50 kcal.mol^{-1}, allowing the attachment of hydrogen atoms into the protein while reducing the procedure. iii) The MOE builder tools were used to create the ligands.

2.7. Statistical analysis

Multivariate analysis, i.e., one-way analysis of variance (ANOVA), was used to evaluate all the data. Triplication was applied for all treatments. Significant group differences were defined as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. GraphPad Prism software version 6.00 (GraphPad Software, USA) was used to create the graphs.

3. Results

3.1. Chemistry

Chromatographic purification of the organic extract obtained from the semi-dried whole plant of *Centaurea pseudosinaica* yielded two sesquiterpene lactones; 17-epichlorohyssopifolin A (**1**) (Fernandez et al., 1995) and chlorjanerin (**2**) and a monoterpene lactone loliolide (**3**). Structures of all isolates were established by measuring 1D and 2D NMR spectra (Table 1) and matching with them published data (Saklani et al., 2011; Sarg et al., 1987; Tanaka and Matsunaga, 1989).

3.2. Biology

3.2.1. Cytotoxicity

The antiproliferative activity of the total extract and the purified lactones was evaluated. The total extract showed potent cytotoxicity against HT-29 cells with an IC_{50} value of 1.32 ± 0.51 μg/mL, while its activity against both HepG2 and PC-3 cells is less but still higher than compounds **2** and **3**. All cancer cells were affected by **1–3**, with IC_{50} values varying between 0.60 ± 0.04 and 5.00 ± 0.6 1 μg/mL against HepG2; 0.20 ± 0.01 and 11.9 ± 1.31 μg/mL against PC-3 and between 0.04 ± 0.01 and 8.90 ± 0.97 μg/mL against HT-29 (Table 2). **1** showed the most potent cytotoxicity against PC3, HT-29, and HepG2 with IC_{50} values of 0.20 ± 0.01, 0.04 ± 0.01, and 0. 60 ± 0.04 μg/mL, respectively. **1** had a stronger impact on all cancer cell lines than the chemotherapy's positive control. Additionally, all lactones and the total extract were evaluated against the HEKa normal cells. **1** had a less detrimental effect on normal cells (IC_{50} = 0.5 ± 0.01 μg/mL) as compared to its impact on PC-3 and HT-29 cells with IC_{50} of 0.20 ± 0.01 and 0.04 ± 0.01 μg/mL, correspondingly (Table 2 and Fig. 2).

3.2.2. Effect of compound *1* on the cell cycle distribution of human cancer cells

The compound's antiproliferative effects were scrutinized using the cell cycle phases. Consequently, using flow cytometry, the impact of 1 on the spread of cell cycle stages in HepG2, PC3, and HT-29 cells was examined after a 48-hour treatment period. Fig. 3 shows that the percentage of HepG2 and HT-29 cells in the G1 phase significantly rose from 41.1 ± 2.1% to 60.1 ± 1.7% and from 39.4 ± 1.4% to 59.4 ± 2.3%, respectively. While after treatment with doxorubicin (positive control), the cells arrested in G2 phase, also, increased the cell distribution in the S phase in PC-3 cells from 41.2 ± 1.4% to 48.4 ± 1.7%, as well as the positive control.

3.2.3. Assessing cell apoptosis with annexin V-FITC

In HepG2, PC-3, and HT-29, the study sought to distinguish between cells dying through necrosis (non-programmed cell death) and cells dying through apoptosis (programmed cell death). After Annexin V-FITC / PI labelling, the apoptosis experiment on *Pseudomonas stutzeri* and *Pseudomonas otitidis* was performed.

Table 1
^{1}H and ^{13}C NMR spectral data ($CDCl_3$, 850 MHz) of compounds **1–3.**

p	1		2		3	
	δ_C	δ_H m *J* (Hz)	δ_C	δ_H m *J* (Hz)	δ_C	δ_H m *J* (Hz)
1	47.5	3.63 ddd (11.2, 9.7, 9.1)	47.4	3.64 ddd (11.2, 9.7, 9.1)	–	–
2	38.1	2.55 ddd (15.4, 11.8, 6.6) 1.58 dd (15.4, 6.6)	37.7	2.52 ddd (15.4, 11.8, 6.4) 1.61 dd (15.4, 6.4)	173.5	–
3	77.1	4.17 brd (6.6)	77.1	4.17 brd (6.4)	112.3	5.80 brs
4	84.7	–	84.5	–	183.1	–
5	57.5	2.31 br t (9.7)	57.4	2.33 br t (9.7)	35.1	–
6	76.0	4.73 dd (11.2, 9.7)	76.3	4.73 dd (11.2, 9.7)	49.3	2.03 dd (13.8, 2.7) 1.33 d (13.8)
7	46.7	3.14 m	46.3	3.16 m	63.9	4.11 m
8	76.2	5.22 ddd (9.7, 5.2, 1.5)	74.4	5.20 ddd (9.7, 5.2, 1.5)	47.7	2.50 dd (14.4, 2.7) 1.45 dd (14.4)
9	34.7	2.66 dd (15.4, 5.2), 2.49 d (15.4)	35.2	2.65 dd (15.4, 5.2) 2.47 d (15.4)	87.2	–
10	141.8	–	142.3	–	24.7	1.60, s
11	137.0	–	136.8	–	29.4	1.30, s
12	168.6	–	167.9	–	24.5	1.33, s
13	122.3	6.23 d (3.2) 5.58 d (3.2)	123.0	6.20 d (3.2) 5.63 d (3.2)		
14	118.6	5.17 brs 5.02 brs	117.7	5.16 brs 5.00 brs		
15	50.1	4.33 d (11.8) 3.96 d (11.8)	50.0	4.34 d (11.8) 3.96 d (11.8)		
16	173.0	–	165.2	–		
17	76.0	–	139.4	–		
18	51.1	3.88 d (11.2) 3.65 d (11.2)	126.9	6.35 s 5.92 s		
19	23.3	1.55 s	62.1	4.36–4.37 (2H)		

