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ORIGINAL ARTICLE

Phytochemical Investigation of Egyptian Spinach Leaves, a Potential Source for Antileukemic Metabolites: *In Vitro* and *In Silico* Study

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Abstract

Spinacia oleracea L., Amaranthaceae, leaves cultivated in Egypt demonstrated a potential antileukemic activity against the chronic myeloid leukemia, K562 cell line. Thus, the aim of this study is to carry out a phytochemical investigation of *S. oleracea* leaves as well as the isolation of its antileukemic phytoconstituents. Phytochemical investigation of *S. oleracea* leaves resulted in the isolation of seventeen known compounds. The biological study revealed that compounds hexaprenol, phytol, and 18-[(1-oxohexadecyl) oxy]-9-octadecenoic acid exhibited a remarkable antiproliferative activity against K562 cells *in vitro*. A mechanistic *in silico* study showed that hexaprenol, phytol, and 18-[(1-oxohexadecyl) oxy]-9-octadecenoic acid exhibited a strong binding affinity towards topoisomerase (docking score −12.50, −9.19, and −13.29 kcal/mol, respectively), and showed as well a strong binding affinity towards Abl kinase (docking score −11.91, −9.35, and −12.59 kcal/mol, respectively). Molecular dynamics study revealed that 18-[(1-oxohexadecyl) oxy]-9-octadecenoic acid produced stable complexes with both topoisomerase and Abl kinase with RMSD values of 1.81 and 1.85 Å, respectively. As a result of our findings, we recommend more *in vivo* and preclinical studies to confirm the potential benefit of spinach leaves for chronic myeloid leukemia patients.

Keywords Spinach · Chronic myeloid leukemia · K562 cell line · Abl kinase · Topoisomerase · Molecular dynamics

Introduction

Chronic myeloid leukemia (CML) is ranked as the fourth predominant cancer in upper Egypt that constituted about 10.2% of all the reported cancer cases, after breast, liver, and bladder cancers that constituted about 34, 23.4, and 16.6% of cases, respectively (Ibrahim et al. 2014). Chronic myeloid leukemia is a myeloproliferative neoplasm that is characterized by uncontrolled myeloid cell divisions in the bone marrow (Shahrabi et al. 2014). Chronic myeloid leukemia arises due to genesis of the BCR-ABL oncogene as a result of the reciprocal translocation between chromosome 9 and chromosome 22 (Deininger et al. 2000). The BCR-ABL oncogene encodes a constitutively activated tyrosine kinase enzyme which activates several proliferatory signaling pathways inside the cells such as RAS, a small GTPase, mitogen-activated protein kinase, signal transducers and activator of transcription, and phosphoinositide-3-kinase pathways (Sattlermc and Griffin 2003). Targeting Abl kinase was reported as a successful strategy to treat CML. Tyrosine kinase inhibitors (TKIs) such as imatinib, ilotinib, dasatinib, bosutinib, and ponatinib are currently used to treat CML

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(Akard 2010; Shamroe and Comeau 2013; Sacha 2014). However, mutations in BCR-ABL and multi-drug resistance make TKIs less effective, so, there is a constant need for alternative therapeutic strategy (Ali 2016).

Several secondary metabolites from natural origin such as alkaloids, flavonoids, terpenoids, polyketides, lignans, saponins, and peptides exhibited a potent anti-CML activity (Khajapeer and Baskaran 2016).

In a previous study to our group investigating the antileukemic potential activities of fifty-six medicinal plants grown and used in upper Egypt, the 75% hydroethanolic extract of *Spinacia oleracea* L., Amaranthaceae, leaves demonstrated a promising antileukemic activity and was one of the most active plants against the chronic myeloid leukemia cell line K562 (Abdelgawad et al. 2021). Moreover, few studies were available in the literature about the phytoconstituents of *S. oleracea* leaves cultivated in Egypt. So, this study was carried out with the aims of isolating the antileukemic metabolites from *S. oleracea* leaves as well as the phytochemical investigation of the plant.

Spinacia oleracea is an annual popular winter vegetable crop that is widely cultivated in Egypt where the leaves are used as fresh, canned, or frozen product (Fekry and Nawar 2017). Spinach is considered as one of the healthy vegetable crops for human consumption. It contains major vitamins such as vitamin C, A, and E, as well as minerals such as iron, calcium, potassium, manganese, and zinc (Toledo et al. 2003). Spinach is a vegetable with a high biological value due to the presence of secondary metabolites from various chemical classes such as flavonoids (Aritomi et al. 1985; Ferreres et al. 1997; Edenharder et al. 2001; Sultana and Anwar 2008), steroids (Dawidar and Amer 1973; Modlin et al. 1994), triterpenoid saponins (Mithöfer et al. 1999), phenolic acids (Bergman et al. 2001; Bunea et al. 2008), carotenoids (Metha and Belemkar 2014), and glycolipids (Wang et al. 2002). Spinach phenolic compounds exhibited a wide range of biological effects including antioxidant (Bergman et al. 2001), anti-inflammatory (Lomnitski et al. 2000), antiproliferative (Nyska et al. 2003), anti-carcinogenic (Nyska et al. 2001), antibacterial (Parekh and Chanda 2008; Altemimi et al. 2017), hepatoprotective (Abdul-Wahab and Jalil 2012), hypolipidemic (Hetta et al. 2017), and CNS suppressant (Das and Guha 2008) properties.

Materials and Methods

General Experimental Procedures

Liquid chromatography analysis was conducted using an Agilent 1100 HPLC system, RP-C18 column (150 × 4.6 mm; particle size 5 μm; Luna) with column oven temperature set at 25 °C and a gradient system of eluent water (A) and methanol (B) used. The gradient condition was as follows: 0–8 min (30% B), 9–11 min (40% B→80% B), 12–15 min (100% B). The flow rate of the solvent was 2 m/min, and the injection volume was 50 μl. All the analysis was carried out at wavelength of 280 nm with a run time of 16 min. HPLC-grade methanol and water solvents were used. Acetic acid was added as a modifier to achieve a final concentration of 0.1% in each solvent. GC/MS analysis was performed with an Agilent 7890B gas chromatograph. Optical activity was measured using an AA-65 series automatic polarimeter (Cambridgeshire, PE26 1NF, England). High-resolution electrospray ionization mass spectrometry (HR-ESIMS) data were acquired using a Bruker BioApex-FTMS with electrospray ionization (ESI). 1D and 2D NMR spectra were recorded on a Bruker 400- and 500-MHz spectrometer.

