



Metabolomic Profiling and Cytotoxic Activity of *Launaea nudicaulis*: Molecular Docking with Topoisomerases

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Abstract

Launaea nudicaulis (L.) Hook. f., Asteraceae, is a wild plant growing in Egypt, used traditionally for treatment of many diseases. LC-HRMS analysis of different polarity soluble extracts allowed the annotation of fifteen compounds: three alkaloids, four flavonoids, three phenolic acids, three coumarins, one sphingolipid, and one triterpene. Chemical investigation led to isolation and identification of caffeic acid, luteolin, luteolin-7-*O*-glucoside, lupeol, β -sitosterol, and palmitic acid. Cytotoxic evaluation for hexane, CH_2Cl_2 , ethyl acetate, and *n*-butanol extracts using MTT assay against three cancer cell lines HL-60, HT-29, and MCF-7 showed a remarkable cytotoxic activity for the CH_2Cl_2 -soluble extract against HL-60 and HT-29 with IC_{50} 5.8 and 8.26 $\mu\text{g/ml}$, respectively, as well the *n*-butanol extract showed good activity against HL-60 and HT-29 with IC_{50} 11.6 and 9.6 $\mu\text{g/ml}$, respectively. Docking study was performed on topoisomerase enzymes (I, $\text{II}\alpha$, and $\text{II}\beta$) and provided a rationale for the biological outcomes where three natural compounds in the plant strongly bound to the proteins, particularly, luteolin-7-(6"-malonylneohesperidoside) with binding affinities of -11.341 , -10.866 , and -10.111 kcal/mol, respectively, and kaempferol-3-*O*-[6"-malonyl- β -D-apiofuranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside] with binding affinities of -10.796 , -10.102 , and -9.916 kcal/mol, respectively. Also, luteolin-7-*O*- β -D-glucopyranoside docked with higher binding affinity to topoisomerase I (-10.367 kcal/mol) compared to topoisomerases $\text{II}\alpha$ and $\text{II}\beta$.

Keywords Cytotoxicity · Flavonoid glucosides · LC-HRMS · Molecular docking · Topoisomerase enzymes

Introduction

Launaea nudicaulis (L.) Hook. F. is a wild plant that belongs to family Asteraceae; many species of the genus *Launaea* are widely distributed from Europe to Africa and Asia

particularly around the Mediterranean woodlands. *L. nudicaulis* has been traditionally known for its numerous biological activities as its milky material is used as laxative while leaves had strong anti-inflammatory effects and are used as antipyretic, which reduce skin itching, bumps, atopic dermatitis, and rheumatism; also its roots were chewed to cure toothache (Cheriti et al. 2012). Biological studies revealed that *L. nudicaulis* different extracts showed insecticidal, antifungal, anticancer (Rashid et al. 2000), antimicrobial (Cheriti et al. 2012), and antioxidant activity (El-Sharkawy et al. 2017). Previous phytochemical studies reported the presence of sphingolipids, steroids, and triterpenoids in the hexane fraction of *L. nudicaulis* (Riaz et al. 2012).

Other classes of secondary metabolites have been isolated and identified from the *L. nudicaulis*, as flavonoids and phenolic derivatives (Mishra et al. 2012), alkaloids (El-Sharkawy et al. 2017), and volatile oils (Al-Mahrezi et al. 2011; Elshamy et al. 2019).

Dereplication technique is an important step in the drug discovery as it improves and speed up the efficiency of drug discovery through quick identification of known secondary

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metabolites that save efforts for the isolation and identification of new metabolites. Dereplication is combined with metabolomic approaches to perform untargeted chemical profiling of natural extracts or to identify a specific class of metabolites (Amin et al. 2022).

Molecular docking or molecular simulation is one of the computational techniques that had been widely applied in drug discovery process. In molecular docking, the desired compounds are docked into the binding sites of the desired target, then the binding affinity is recorded (Elwekeel et al. 2022a, b). Molecular docking is a very useful technique when directed to analysis and exploring experimental results thus providing new ways for future work.