Table 2
Cytotoxic effect of the total extract and lactones **1–3** obtained from C. *pseudosinaica.*

	IC_{50} (mean ± SD; n = 3) (μg/mL)			
	HepG2	PC-3	HT-29	HEKa
Comp# 1	0.60 ± 0.04	0.20 ± 0.01	0.04 ± 0.01	0.50 ± 0.01
Comp# 2	5.00 ± 0.61	5.30 ± 0.65	2.50 ± 0.28	2.50 ± 0.30
Comp# 3	4.60 ± 0.51	11.90 ± 1.31	8.90 ± 0.97	3.10 ± 0.10
Total extract	4.10 ± 0.37	2.80 ± 0.23	1.32 ± 0.12	0.2 ± 0.02
Doxorubicin	0.80 ± 0.10	1.20 ± 0.60	1.70 ± 0.02	0.7 ± 0.04

Using flow cytometry, the proportion of cells expiring through apoptosis (programmed cell death), as opposed to necrosis (non-programmed cell death) was calculated. Sesquiterpenid **1** caused high apoptosis percentage in prostate cancer cells (PC-3) by 91.50 %, followed by hepatocellular carcinoma cells (HepG2) by 79.72 % after being treated with **1**. While the higher percentage of necrosis was observed in colorectal cancer cells (HT-29) by 23.60% compared to cell control and other response of cells to **1** (Fig. 4). These findings imply that the induction of apoptosis in the examined cancer cells is the cause of the cytotoxicity displayed by **1**.

*3.2.4. Antibacterial effects of the total extract and lactones **1–3***

The data in Table 3 indicated that the total extract exhibited moderate activity against three gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*) with an inhibition zone diameter of almost 14 mm. The activity of the total extract was improved against the two tested Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). The isolated lactones (**1–3**) demonstrated mild to moderate action against the tested Gram-negative bacteria, with inhibition zone width falling between 08 ± 1.20 and 10 ± 1.50 mm. **3** showed potent activity against the two tested Gram-positive bacteria *B. subtilis* (inhibition zone = 15 ± 1.70 mm) and *S. aureus* (inhibition zone = 16 ± 1.90 mm) (Table 3).

*3.2.5. Antifungal effects of the total extract and compounds **1–3***

The total extract exhibited potent antifungal activity against all test fungi (i.e., *Candida albicans*, *C. tropicals*, *Aspergillus flavus*, and *A. niger*) with the inhibition zone diameter ranging from 12 to 18 mm. Sesquiterpenoids **1–3** showed moderated antifungal activity against *C. albicans* and *C. tropicals* with inhibition zone diameter in a range of 8–12 mm. Also, they showed moderate antifungal activity against *A. flavus* and *A. niger* with inhibition zone diameter in a range between 11 and 14 mm.

*3.2.6. Antioxidant effects of the total extract and compounds **1–3** against DPPH*

The free radical scavenging activity of the isolates **1–3** and the total extract was assessed by employing of DPPH assay. The total extract showed the best activity with an IC_{50} value of 23.6 μg/mL. **3** showed an antioxidant effect with an IC_{50} value of 55.9 μg/mL. In comparison, lactones **1** and **2** showed moderate effects with almost similar IC_{50} value of 121.0 μg/mL compared with positive control vitamin C, which has an IC_{50} value of 14.2 μg/mL.

3.3. Molecular modeling

The docking simulation was carried out to predict the impact of our extracted compounds on the EGFR enzymes. PDB: 4I23 is thought to be one of the two different wild types of EGFR enzymes