Sephadex LH-20 (Mitsubishi Kagaku, Tokyo, Japan) and silica gel (60–120-μM mesh, Merck, Darmstadt, Germany), reversed phase silica (40–63 μM, Sorbent Technologies, 5955 Peachtree Corners East, Suite A, Norcross, GA 30071 USA), and Diaion® HP-20 (250 μm, Supelco, Bellefonte, PA 16823-00048, USA) were used for column chromatography (CC). SPE cartridges silica gel and C18 (Supelco Inc., Bellefonte, PA, USA) were used in the fractionation work. Fractions from CC were monitored using precoated aluminum sheets (silica 60 F254, 0.25 mm (Merck, Darmstadt, Germany)), with detection provided by UV light (254 and 366 nm) and by spraying with 2% *p*-anisaldehyde-H_2SO_4 reagent followed by heating for 5–10 min (105 °C).

Plant Material

The fresh leaves of *Spinacia oleracea* L., Amaranthaceae, were purchased from the Sara Organic Food Farm (https://www.sarasorganicfood.com/), Egypt, in June 2018. The plant material was authenticated as *S. oleracea* L. (Baladi cultivar) by Hesham Elfayoumi, lecturer at Plant Taxonomy Department, Faculty of Science, Fayoum University, and voucher specimen (FUPD-48) was kept at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Fayoum University, Egypt.

Extraction and Isolation Procedure

The shade dried leaves (1.6 kg) were ground and extracted six times with 75% ethanol at room temperature. The combined 75% hydroethanolic extract was concentrated in vacuum to afford a crude extract (303.8 g), that was suspended in water, and fractionated successively with hexane, dichloromethane, ethyl acetate, and *n*-butanol to afford fractions of 31, 2.9, 7.3, and 25.3 g, respectively, and aqueous mother liquor 235 g. The obtained fractions were subjected to biological testing against leukemia K562 cell line.

Phytochemical Study of the Hexane Fraction [Fr-A]

The hexane fraction of *S. oleracea* leaves was saponified according to the published procedure (Finar 1973) and the fatty acid methyl ester (FAME) and unsaponifiable matter (USM) were then subjected to GC/MS analysis.

Unsaponifiable matter (1.8 g) was chromatographed on silica gel column by gradient elution with hexane/EtOAc (2.5% gradient) to afford six fractions (A1–A6). Fr-A-2 (68.7 mg) was chromatographed on silica gel column by gradient elution with hexane/EtOAc (2.5% gradient) to afford compounds **1** (19.1 mg) and unseparated mixture of compounds **4** and **5** (34 mg). Fr-A-3 (284.44 mg) was chromatographed on silica gel column by gradient elution with hexane/EtOAc (2.5% gradient) to afford compounds **2** (133.9 mg), **3** (31.4 mg), and **6** (86.4 mg). Fr-A-4 (742.27 mg) was chromatographed on silica gel column by gradient elution with hexane/EtOAc (2.5% gradient) to afford unseparated mixture of compounds **7** and **8** (23.4 mg), unseparated mixture of compounds **9** and **10** (11.4 mg), and compound **11** (22.2 mg). Fr-A-6 was one pure compound **12** (15.7 mg) on TLC.

Phytochemical Study of the EtOAc Fraction [Fr-B]

EtOAc fraction (6.8 g) was subjected to column chromatography on Sephadex LH-20 with methanol as eluent to afford 9 subfractions (B1–B9). Fr-B-5 (2.9 g) was chromatographed on reversed phase silica gel (RP-SPE) cartridge by gradient elution with water/methanol to afford 3 subfractions. Fr-B-5-1 (1.9 g) was chromatographed on silica gel column eluting with dichloromethane/methanol gradient to afford 6 subfractions. Fr-B-5-1-3 (123 mg) was chromatographed on silica gel column chromatography eluting with dichloromethane/methanol gradient to afford 5 subfractions. Subfraction Fr-B-5-1-3-2 (15.3 mg) was purified using HPLC RP column eluting by water/methanol gradient to afford compound **13** (3 mg) at retention time of 9.16 min (Fig. S1, Supplementary materials). Subfraction Fr-B-5-1-3-4 (82.18 mg) was purified using HPLC RP column eluting by water/methanol gradient to afford compounds **14** (11 mg) and **15** (4 mg) at retention times 7.51 and 8.02 min, respectively (Fig. S2, Supplementary materials). Fr-B-5-1-5 was a single spot by TLC under UV light and by spraying with *p*-anisaldehyde and afforded compound **16** (16.9 mg). Fr-B-5-2 (130.3 mg) was chromatographed on reversed phase silica gel (RP-SPE) cartridge by gradient elution with water/methanol to afford compound **17** (3.4 mg).

Cytotoxicity Assay

K562 cells from the American Type Culture Collection (ATCC) were plated in a clear 384-well plate at an initial density of 2500 cells/well in 40 µl of growth medium (DMEM with 10% FBS and 1% pen/strep). Next day, the test agents were added in quadruplicate at the specified concentration and the treatment continued for 48 h and the cell viability was finally assessed using WST-8 assay Cell Counting Kit from Bimake, according to manufacturer's instructions. The results were calculated by measuring the absorbance at 450 nm using a Spectra Max M5 plate reader (Molecular Devices). Cell viability was calculated in comparison to DMSO as a negative control, Taxol and doxorubicin as a positive control (Kageyama et al. 2018). The extract and fractions were screened primary at concentration of 20 µg/ml and the percentage inhibitions were calculated. Isolated compounds were screened at six concentrations (5, 10, 25, 50, 75, and 100 µg/ml) and IC_{50}s were calculated.

Molecular Docking

All docking simulations were conducted using MOE 2019 software (https://www.chemcomp.com). The receptors and the ligands were prepared using the standard structure optimization protocol of the software. The receptors were obtained from the protein data bank, PDB IDs: 3QX3, 3QRJ, 1M17, 2SRC, and 6QS9 for topoisomerase, Abl Kinase, EGFR-tyrosine kinase, SRC kinase, and albumin, respectively. Then they were energy minimized under AMBER12: EHT force field. The active sites were set as where the co-crystalized ligand was bound. The docking was performed using a molecular structure of compounds isolated from *S. oleracea* leaves using the general protocol of MOE DOCKTITE Wizard. Triangle matcher and London dG were utilized as the placement method and scoring algorithm, respectively. The validation of docking experiments was achieved through the re-docking of the co-crystalized ligands into their corresponding active sites and then the root mean square deviation (RMSD) was calculated. The docking results were visualized, and the docking scores were reported in kcal/mol.