Cancer constitutes one of the serious health problems worldwide and it is the second reason for death in the world. Early diagnosis and improvements in the methods of treatment had reduced the percentage of mortality. Cytotoxic chemotherapeutics had been identified as the systemic method for treatment of cancer; these drugs mostly act as DNA-damaging agents, which are responsible for inhibition of cell division. The topoisomerase enzymes type I and type II are important in DNA metabolism, as these enzymes are responsible for adjustment of DNA supercoils which is vital element in transcription and replication cellular process. Both enzymes had been recognized as clinically significant targets for chemotherapy in cancer treatment through their inhibition (Okoro and Fatoki 2022). During the last 30 years, enzyme inhibitors with diverse structures have been established using drug screening and molecular simulation programs. Doxorubicin and etoposide are inhibitors commonly used in the clinic.

In this work, metabolomic profiling using LC-HRMS analysis of different extracts, as well as the isolation and identification of secondary metabolites, were carried out, together with their cytotoxic evaluation against three cancer cell lines. Molecular docking studies for the secondary metabolites dereplicated in the plant against topoisomerase enzymes (I, II α , and II β) were estimated to explore the cytotoxic potential of the plant.

Materials and Methods

The whole plant of *Launaea nudicaulis* (L.) Hook. f., Asteraceae, was collected in April 2017 from Cairo, Alexandria desert road near Mirage city, Egypt. The plant was identified by Prof. Dr. Abdelhalim Mohamed (Plant Taxonomy Department, Agricultural Research Center, Dokki, Giza, Egypt). Voucher specimen (2017-BUPD-74) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University.

Three cell lines (MCF-7, human breast adenocarcinoma; HL-60, human promyelocytic leukemia; and

HT-29, human colon carcinoma) were used for cytotoxicity evaluation using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Tolosa et al. 2015). Cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD; HPACC, Salisbury, UK). MCF-7 cells were cultured at 37 °C, 5% (v/v) CO₂ in RPMI1640 medium, supplemented with 5% (v/v) fetal bovine serum (FBS), 1% (w/v) L-glutamine, 1% sodium pyruvate and 0.4% (w/v) antibiotics (50 U/ml penicillin, 50 mg/ml streptomycin), HL-60 cells were grown in 5% (v/v) CO₂ in RPMI 1640 medium at 37 °C, supplemented with 10% (v/v) FBS, 1% (w/v) L-glutamine, and 0.4% (w/v) antibiotics (50 U/ml penicillin and 50-mg/ml streptomycin). The HT-29 cells were cultured at 37 °C, 5% (v/v) CO₂ in Dulbecco's Modified Eagle Medium with high glucose (4.5 g/l) supplemented with 10% (v/v) FBS, 1% (w/v) L-glutamine, and 0.4% (w/v) antibiotics (50 U/ml penicillin, 50 mg/ml streptomycin) and routinely subcultured twice per week. Cell proliferation was evaluated in cell lines by the MTT assay in triplicates as previously described (Tolosa et al. 2015). Dose–response curves were generated and the IC₅₀ values were defined as the concentration of extract required to inhibit cell proliferation by 50%. Doxorubicin was used as a positive control.

Air-dried powdered plant material (1 kg) was extracted by maceration with 70% ethyl alcohol three times at room temperature then concentrated under reduced pressure using rotary evaporator to afford a crude alcoholic extract (50 g), which was dissolved in water and subjected to a partition with immiscible organic solvents hexane, CH₂Cl₂, EtOAc, and *n*-butanol successively to afford 2.7 g, 6.4 g, 2.4 g, and 2 g, respectively. The alcoholic extract and the fractions were used for LC-HRMS analysis and cytotoxic evaluation. For isolation, powdered plant material (5 kg) was defatted by extraction with hexane (3 $\times$ 3 l) at room temperature, then it was concentrated under reduced pressure using rotary evaporator to afford the hexane extract (10 g), the defatted plant powder was dried then extracted with methanol three times and evaporated under vacuum to afford the methanolic extract (120 g).

For the metabolomics analysis procedure, all different extracts were subjected to metabolomic analysis using analytical LC-HRMS according to the reported method in (Elwekeel et al. 2022a, b). The positive and negative ionization mode data sets from each of the respective plant extract were dereplicated against Dictionary of Natural Products and METLIN databases. Details for the isolation and purification procedures of major compounds are described as part of the supplementary data.