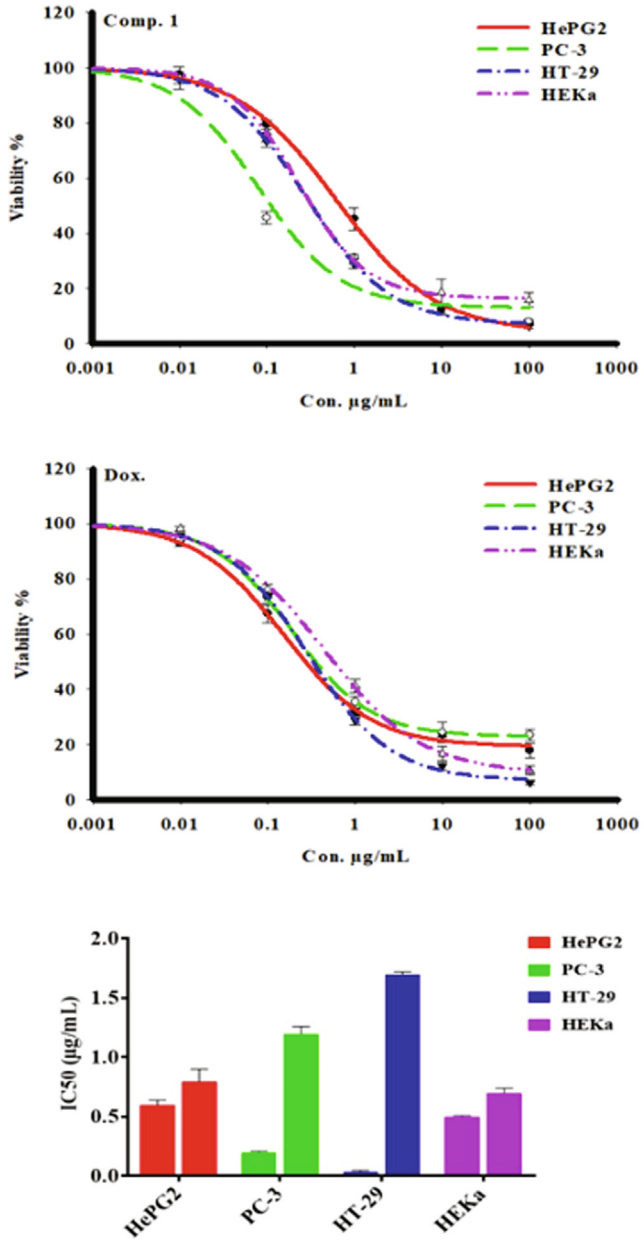

Fig. 2. The cytotoxicity curves of sesquiterpenoid **1** against human cancer cells.

since it may afford a prediction for future experimental studies to show the effects of these extracts on the EGFR enzymes (Ayati et al., 2020; Lakshmi et al., 2018). By way of the extracts demonstrated anticancer activity, particularly against the HT-29 cell line. Through molecular docking experiments with the MOE "2015.10" program, we further investigated the ways that 17-epichlorohyssopifolin A (**1**), chlorjanerin (**2**), and the monoterpenoid loliolide (**3**) bind to the EGFR protein. The ATP-binding region of the EGFR kinase domain was taken into consideration for docking experiments in this study because small molecule inhibitors engage with the kinase domain rather than the extracellular domain. Whereas **1** displayed good binding, S = -6.5590 kcal/mol, Rmsd = 1.4528 above two H-donor and H-acceptors interaction among O 20 of the secondary hydroxyl group with both of Met 766 and Lys 754 crosswise distance 4.04 and 3.22 Å, respectively (Table 4 and Fig. 5).

Meanwhile, chlorjanerin analog (**2**) offered attractive binding, S = –6.6277 kcal/mol, Rmsd 1.1688 across three H-bonds, H-donor over O20 of the secondary hydroxyl group with Thr 790 through intermolecular distance 3.05 Å, two H-acceptors through Lys 754 with both of O 17 of the carbonyl group and chlorine atom through bond distances 2.87, 3.18 Å, respectively (Fig. 6).

Moreover, **3** analogs disclosed negligible binding, S = –5.3249 k cal/mol, Rmsd = 10.7858 above interaction between O13 of the secondary hydroxyl group with Asp 855 through H-donor with distance (3.21 Å) (Fig. 7).

Furthermore, the doxorubicin drug demonstrated higher binding, S = –8.5632 kcal/mol, Rmsd = 1.3133 over one H-donor and one H-acceptor, composed by the anomeric carbon with Asp 855 and both O20 with Met 793 through distances (3.28 and 2.88, individually) (Fig. 8).

4. Discussion

Exploration of the plant extracts' biological activity is a scaffold for identifying natural compounds. Natural products have shown a safety profile and acquired wide acceptance by the scientific community. Also, they are of particular interest as lead bioactive molecules for the management of several diseases (Hörl, 2010; Abdou et al., 2013; Akanda et al., 2019; Lass and Belluzzi, 2019; Shi et al., 2019; 19; Kang et al., 2018). The increasing need for antibiotics that keep pace with the evolution in the world of pathogenic microbes, the urgent need for nutraceutical products that have strong antioxidant activities, and the necessity of finding less painful antitumor agents imply extensive bioactive screening of plant species in the environments which are a gift from the Almighty. One of the most common plant families is the Asteraceae family, which in turn comprises many genera. Within the Asteraceae family, the genus *Centaurea* is a productive source of bioactive extracts and/or secondary metabolite individuals. Several *Centaurea* species have been classified as medicinal plants, traditionally used for treating and managing diseases. Natural metabolites originating from restorative plant-based therapies exhibit substantial bioeffects and drug-like characteristics owing to their exceptional chemical diversity. They are promising candidates for finding and developing effective remedies. Sesquiterpene lactones are naturally occurring bioactive metabolites frequently identified from members of the *Centaurea* genera. Several scientific reports relate the antiproliferative or the proapoptotic activity to the presence of α-methylene-γ-lactone ring (Chicca et al., 2011).