Molecular Dynamics

To conduct the required molecular dynamics (MD) simulations, Groningen Machine for Chemical Simulations (GROMACS) 5.1.1 software was employed (Abraham et al. 2015). To validate the retrieved binding modes from the docking study, two MD simulation experiments were conducted. The two simulation experiments were performed on the most active compound **3** in complex with Abl kinase and topoisomerase. GROMOS96 force field was implemented to generate the ligand topology using the GlycoBioChem PRODRG2 Server (Schüttelkopf and Van Aalten 2004). Later on, complex topology was generated through joining both ligand and enzymes. As already published in the literature, the typical scheme for enzyme-ligand simulations by

GROMACS was applied, starting with system solvation using a single point charge (SPC) water model and ending with neutralization by adding the suitable number of counter ions (El Hassab et al. 2020, 2021, 2022a, 2022b).

The two solvated neutralized systems were energy minimized under GROMOS9643a1 force field using the steepest descent minimization algorithm with a maximum of 50,000 steps and 10 kJ/mol force under. All the systems were equilibrated to the used temperature (310 K) and pressure (1 atm) using NPT (isothermal-isobaric ensemble) for 2 ns preceded by NVT (canonical ensemble) for 1 ns. To compute the long-range electrostatic values, the particle mesh Ewald (PME) method with a 12-Å cut-off and 12-Å Fourier spacing was implemented. All the systems were subjected to a production stage of 50 ns. Every two consecutive steps were separated by 2 fs and the structural coordinates were saved every 20 ps. The V-rescale weak coupling method (modified Berendsen thermostat) and the Parrinello-Rahman method were used to regulate the temperature (310 K) and the pressure (1 atm) throughout the simulation (Parrinello and Rahman 1981; Berendsen et al. 1984). The root mean square deviation (RMSD) of the entire system was calculated from the generated trajectories from the production step.

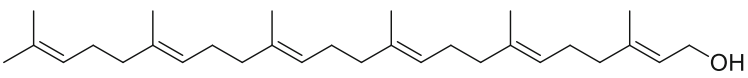
Results and Discussion

Spinacia oleracea was selected based on the results of our previous screening of certain Egyptian leafy vegetables for antileukemic activity. The total 75% ethanolic extract of *S. oleracea* leaves exhibited a strong antiproliferative activity against K562 cell with 88.9% percentage of inhibition at a concentration of 10 mg/ml. The hexane, dichloromethane, ethyl acetate, *n*-butanol, and the aqueous fractions were tested at a concentration of 20 μg/ml and percentage inhibitions were calculated as 23, 19, 19, 20, and 18%, respectively, compared to doxorubicin and Taxol (86 and 79% at 10 μM, respectively). Based on the biological screening results as well as TLC screening of the bioactive fractions, the hexane and ethyl acetate fractions were selected for further phytochemical study with the aim of isolating the bioactive compounds.

Phytochemical Study of Hexane Fraction

Twenty-five compounds were identified in the saponifiable matter (Table 1) with five majors identified as methyl palmitate, (*Z*)-methyl hexadec-11-enoate, methyl oleate, methyl linoleate, and methyl linolenate at retention times of 33.1, 33.3, 37.5, 37.7, and 38.2 min, respectively. Moreover, twelve compounds were identified in the unsaponifiable matter (Table 2) with seven majors identified as palmitic acid, phytol, oleic acid, linoelaidic acid, linolenic acid, stigmasterol, and γ-sitosterol at retention times of 34.2, 37.3, 38.7, 39.0, 39.5, 66.1, and 68.6 min, respectively.

The unsaponifiable matter was subjected to column chromatographic separations which resulted in the isolation of twelve compounds (Fig. S3, Supplementary materials), identified as hexaprenol (**1**) (Grigor'eva et al. 1990), phytol (**2**) (Arigoni et al. 1999), 18-[(1-oxohexadecyl) oxy]-9-octadecenoic acid (**3**), 24-methylenecycloartanol (**4**) (El-Feky et al. 2020), (2*E*,6*E*)-3,7,11,15,19 pentamethylicosa-2,6-dien-1-ol (**5**) (Toyoda et al. 1969), palmitic acid (**6**) (Di Pietro et al. 2020), (Schulz et al. 2000), γ-sitosterol (**7**) (Jain et al. 2009), stigmasterol (**8**) (Jain et al. 2009), 25,26-dihydroelasterol (**9**) (Doshi et al. 2015), 22,23-dihydrospinasterol (**10**) (Hetta et al. 2017), spinasterol (**11**) (Ragasa and Lim 2005), and lutein (**12**) (Prapalert et al. 2016). This is the first report of compounds **1** and **3** in *S. oleracea* leaves while compounds **2**, **4**–**12** were previously isolated from spinach leaves (Wolf et al. 1962; Modlin et al. 1994; Drews 1996; Ligor and Buszewski 2012; Hetta et al. 2017).



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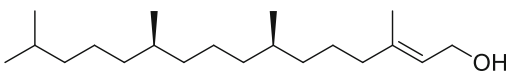

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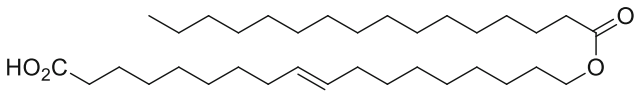

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Table 1 Results of GC/MS analysis of fatty acid methyl ester (FAME) of the hexane fraction of *Spinacia oleracea* leaves