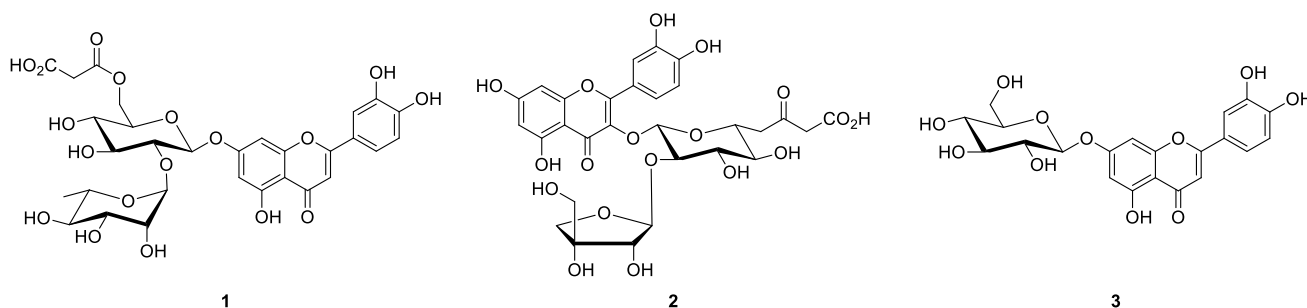
Molecular docking studies were performed using the Molecular Operating Environment (MOE) (version 2015.10) as the computational software. The X-ray crystallographic structure of human topoisomerase I (PDB ID: 1T8I) (Staker

et al. 2005), human topoisomerase II α (PDB ID: 5GWK) (Wang et al. 2017), and human topoisomerase II β (PDB ID: 3QX3) (Wu et al. 2011) were obtained from the Protein Data Bank <https://www.rcsb.org/structure> to investigate binding affinities and interactions of compounds toward the receptors active sites. Hydrogen atoms and missed bonds and connections were added, and the potential of the receptor atoms was fixed. To evaluate the accuracy of docking, a re-docking process of the co-crystallized ligand with each enzyme (camptothecin for topoisomerase I “1T8I” and etoposide for both topoisomerase II α “5GWK”, and topoisomerase II β “3QX3”) was performed using the Amber10: EHT force field, London ΔG scoring function for the placement of poses, and the GBVI/WSA ΔG scoring function for poses refinement to detect the binding energy score, amino acid interactions, and relative mean square deviation (rmsd). The re-docking process succeeded in regenerating the orientation of the co-crystallized ligand with rmsd values of 1.0, 0.7, and 1.3 Å. Three dereplicated metabolites in *L. nudicaulis*, luteolin-7-(6''-malonylneohesperidoside) (**1**), kaempferol-3-*O*- β -D-(6''-*O*-malonyl)-glucopyranoside-(1 $\rightarrow$ 2)-apiofuranosyl (**2**), and luteolin-7-*O*- β -D-glucopyranoside (**3**), were imported to MOE and underwent 3D protonation, then they were partially charged and energy-minimized with force field Amber10: EHT, and a virtual ligand database was created. The entire energy-minimized ligand database was docked with the prepared catalytic domains of each enzyme using the same parameters as in the re-docking process. For each docked compound, 30 docked poses were chosen, followed by refinement into the best 5 docked poses. The binding interactions of the docked compounds with each receptor and docking score were studied using 2D and 3D pictures.

identified, as aspidofractinin, *N*-(2-methylpropylamide)-2*E*,4*E*-dodecadienamide, and ruscopine; three coumarins were annotated as umbelliferone, aesculetin, and 3,4-dihydroscopoletin; three flavonoid glycosides were dereplicated as luteolin 7-(6''-malonylneohesperidoside), kaempferol-3-*O*-[6''-malonyl- β -D-apiofuranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside], and luteolin-7-*O*- β -D-glucopyranoside. Other identified compounds were luteolin, caffeic acid, 1-*O*-caffeoylgalactoside, and chicoric acid methyl ether. Finally, the sphingolipid nidicaulin B and the triterpene lupeol (Supplementary data; Table S1, Figure S2) completed the metabolomics analysis of the different extracts with annotation for each dereplicated compound.