Aguerin B, a sesquiterpene lactone isolated from *C. deflexa* displayed antiproliferative activity against human pancreatic and colonic cancer cells through induction of apoptotic cell death (Chicca et al., 2011). On both acute lymphoblastic leukemia (CCRF-CEM) and its multidrug-resistant subline CEM/ADR5000, aguerin B and cynaropicrin demonstrated strong action (Formisano et al., 2017). Interestingly, prior research showed intriguing information about the structure-action correlations in the family of acylated guaiane sesquiterpenes (Formisano et al., 2017). From *C. omphalotricha*, 3-acetyl cynaropicrin, and 4′-acetyl cynaropicrin were reported. These metabolites exhibited cytotoxic effects against human leukemia cells, U937 (IC_{50} = 5.1 ± 0.4 µmol/L) and HL-60 (IC_{50} = 2.0 ± 0.9 µmol/L) (Kolli et al., 2012). Solstitialin A and 15-dechloro-15-hydroxychlorojanerin isolated from *C. solstitialis* exhibited potent antiproliferative activity towards mouse brain cancer cells C6 cells and human uterus carcinoma HeLa cells (Erenler et al., 2016).

Cynaropicrin and aguerin B from *C. behen* L. were characterized by the highest cytotoxic activities against A2780 cells. The anti-angiogenic study of these two sesquiterpene lactones showed a remarkable inhibitory effect on the proliferation and migration of HUVECs. Moreover, in the CAM test, cynaropycrin and aguerin B exhibit strong angio-inhibitory effects (Shakeri et al., 2018). Using

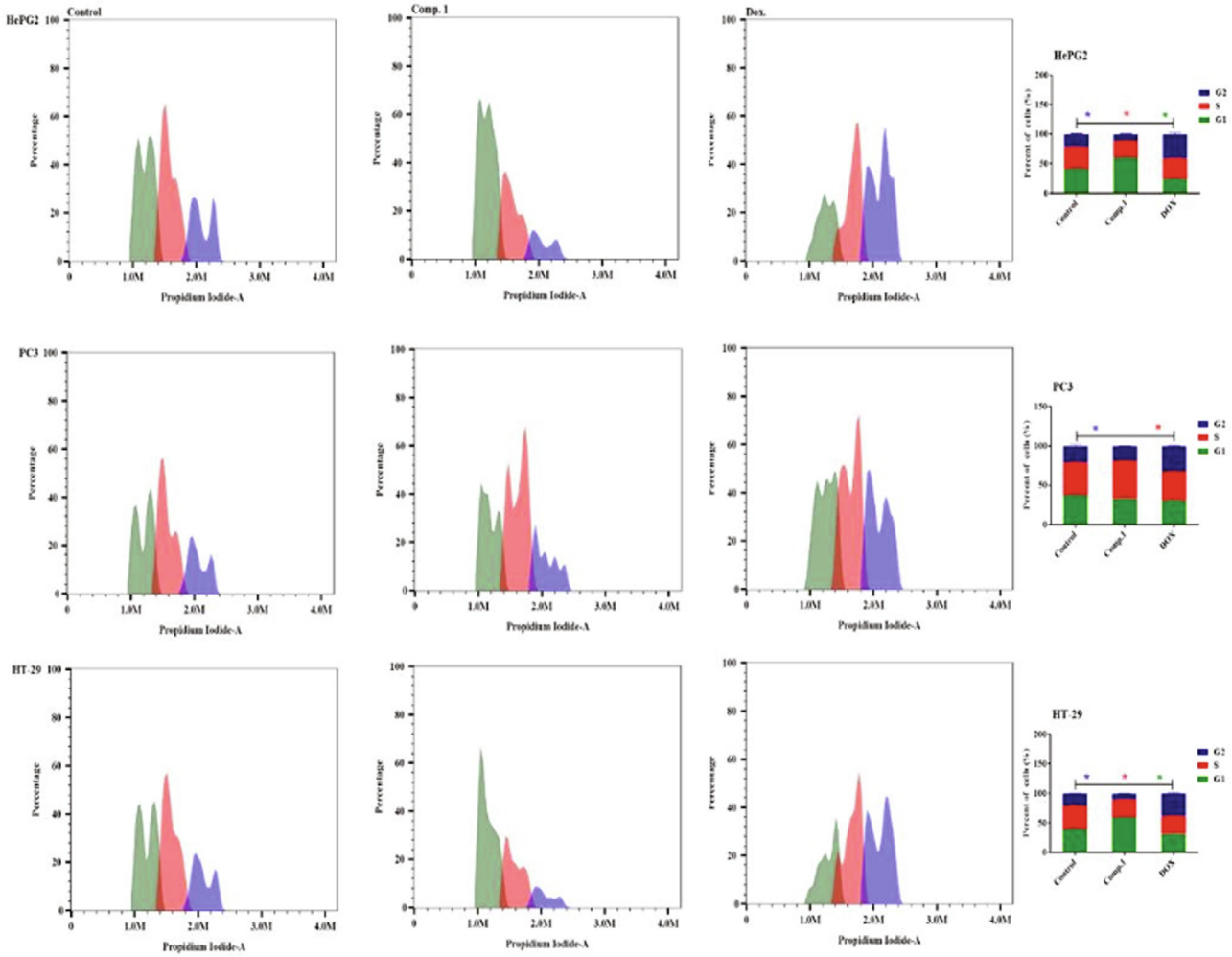

Fig. 3. Cell cycle phases (mean ± SD; n = 3) of HepG2, PC-3, and HT-29 after compound **1** exposure. After 48 h of exposure to **1**, DNA cytometry analysis was used to identify the cell cycle distribution. ** $p < 0.01$ and *** $p < 0.001$.

the in vitro MTT test on three human cancer cell lines, sesquiterpene lactones extracted from *C. papposa* (Coss.) showed that they could be potential anticancer drugs for cervical cancer (Garfakou et al., 2021). In the current study, the total extract and isolated lactones showed significant cytotoxicity, whereas compound **1** showed highly potent effects against all cancer cells comparing with the positive control. Compound **1** is like the previously isolated sesquiterpene lactone aguerin B in that they induce apoptotic cell death (Chicca et al., 2011). There are many shreds of evidence that the apparent cytotoxic activity exerted by the *Centaurea* sesquiterpene lactone is related to the presence of the α-methy lene-γ-lactone ring.