Comp. no.	Compound name	RT (min)	Mol weight (amu)	Peak area (%)
1.	Methyl myristate	27.8	242.225	0.70
2.	Methyl pentadecanoate	30.4	256.24	0.57
3.	Phytone	30.7	268.277	0.47
4.	(*Z*)-9-Hexadecenoic acid, methyl ester	32.7	268.24	0.51
5.	Methyl palmitate	33.1	270.256	24.09
6.	(*Z*)-Methyl hexadec-11-enoate	33.3	268.24	2.40
7.	7,10,13-Hexadecatrienoic acid, methyl ester	33.4	264.209	0.77
8.	Palmitic acid	34.2	256.24	0.28
9.	Methyl margarate	35.4	284.272	0.51
10.	Hexadecanoic acid, 2-hydroxy-, methyl ester	36.4	286.251	1.00
11.	Methyl isostearate	36.7	298.287	0.28
12.	Methyl oleate	37.5	296.272	8.70
13.	Methyl lineoleate	37.7	294.256	13.99
14.	11,14-Octadecadienoic acid, methyl ester	37.9	294.256	0.61
15.	Methyl linolenate	38.2	292.24	17.43
16.	Methyl 9-*cis*,11-*trans*-octadecadienoate	40.2	294.256	0.56
17.	*cis*-13-Eicosenoic acid, methyl ester	41.9	324.303	0.55
18.	Methyl arachidate	42.0	326.318	0.65
19.	Methyl behenate	46.1	354.35	1.46
20.	Methyl tricosanoate	48.0	368.365	0.49
21.	Methyl lignocerate	49.9	382.381	1.60
22.	Methyl 2-hydroxy-tetracosanoate	52.8	398.376	1.65
23.	Methyl hexacosanoate	53.4	410.412	0.66
24.	Methyl montanate	57.3	438.444	0.66
25.	Stigmasta-3,5-diene	58.9	396.376	0.83
Saturated fatty acids			**35%**	
Unsaturated fatty acids			**46.4%**	
Unidentified compounds			**18.6%**	

Table 2 Results of GC/MS analysis of unsaponifiable matter of the hexane fraction of *Spinacia oleracea* leaves

Comp. no.	Compound name	RT (min)	Mol weight (amu)	Peak area (%)
1.	Dihydroactinolide	26.9	180.115	0.55
2.	Phytone	30.7	268.277	0.83
3.	Palmitic acid	34.2	256.24	11.07
4.	Loliolid	34.8	196.11	1.64
5.	Phytol	37.3	296.308	33.37
6.	Oleic acid	38.7	282.256	3.24
7.	Linoelaidic acid	39.0	280.24	10.12
8.	Linolenic acid	39.5	278.225	24.79
9.	Nonacos-1-ene	45.4	406.454	0.22
10.	1-Tetracosene	49.3	406.454	0.57
11.	Stigmasterol	66.1	412.371	2.56
12.	gamma-Sitosterol	68.6	414.386	1.79
Total hydrocarbons			**83%**	
Sterols			**4.35%**	
Unidentified compounds			**12.65%**	

Phytochemical Investigation of the Ethyl Acetate Fraction

Five flavonoids (Fig. S4, Supplementary materials) were isolated from the ethyl acetate fraction identified as isoswertisin-2″-*O*-xyloside (**13**) (Bakhtiar et al. 1990), vitexin 2″-*O*-xyloside (**14**) (Isayenkova et al. 2006), margaritene (**15**) (Larionova et al. 2010), vitexin-2″-*O*-rhamnoside (**16**) (Nikolov et al. 1982), and 3-*O*-gylcoside identified as isorhamnetin 3-*O*-β-D-xylopyranosyl (1→6)-β-D-glucopyranoside (**17**) (Sakar et al. 1980; Moustapha et al. 2011). This is the first report for compounds **13**–**17** in *S. oleracea* leaves.

Biological Study

The antileukemic activity of the major constituent of the saponifiable matter of hexane fraction, the linolenic acid, was previously reported in many research articles (Beaulieu et al. 2011; Ge et al. 2009; Harada et al. 2002; Jóźwiak et al. 2020; Liu and Leung 2014; Mainou-Fowler et al. 2001; Moloudizargari et al. 2018; Valencia-Serna et al. 2013). Methyl oleate (Saab et al. 2011) and methyl linolenate (Ge et al. 2009) were also reported for their *in vitro* antileukemic activity against K562 cells. Thus, the activity of saponifiable matter may be attributed to the presence of those compounds.

The compounds isolated from USM, hexaprenol (**1**), phytol (**2**), and 18-[(1-oxohexadecyl) oxy]-9-octadecenoic acid (**3**) (NMR spectrum Figs. S5-S7, Supplementary materials), exhibited antiproliferative activity against K562 cells with IC_{50} of 44.89, 33.28, and 70.58 μg/ml, respectively, compared to doxorubicin and Taxol with IC_{50} of 11.41 and 1.70 μg/ml, respectively. The antileukemic activity of compound **2** against K562 cells was previously reported (Anuchapreeda et al. 2020) while this is the first report of the activity of compounds **1** and **3** against K562 cells. The compounds isolated from the ethyl acetate fraction showed no antiproliferative activity against K562 cell line. However, some of these compounds have several applications that could be beneficial in the management of CML disease. For example, vitexin-2″-*O*-rhamnoside (**16**), which is a major compound in the EtOAc fraction, reported for its antioxidant and anti-apoptotic activities (Wei et al. 2014). Margaritene (**15**) also was reported for its antioxidant activity (Lou et al. 2015). Additionally, 22,23-dihydrospinasterol (**10**) and spinasterol (**11**) were recently reported for their moderate antioxidant activity (Ahmed et al. 2022).

In a clinical study conducted on 47 CML patients, the oxidative stress was reported to be associated with the pathophysiology of CML (Ahmad et al. 2008). Thus, the use of antioxidants could be beneficial for CML patients. Spinach leaves extract was reported for its antioxidant activity in several clinical studies (Cao et al. 1998; Castenmiller et al. 1999; Pool-Zobel et al. 1997; Porrini et al. 2002). Therefore, the antioxidant activity of the spinach extract or its isolated compounds in addition to the antileukemic activities of the isolated compounds might work in symphony to improve CML disease, but this still needs further *in vivo* and preclinical investigations.

Molecular Docking

Multitarget therapies are crucial in the field of complex diseases, such as cancers and inflammatory and thrombotic diseases which can be affected by several cellular pathways (Skok et al. 2019). Targeting two different pathways involved in the development of a disease can represent logic solution for this challenge and can reduce the potential for the development of resistance (Skok et al. 2019).

Recent research showed a correlation between CML cell line (K562) and several target proteins whose inhibition leads to antiproliferative effect in this cell line. Four target proteins are named: human topoisomerase II beta in complex with DNA and etoposide (PDB ID–3QX3), epidermal growth factor receptor complexed with erlotinib (PDB ID–1M17), human ABL1 kinase (PDB ID–3QRJ), and SRC kinase (PDB ID–2SRC) were reported as docking targets in the K562 cell line (James et al. 2017; Zamakshshari et al. 2019).