Depending on the physicochemical properties and spectroscopic data (^1H , and DEPT-Q NMR) (Supplementary data: Figure S4–S15) as well as comparison with the reported data and the results of metabolomic analysis, six compounds L1–6 (Supplementary data: Figure S3) were isolated and identified as caffeic acid (L1) (Atoui et al. 2005), luteolin (L2) (Elwekeel et al. 2022a, b), luteolin-7-*O* glucoside (L3) (Lin et al. 2015), β -sitosterol (L4) (El-Hawary et al. 2016), lupeol (L5) (Silva et al. 2018), and palmitic acid (L6) (Di Pietro et al. 2020).

The cytotoxic activity was evaluated for hexane, CH_2Cl_2 , EtOAc, and *n*-butanol extracts against three human cancer cell lines, i.e., HL-60, HT-29, and MCF-7 using MTT assay; the results was expressed as IC_{50} (Table 1). The results revealed that the DCM extract displayed a significant cytotoxic activity toward HL-60 and HT-29 cells with IC_{50} values of 5.8 and 8.2 $\mu\text{g/ml}$, respectively, followed by the *n*-butanol extract with IC_{50} values of 11.6 and 9.6 $\mu\text{g/ml}$, respectively. Also, the EtOAc extract showed a good activity against HT-29 with



Results and Discussion

Chemical profiling of the secondary metabolites of the ethyl alcohol extract of *L. nudicaulis* together with hexane, CH_2Cl_2 , ethyl acetate, and *n*-butanol fractions was carried out using LC-HRMS which resulted in dereplication of 15 compounds belonging to different chemical classes (Supplementary data; Figure S1a–S1d); three alkaloids were

IC_{50} value 10.7 $\mu\text{g/ml}$ and moderate activity against HL-60 with IC_{50} value 12.40 $\mu\text{g/ml}$. CH_2Cl_2 , *n*-butanol, and EtOAc extracts showed a moderate activity against MCF-7 with IC_{50} values 15.1, 18.2, and 19.5 $\mu\text{g/ml}$, respectively, while the hexane extract exhibited a weak cytotoxic activity against the tested cell lines. Previous study on the anticancer activity of the ethanol extract of *L. fragilis* and *L. nudicaulis* against six cell lines using sulforhodamine B (SRB) assay revealed

Table 1 IC₅₀ values of different extracts of *Launaea nudicaulis* against different human cell lines: MCF-7, HL-60, and HT-29

IC ₅₀ µg/ml			Plant extract
HT-29	HL-60	MCF-7	
32.65 ± 3.52	46.84 ± 2.02	50.60 ± 4.01	Hexane
8.20 ± 0.34	5.80 ± 0.01	15.10 ± 2.26	CH ₂ Cl ₂
10.70 ± 0.50	12.40 ± 1.23	19.50 ± 2.20	Ethyl acetate
9.60 ± 0.64	11.60 ± 0.92	18.20 ± 1.86	<i>n</i> -Butanol
1.14 ± 0.12	2.04 ± 0.22	3.62 ± 0.10	Doxorubicin

IC₅₀ concentration of the extract causing 50% inhibition of cell proliferation

Data were expressed as mean values ± standard deviation for triplicate assay

MCF-7 human breast adenocarcinoma, HL-60 human promyelocytic leukemia, HT-29 human colon carcinoma