The antibacterial activity of sesquiterpene lactones isolated from the genus *Centaurea* was reported. For example, the antibacterial activity of two amino acid conjugated sesquiterpene lactones, namely, centaureolide A and B, isolated from *C. pungens* against several Gram-positive and Gram-negative strains were estimated, and a resistance to the tested compounds by *Pseudomonas fragi* was reported (Labed et al., 2019). Deacylcynaropicrin 8-O-[3′-hydroxy-2′-methylpropionate] isolated from *C. rhizantha* exhibited moderate antibacterial potency against *Staphylococcus aureus* (MIC/MBC = 500 μg/mL) (Shakeri et al., 2019). In this work, studying the antibacterial effects of the total extract, two sesquiterpene lactones (**1**, **2**) and a monoterpene lactone (**3**) reflected the superior activity of the total extract. The loliolide (**3**), a famous worldwide monoterpene, was reported from a diversity of organisms, including insects, algae, and plants in both marine and terrestrial environments. The activity of the total extract exceeds that of the sesquiterpene lactones, which refers to the presence of other ingredients caused by the observed activity. The weak to moderate activity against Gram-negative bacteria and the significant activity against Gram-positive bacteria of loliolide (**3**) observed in this context align with those previously reported in the literature (Grabarczyk et al., 2015).

The antifungal activity of sesquiterpene lactones isolated from the *Centaurea* species was previously reported. Vajs et al. (1999) reported five sesquiterpene lactones from *C. nicolai* Three of them, namely, salograviolide A and its 9-O-acetyl and 3-O-deacetyl-9-O-acetyl showed antifungal effect against six fungi; *Phomopsis helianthi, Fusarium tricinctum, Cladosporium cladosporoides, Penicillium ochrochloron, Aspergillus ochraceus,* and *Aspergillus niger*, however neither of these three lactones showed antifungal effect against *Trichoderma viride* (Vajs et al., 1999). Cnicin, 4′- acetylcnicin, 8 α-[(4-acetoxy-5-hydroxy)-angelate] salonitenolide), a sesquiterpene lactone isolated from *C. thessala* appreciated antifungal activity when tested against a set of nine fungal species, using the (Skaltsa et al., 2000). In the current work, the total extract exhibited antifungal activity against all test fungi (i.e., *Aspergillus niger (A. niger), Aspergillus flavus (*A. flavus), *Candida albicans (*C. albicans), and *Candida tropicals (C. tropicals)* and) with a diameter of the inhibition zone in the range of 12–18 mm. The positive control was amphotericin B and the diameters of inhibition zones