Table 3 Affinity binding docking scores (kcal/mol) of compounds isolated from *Spinacia oleracea* leaves against several targets

Compound no.	Topoisomerase (3QX3)	SRC kinase (2SRC)	Abl kinase (3QRJ)	EGFR-tyrosine kinase (1M17)	Albumin (6QS9)
1	−12.50	−9.13	−11.91	−9.33	−9.36
2	−9.19	−9.62	−9.35	−8.34	−9.41
3	−13.29	−9.21	−12.59	−9.44	−11.32
Etoposide	−11.77				
Adenylyl-imidodiphosphate		−8.97			
Rebastinib			−13.43		
Erlotinib				−12.73	
Ketoprofen					−7.54

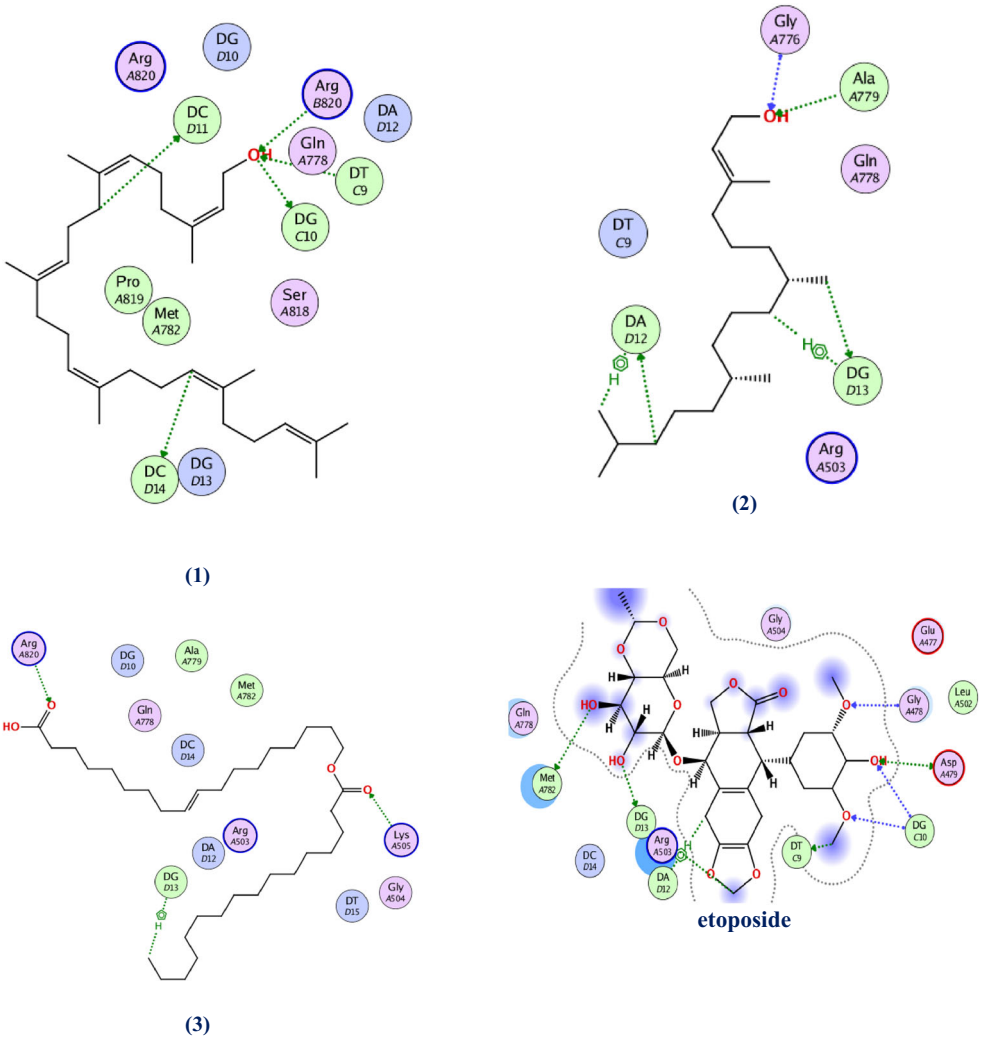

Fig. 1 The 2D interaction diagrams of compounds (**1**, **2**, and **3**) and etoposide with topoisomerase

The active isolated compounds were docked into the active site of the selected targets and the resulted docking scores are reported in Table 3. Hexaprenol (**1**), phytol (**2**), and 18-[(1-oxohexadecyl) oxy]-9-octadecenoic acid (**3**) showed a strong binding affinity towards all the selected targets (Table 3). The best docking results were obtained with topoisomerase and Abl kinase enzymes and in turn their docking results were selected for further analysis. As depicted in Fig. 1, compound **1** showed a strong binding pattern towards the topoisomerase enzyme through forming several interactions with DT9, DG10, DC11, and DC14 in the DNA in addition to one interaction with Arg820. Similarly, compound **2** formed six interactions with DA12, DG13, Gly776, and Ala779, while compound **3** interacted with DG13, Lys505, and Arg820. The interaction pattern and docking scores of the three isolated compounds with topoisomerases is very similar to the crystal reference etoposide (Table 3, Fig. 1). Moreover, the three isolated compounds achieved acceptable docking scores with Abl kinase, having binding scores of −11.91, −9.35, and −12.59 kcal/mol for compounds **1**, **2**, and **3**, respectively (Table 3). Moreover, compound **1** formed four interactions with Phe359 and His361, while compound **2** formed four interactions with Met290, Asn358, and Ile360 (Fig. 2). At last, compound **3** interacted with Lys271, Met290, and Phe382 (Fig. 2).

Molecular docking provided a mechanistic information on the possible antiproliferative activity against K562 cells through binding with Abl kinase and topoisomerase.