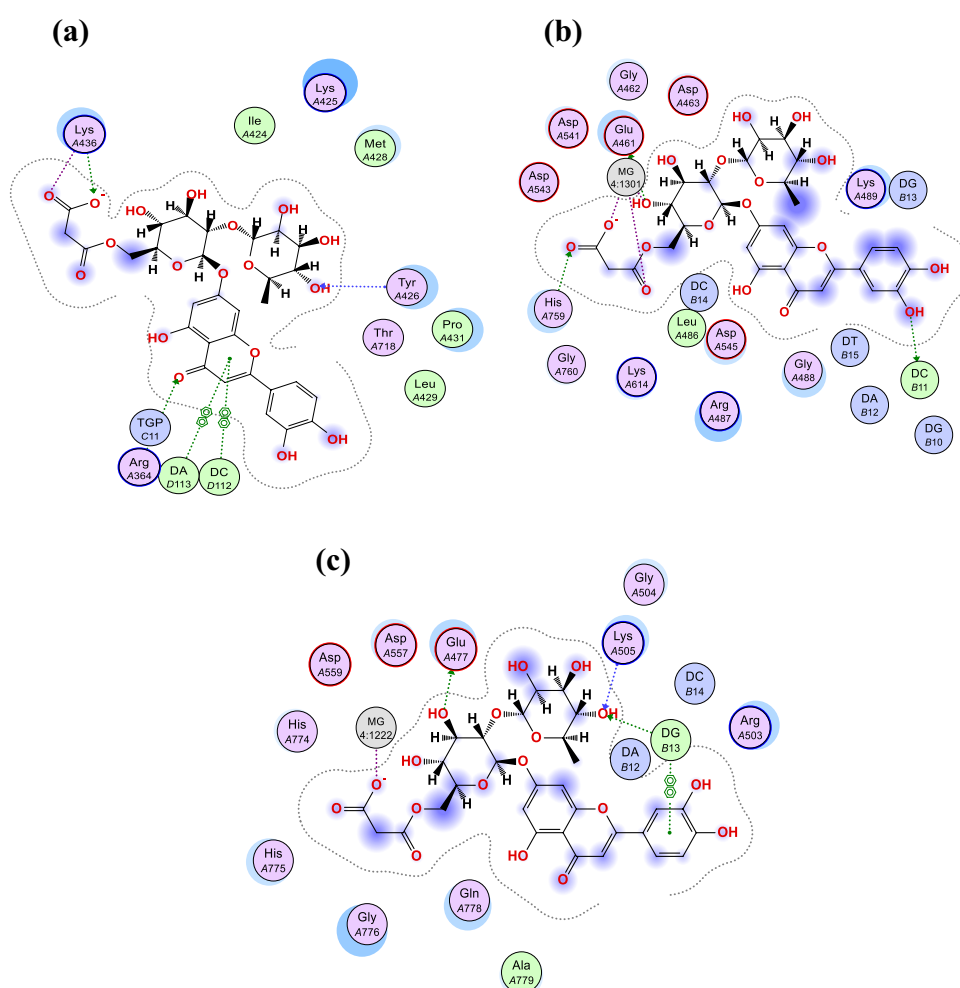
Doxorubicin: positive control

that the ethanol extract of *L. nudicaulis* exhibited cytotoxic activity against lung carcinoma cell line (H1299) (El-Darier et al. 2021). LC/MS analysis of different extracts disclosed that the activity of the CH₂Cl₂-soluble fraction could be attributed, in part, to the alkaloid content, since aspidofractinine alkaloid showed potent cytotoxic activity against BGC-823 cells (human gastric carcinoma), HepG2 cells (Human hepatocellular carcinoma), MCF-7 cells (human breast cancer), SGC-7901 cells (human gastric adenocarcinoma), SK-MEL-2 (human skin cancer), and with SK-OV-3 (ovarian) compared with doxorubicin (Wang et al. 2017). The polar extracts EtOAc and *n*-butanol showed a rich content of phenolic acids, such as caffeic acid, chicoric methyl ether (caffeic acid derivative), and 1-*O*-caffeoylgalactose previous studies revealed that caffeic acid was active as anticancer against colon cancer cell lines (Hashim et al. 2008), while chicoric acid was active against human gastric cancer progress (Sun et al. 2019). Flavonoids as luteolin and luteolin-7-*O*-glucoside, which were detected in the plant extract, have been described as cytotoxic agents against many cancer cell lines (Seelinger et al. 2008). So, the cytotoxic effect of EtOAc and *n*-butanol extracts could be attributed to the presence of phenolic constituents, active redox agents, and fully recognized as antioxidants or scavengers.