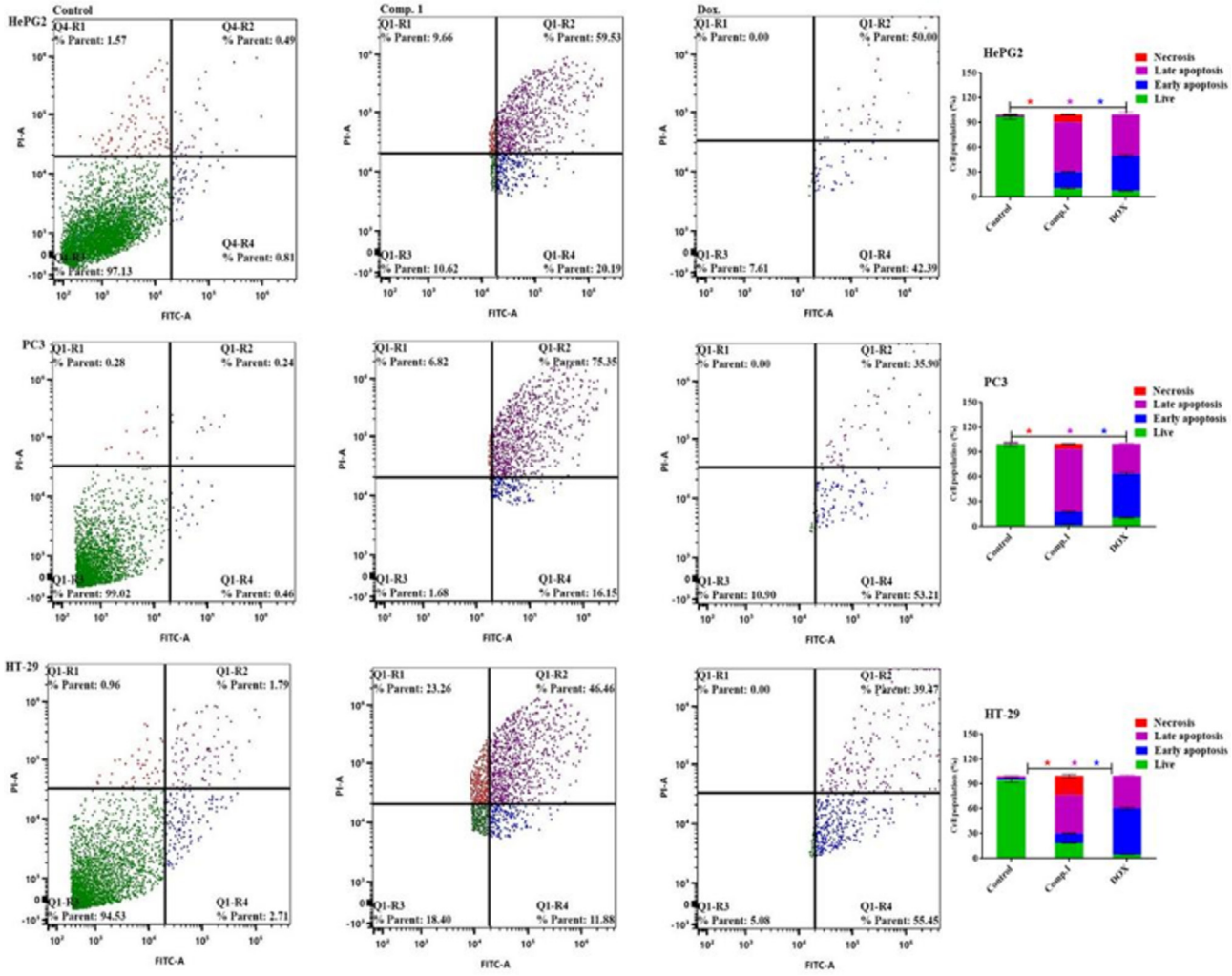

Fig. 4. The apoptotic effect of compound **1** (mean ± SD; n = 3) on the cell apoptosis in HepG2, PC-3, and HT-29 cancerous cells by Annexin V-FITC/PI labeling by flow cytometry. Exposure to treatments for 48 h. ** $p < 0.01$ and *** $p < 0.001$.

Table 3
Activity of the total extract and compounds **1**–**3** against certain strains of Gram-positive and Gram-negative bacteria.

Tested samples	**Gram-negative**			**Gram-positive**	
	Escherichia coli	*Pseudomonas aeruginosa*	*Proteus vulgaris*	Bacillus subtilis	Staphylococcus aureus
	Inhibition zone (mm)				
Comp#1	09 ± 1.30	08 ± 1.20	08 ± 1.10	12 ± 1.60	11 ± 0.43
Comp#2	09 ± 1.30	08 ± 1.30	09 ± 1.30	12 ± 1.60	12 ± 0.33
Comp#3	10 ± 1.40	09 ± 1.20	10 ± 1.50	15 ± 1.70	16 ± 1.90
Total extract	14 ± 1.80	14 ± 1.80	14 ± 1.70	17 ± 1.90	18 ± 2.00
Amoxicillin	33 ± 2.90	39 ± 2.40	30 ± 4.00	23 ± 6.40	20 ± 1.90

Table 4
Docking data of the extracted compounds **1**–**3.**

Code	S (Kcal/mol)	Rmsd	Interaction with ligand	Types of Interactions	Distance (Å)
Comp# 1	−6.5590	1.4528	O 20 with Met 766	H-donor	4.04
			O 20 with Lys 754	H-acceptor	3.22
Comp# 2	−6.6277	1.1688	O 20 with Thr 790	H-donor	3.05
			O 17 with Lys 754	H-acceptor	2.87
			Cl 22 with Lys 754	H-acceptor	3.18
Comp# 3	−5.3249	0.7858	O 13 with Asp 855	H-donor	3.21
Doxorubicin	−8.5632	1.3133	C 32 with Asp 855	H-donor	3.28
			O20 with Met 793	H-acceptor	2.88