The *in silico*–based safety analysis of the antileukemic compounds was tested by measuring their binding affinity to albumin (PDB ID–6QS9) (Behzadi et al. 2019). The affinity of cytotoxic compounds to bind to albumin has a great impact on their pharmacodynamics and pharmacokinetic properties; for example, the anticancer agent chlorambucil is 99% bound to albumin and yet has a short half-life of 1.3 ± 0.90 h (Sparreboom and Loos 2004). In accordance with this

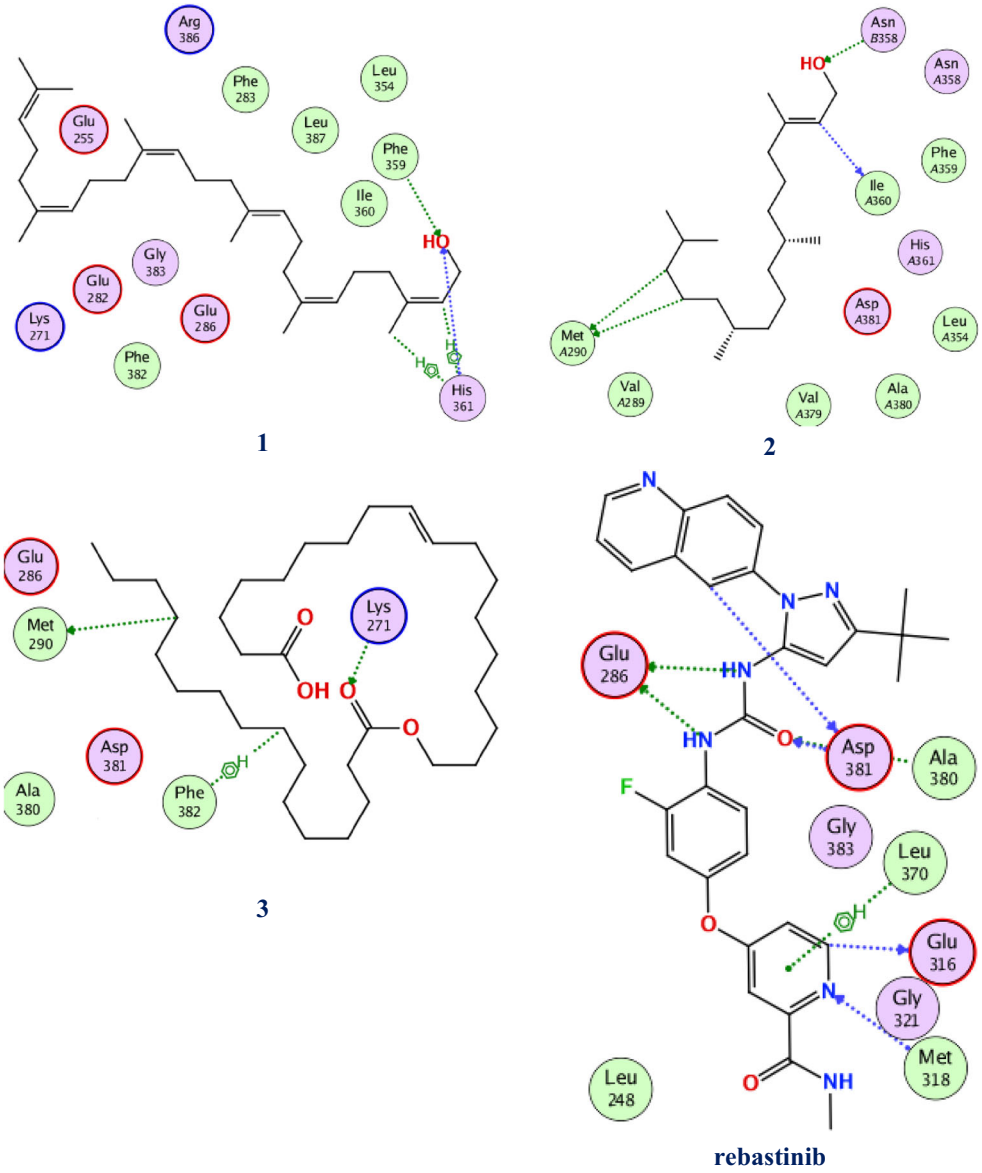

Fig. 2 The 2D interaction diagrams of compounds (**1**, **2**, and **3**) and rebastinib with Abl kinase

information, compounds **1** and **2** are safer *in silico* and with greater half-life than compound **3** (Table 3).

Molecular Dynamics

Molecular dynamics (MD) simulation has been an inevitable technique in studies involving *in silico* drug discovery. MD provides many important parameters, data, and figures necessary in various computational and molecular modelling studies. One of the most common applications of the MD is the precise determination of the binding stability between a ligand and its target. Therefore, it was logistic to take the advantage of the MD to further endorse our docking results. Two MD simulation experiments were conducted on compound **3** bound to Abl kinase and topoisomerase. Interestingly, the calculated RMSD for compound **3** with Abl kinase and topoisomerase reached 1.85 and 1.81 Å respectively at their maximum deviations (Fig. 3). The ability of compound **3** to produce such a lower RMSD value is a powerful indicator of its ability to produce stable complexes with Abl kinase and topoisomerase. The MD results supported the docking results and highlighted the ability of the isolated compounds as antileukemic agents.

Conclusions

The hexane fraction of Egyptian Spinach leaves as well as its isolated compounds, hexaprenol (**1**), phytol (**2**), and 18-[(1-oxohexadecyl) oxy]-9-octadecenoic acid (**3**), showed

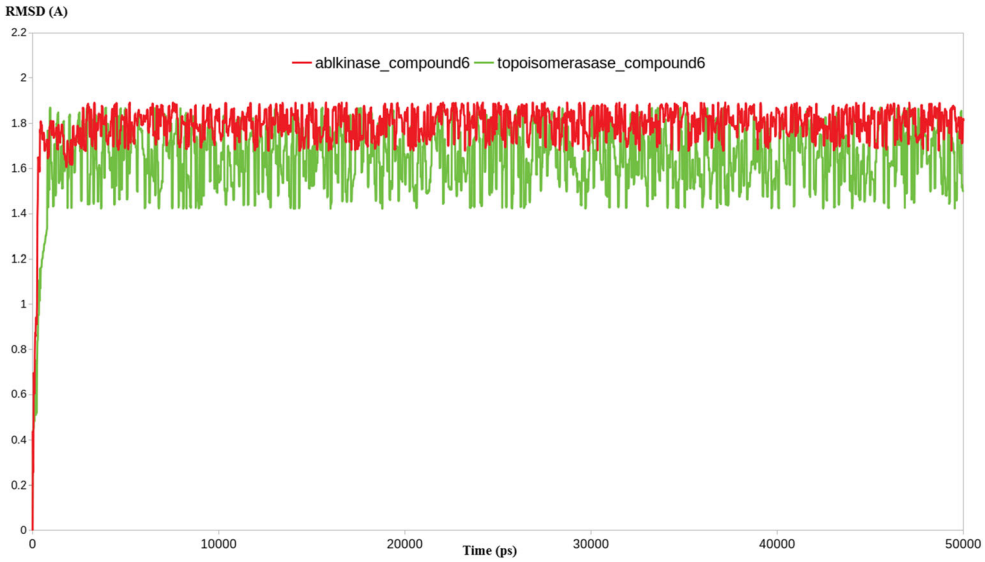

Fig. 3 The RMSD of compound **3** with Abl kinase (red) and topoisomerase (green)