Since the isolated compounds L1–L6 have been reported previously to have cytotoxic properties against various cell lines (Baskar et al. 2011; Rajavel et al. 2017) with some of them identified as inhibitors of topoisomerase I (topo I) and/or topoisomerase II (topo II) (Webb and Ebeler 2004), molecular docking study of both isolated and detected metabolites in *L. nudicaulis* extracts has been accomplished to investigate binding modes and interactions of the compounds with topoisomerases. Analysis of binding free energies of the compounds

docked to topo I (1T8I) (Supplementary data: Table S2) demonstrated that the detected metabolite luteolin-7-(6"-malonylneohesperidoside) (**1**) exhibited the lowest binding energy (− 11.341 kcal/mol) (Fig. 1a). This flavonoid intercalated with DNA where the planar aromatic flavonoid nucleus is stacked between the diphosphate cytosine (DC112) and diphosphate adenosine (DA113); binding mode of this compound with topo I depicted three hydrogen bonds with side chains of Arg364 and Lys436 and backbone of Tyr426, in addition to an ionic interaction between malonyl group and Lys436. Similarly, kaempferol-3-*O*-[6"-malonyl-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside] (**2**) (− 10.796 kcal/mol) exhibited hydrogen bond interaction between the apiose moiety and Tyr426 in addition to stabilization through π-π stacking of the flavonoid nucleus with four DNA bases (Supplementary data: Figure S16a). Among the isolated compounds, luteolin-7-*O*-β-D-glucopyranoside (**3**) showed the highest binding affinity (− 10.367 kcal/mol) with four π-π stacking interactions with DNA and two hydrogen bonds formed between both 2"-OH and 6"-OH of the glucose unit and Asp533 (Supplementary data: Figure S16b). Docking simulation of the compounds to topo IIα (PDB: 5gwk) and topo IIβ (PDB: 3QX3) revealed again luteolin-7-(6"-malonylneohesperidoside) (**1**) with the lowest energy of binding (− 10.866 and − 10.111 kcal/mol, respectively) where the docking interaction with topo IIα exhibited stabilization of the compound in the binding site through formation of hydrogen bonding with DNA base in addition to two hydrogen bondings with the enzyme residues Glu461 and His759 and two ionic contacts with Mg ion that further contributed to the high binding affinity (Fig. 1b) while its proposed binding mode to topo IIβ showed the planar aromatic nucleus stacked with diphosphate guanosine (DG13) and stabilized by additional formation of hydrogen bonding, in addition to two hydrogen bonds with the amino acids Glu477 and Lys505 (Fig. 1c). Another noticeable compound is kaempferol-3-*O*-[6"-malonyl-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside] (**2**) with topo IIα and topo IIβ binding affinity values of − 10.102 and − 9.916 kcal/mol, respectively. Its interactions with topo IIα (Supplementary data: Figure S17a) revealed the presence of both π-stacking interaction and hydrogen bonding with DNA base pairs, along with two hydrogen bonds with Glu461 and Lys489 and two contacts with Mg ion, while binding mode to topoisomerase IIβ depicted three hydrogen bonds with Glu477, Asp557, and Gly776 along with three ionic interactions with Mg ion (Supplementary data: Figure S17b). The higher binding affinity of luteolin-7-*O*-β-D-glucopyranoside (**3**) to topoisomerase I (− 10.367 kcal/mol) compared to topoisomerases IIα and IIβ (− 7.048 and − 6.923 kcal/mol, respectively) suggests that presence of more than one sugar moiety substituted

Fig. 1 2D diagram of the binding interactions of luteolin-7-(6''-malonylneohesperidoside) with the active site residues of the enzymes **a** topoisomerase I (1T8I), **b** topoisomerase II α (5GWK), and **c** topoisomerase II β (3QX3). Pink circles with red and blue borders indicate polar acidic and basic amino acids, respectively, green circles indicate nonpolar amino acids, and light blue circles indicate DNA nucleotides



into the flavonoid nucleus may have a role in stabilization of the inhibitor binding to topoisomerases II α and II β . Altogether, the provided docking results endorse the studied compounds as candidates for further investigations to develop anticancer therapies.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s43450-023-00371-0>.

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