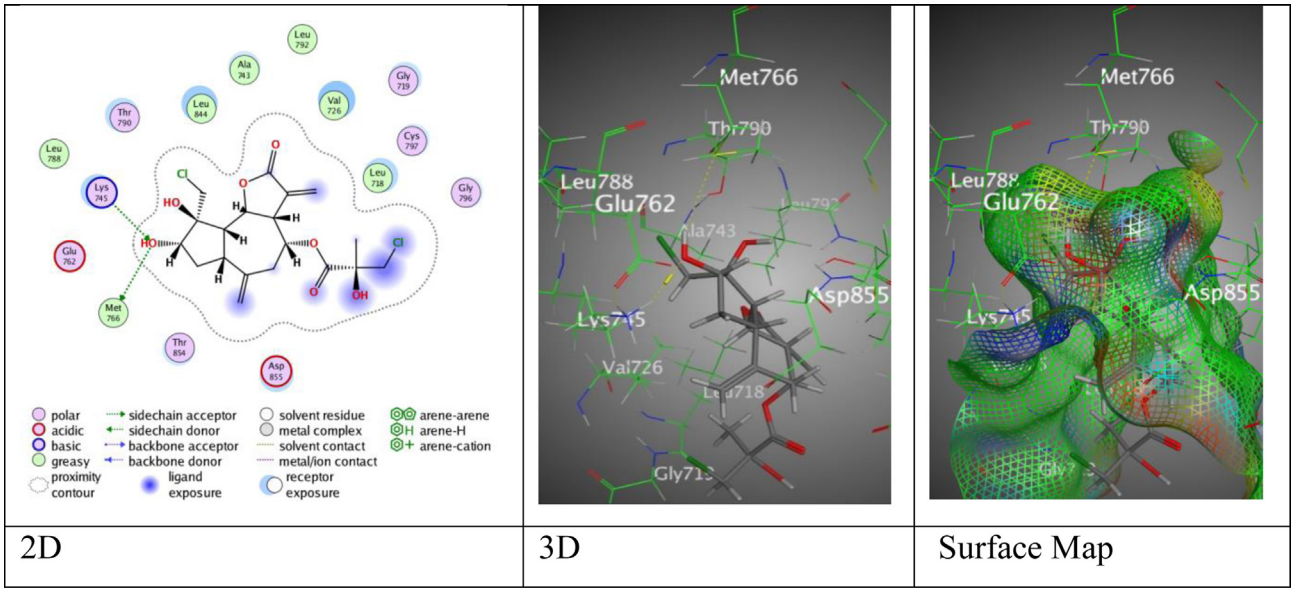

Fig. 5. Binding images of 17-epichlorohyssopifolin A (**1**) with (PDB ID: 4I23).

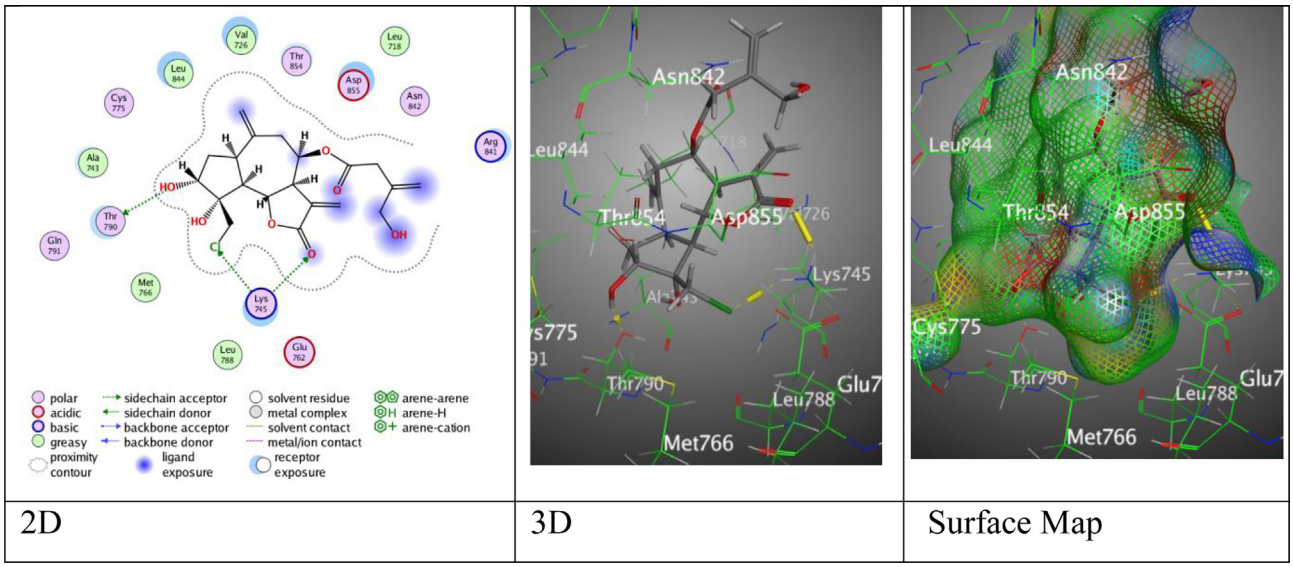

Fig. 6. Binding images of chlorjanerin (**2**) with (PDB ID: 4I23).

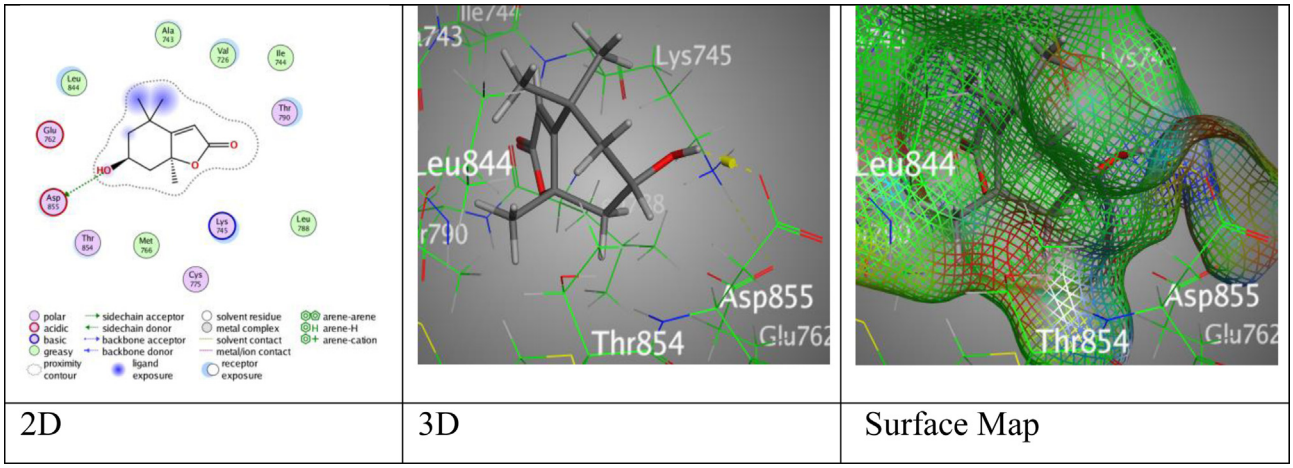

Fig. 7. Binding images of loliolide (**3**) with (PDB ID: 4I23).