remarkable antiproliferative activity against leukemia K562 cell line. The molecular docking study revealed that this activity is supposed to be through targeting Abl kinase and topoisomerase, and this still needs to be proved by *in vitro* assay of these compounds against the mentioned targets. As a result of our findings, we recommend more *in vivo* and preclinical investigations to confirm the potential benefit of spinach leaves for CML patients.

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Author Contribution SMA contributed to the plant material collection, extraction, and chemical assays. MAI, PB, and JZ participated in the experimental biological assays. MHH, GAF, HIE-A, MAI, and SAR conceived, designed, and contributed to the formal analysis of the study. MW participated in the GC/MS analyses and interpretation of the data. The first draft of the manuscript was written by SMA, and all authors commented on this version. All authors have read the final manuscript and approved the submission.

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Declarations

Conflict of Interest The authors declare no competing interests.

References

Abdelgawad SM, Hetta MH, Fawzy GA, El-Askary HI (2021) *In vitro* antileukemic activity of extracts of some medicinal plants from upper Egypt in human chronic leukemia K562 cell line. Trop J Nat Prod Res 5:2115–2122. https://doi.org/10.26538/tjnpr/v1i4.5

Abdul-Wahab FK, Jalil TZA (2012) Study of Iraqi spinach leaves (phytochemical and protective effects against methotrexate-induced hepatotoxicity in rats). Iraqi J Pharm Sci 21:8–17. https://doi.org/10.31351/vol21iss2pp8-17

Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, Lindahl E (2015) GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX 1:19–25. https://doi.org/10.1016/j.softx.2015.06.001

Ahmad R, Tripathi AK, Tripathi P, Singh R, Singh S, Singh RK (2008) Oxidative stress and antioxidant status in patients with chronic myeloid leukemia. Indian J Clin Biochem 23:328–333. https://doi.org/10.1007/s12291-008-0072-9

Ahmed M, Sajid AR, Javeed A, Aslam M, Ahsan T, Hussain D, Mateen A, Li X, Qin P, Ji M (2022) Antioxidant, antifungal, and aphicidal activity of the triterpenoids spinasterol and 22,23-dihydrospinasterol from leaves of *Citrullus colocynthis* L. Sci Rep 12:4910. https://doi.org/10.1038/s41598-022-08999-z

Akard LP (2010) Second-generation BCR-ABL kinase inhibitors in CML. N Engl J Med 363:1672–1673. https://doi.org/10.1038/nrc2126

Ali MA (2016) Chronic myeloid leukemia in the era of tyrosine kinase inhibitors: an evolving paradigm of molecularly targeted therapy. Mol Diagn Ther 20:315–333. https://doi.org/10.1007/s40291-016-0208-1

Altemimi A, Lakhssassi N, Abu-Ghazaleh A, Lightfoot DA (2017) Evaluation of the antimicrobial activities of ultrasonicated spinach leaf extracts using rapd markers and electron microscopy. Arch

Microbiol 199:1417–1429. https://doi.org/10.1007/s00203-017-1418-6

Anuchapreeda S, Chueahongthong F, Viriyaadhammaa N, Panyajai P, Anzawa R, Tima S, Ampasavate C, Saiai A, Rungrojsakul M, Usuki T, Okonogi S (2020) Antileukemic cell proliferation of active compounds from Kaffir lime (*Citrus hystrix*) leaves. Molecules 25: 1300. https://doi.org/10.3390/molecules25061300

Arigoni D, Eisenreich W, Latzel C, Sagner S, Radykewicz T, Zenk MH, Bacher A (1999) Dimethylallyl pyrophosphate is not the committed precursor of isopentenyl pyrophosphate during terpenoid biosynthesis from 1-deoxyxylulose in higher plants. Proc Natl Acad Sci 96:1309–1314. https://doi.org/10.1073/pnas.96.4.1309

Aritomi M, Komori T, Kawasaki T (1985) Flavonol glycosides in leaves of *Spinacia oleracea*. Phytochemistry 25:231–234. https://doi.org/10.1016/S0031-9422(00)94534-5

Bakhtiar A, Gleye J, Moulis C, Fouraste I, Stanislas E (1990) *C*-Glycosylflavones from *Galipea trifoliata*. Phytochemistry 29: 1339–1340. https://doi.org/10.1016/0031-9422(90)85461-N

Beaulieu A, Poncin G, Belaid-Choucair Z, Humblet C, Bogdanovic G, Lognay G, Boniver J, Defresne MP (2011) Leptin reverts pro-apoptotic and antiproliferative effects of α-linolenic acids in BCR-ABL positive leukemic cells: involvement of PI3K pathway. PLoS One 6:e25651. https://doi.org/10.1371/journal.pone.0025651

Behzadi E, Sarsharzadeh R, Nouri M, Attar F, Akhtari K, Shahpasand K, Falahati M (2019) Albumin binding and anticancer effect of magnesium oxide nanoparticles. Int J Nanomed 14:257–270. https://doi.org/10.2147/IJN.S186428

Berendsen HJ, Jv P, Van Gunsteren WF, DiNola A, Haak JR (1984) Molecular dynamics with coupling to an external bath. Chem Phys 81:3684–3690. https://doi.org/10.1063/1.448118

Bergman M, Varshavsky L, Gottlieb HE, Grossman S (2001) The antioxidant activity of aqueous spinach extract: chemical identification of active fractions. Phytochemistry 58:143–152. https://doi.org/10.1016/S0031-9422(01)00137-6

Bunea A, Andjelkovic M, Socaciu C, Bobis O, Neacsu M, Verhé R, Van Camp J (2008) Total and individual carotenoids and phenolic acids content in fresh, refrigerated and processed spinach (*Spinacia oleracea* L.). Food Chemi 108:649–656. https://doi.org/10.1016/j.foodchem.2007.11.056