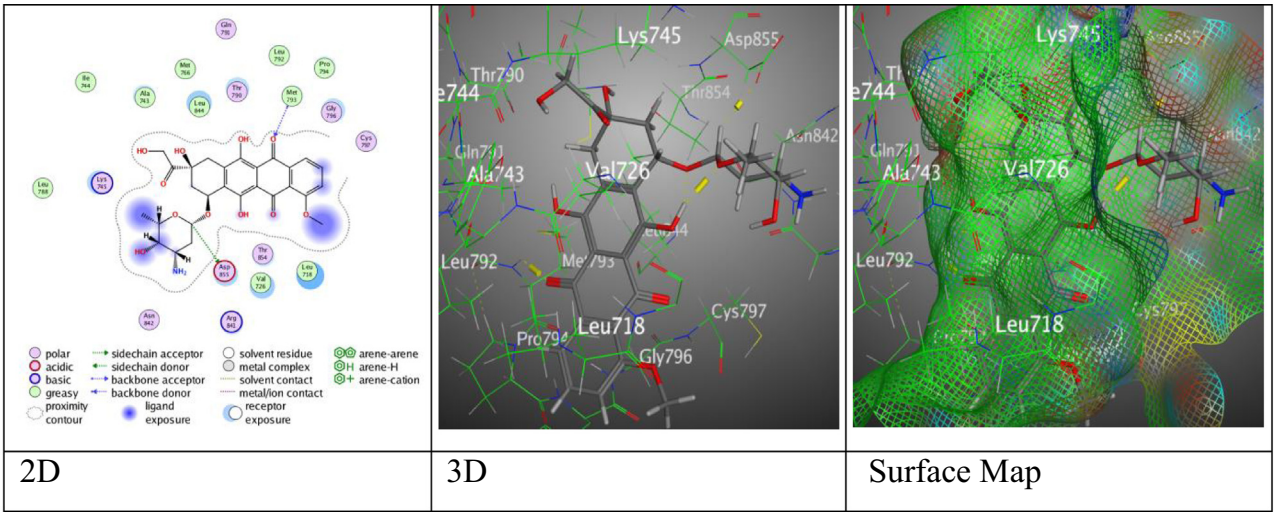

Fig. 8. The binding of doxorubicin with (PDB ID: 4I23).

were 41 ± 0.70, 33 ± 1.11, 29 ± 0.90, and 25 ± 0.87 mm against *A. niger, A. flavus, C. albicans, and C. tropicalis,* respectively. The potent effect of the total extract and the weak effect of the compounds **1–3** may be due to the other components that were not yet isolated.

As aforementioned in the results section, the antioxidant activity of the total extract is higher than those estimated for compounds **1–3**. Several scientific reports attributed the significant antioxidant activity noted within species of the *Centaurea,* such as *C. cyanus* L. and *C. bornmuelleri* Hausskn, to their richness in phenolics (Noman et al., 2022). The increased worldwide interest in searching for new nutraceutical products directed attention to Centaurea plants as potential sources of food additives (Noman et al., 2022).

The following summaries were given as the results of molecular docking: 1) The extracted compounds' docking scores produced satisfactory results with ranges between −5.3249 and −6.6277 Kcal/mol, 2) The most diverse kinds of bindings, such as H-donor and H-acceptor, were revealed in the extracted compounds **1–3** and better support for the optimal binding with numerous amino acids 4I23 was given. 3) The most protein amino-acids, including "Met 766, Lys 754, Thr 790, and Asp 855", were attached to the extracted compounds **1–3**, which provided strong proof for the binding processes, 4) The surface map, 2D, and 3D images that were produced clearly showed the close connections between the various kinds of 4I23 amino acids and the extracted compounds.

5. Conclusions

Three cytotoxic sesquiterpene lactones were isolated and identified as 17-epichlorohyssopifolin A (**1**), chlorjanerin (**2**), and loliolide (**3**). The cytotoxic effects of the isolated compounds were examined against three human cancer cells (HepG2, PC-3, and HT-29). Compounds **1–3** and the total organic extract demonstrated cytotoxicity against the cancer cells with IC_{50} values ranging between 0.6 ± 0.04 and 5.0 ± 0.61 µg/mL against HepG2; 0.2 ± 0.01 and 11.9 ± 0.1.31 µg/mL against PC-3 and 0.04 ± 0.013 and 8.9 ± 0.97 µg/mL against HT-29. Compound **1** showed the most potent cytotoxic activity against PC-3, HT-29, and HepG2 with an IC_{50} value of 0.2 ± 0.01, 0.04 ± 0.013, and 0.6 ± 0.04 µg/mL, respectively. Compound **1** showed a slightly safer effect on the normal cells HEKa compared to its effect against PC-3 and HT-29 and was safe compared to the other tested substances. The compounds' antimicrobial efficacy was assessed against a collection of Gram-negative and Gram-negative microorganisms. Finally, the antifungal effect of the isolated compounds was examined. These findings can explain the antiproliferative effect of compound **1.**

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.04.017.

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