Cao G, Russell RM, Lischner N, Prior RL (1998) Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. J Nutr 128:2383–2390. https://doi.org/10.1093/jn/128.12.2383

Castenmiller JJ, West CE, Linssen JP, van het Hof KH, Voragen AG (1999) The food matrix of spinach is a limiting factor in determining the bioavailability of β-carotene and to a lesser extent of lutein in humans. J Nutr 129:349–355. https://doi.org/10.1093/jn/129.2.349

Das S, Guha D (2008) CNS depressive role of aqueous extract of *Spinacia oleracea* L. leaves in adult male albino rats. Indian J Exp Biol. 46:185–190

Dawidar A, Amer M (1973) Sterol content of *Spinacia oleracea*. Phytochemistry 12:1180–1181. https://doi.org/10.1016/0031-9422(73)85043-5

Deininger MW, Goldman JM, Melo JV (2000) The molecular biology of chronic myeloid leukemia. Blood Am J Hematol 96:3343–3356. https://doi.org/10.1182/blood.V96.10.3343

Di Pietro ME, Mannu A, Mele A (2020) NMR determination of free fatty acids in vegetable oils. Processes 8:410. https://doi.org/10.3390/pr8040410

Doshi GM, Nalawade VV, Mukadam AS, Chaskar PK, Zine SP, Somani RR, Une HD (2015) Structural elucidation of chemical constituents from *Benincasa hispida* seeds and *Carissa congesta* roots by gas chromatography: mass spectroscopy. Pharmacogn Res 7:282–293. https://doi.org/10.4103/0974-8490.157179

Drews HJ (1996) Analysis of free sugars and chlorophyll in spinach from a local retail market. Master's Thesis, University of Tennessee, 1996. https://trace.tennessee.edu/utk_gradthes/4424. (5, 1996)

Edenharder R, Keller G, Platt KL, Unger KK (2001) Isolation and characterization of structurally novel antimutagenic flavonoids from spinach (*Spinacia oleracea*). J Agric Food Chem 49:2767–2773. https://doi.org/10.1021/jf0013712

El Hassab MA, Shoun AA, Al-Rashood ST, Al-Warhi T, Eldehna WM (2020) Identification of a new potential SARS-COV-2 RNA-dependent RNA polymerase inhibitor via combining fragment-based drug design, docking, molecular dynamics, and MM-PBSA calculations. Front Chem 8:915. https://doi.org/10.3389/fchem.2020.584894

El Hassab MA, Ibrahim TM, Shoun AA, Al-Rashood ST, Alkahtani HM, Alharbi A, Eskandrani RO, Eldehna WM (2021) *In silico* identification of potential SARS COV-2 2′-*O*-methyltransferase inhibitor: fragment-based screening approach and MM-PBSA calculations. RSC Adv 11:16026–16033. https://doi.org/10.1039/D1RA01809D

El Hassab MA, Eldehna WM, Al-Rashood ST, Alharbi A, Eskandrani RO, Alkahtani HM, Elkaeed EB, Abou-Seri SM (2022a) Multi-stage structure-based virtual screening approach towards identification of potential SARS-CoV-2 NSP13 helicase inhibitors. J Enzyme Inhib Med Chem 37:563–572. https://doi.org/10.1080/14756366.2021.2022659

El Hassab MA, Hemeda LR, Elsayed ZM, Al-Rashood ST, Abdel-Hamid Amin MK, Abdel-Aziz HA, Eldehna WM (2022b) Computational prediction of the potential target of SARS-CoV-2 inhibitor plitidepsin via molecular docking, dynamic simulations and MM-PBSA calculations. Chem Biodivers 19:e202100719. https://doi.org/10.1002/cbdv.202100719

El-Feky AM, Elbatanony MM, Naser AFA, Kutkat OM, El-Sayed AE, Hamed MA (2020) Phytoconstituents and *in vitro* anti-oxidant, anti-viral, anti-hyperlipidemic and anticancer effects of *Chlorella vulgaris* microalga in normal and stress conditions. Pharma Chem. 12:9–20 http://derpharmachemica.com/

Fekry WA, Nawar DA (2017) Improving the growth, productivity and quality of spinach plants (*Spinacia oleracea* L.). Zagazig J Agric Res 44:2473–2484. https://doi.org/10.21608/ZJAR.2017.51328

Ferreres F, Castañer M, Tomás-Barberán FA (1997) Acylated flavonol glycosides from spinach leaves (*Spinacia oleracea*). Phytochemistry 45:1701–1705. https://doi.org/10.1016/S0031-9422(97)00244-6

Finar I (1973) Organic Chemistry Vol 1, 6th edn. ELBS

Ge H, Kong X, Shi L, Hou L, Liu Z, Li P (2009) Gamma-linolenic acid induces apoptosis and lipid peroxidation in human chronic myelogenous leukemia K562 cells. Cell Biol Int 33:402–410. https://doi.org/10.1016/j.cellbi.2009.01.014

Grigor'eva NY, Yudina O, Daeva E, Moiseenkov A (1990) Synthesis of modified hexaprenol WC 5 OH from glutaraldehyde derivatives. Bull Acad Sci 39:76–84. https://doi.org/10.1007/BF00963008

Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y (2002) Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res 22:2587–2590

Hetta MH, Moawad AS, Hamed MAA, Sabri AI (2017) *In-vitro* and *In-vivo* hypolipidemic activity of spinach roots and flowers. Iran J Pharm Sci 16:1509. https://doi.org/10.22037/IJPR.2017.2113

Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H (2014) Cancer incidence in Egypt: results of the national population-based cancer registry program. J Cancer Epidemiol 2014:437971. https://doi.org/10.1155/2014/437971

Isayenkova J, Wray V, Nimtz M, Strack D, Vogt T (2006) Cloning and functional characterisation of two regioselective flavonoid glucosyltransferases from *Beta vulgaris*. Phytochemistry 67:1598–1612. https://doi.org/10.1016/j.phytochem.2006.06.026

Jain P, Bari S, Surana S (2009) Isolation of stigmasterol and γ-sitosterol from petroleum ether extract of woody stem of *Abelmoschus*

manihot. Asian J Biol Sci 2:112–117. https://doi.org/10.3923/ajbs.2009.112.117

James AR, Unnikrishnan B, Priya R, Joseph MM, Manojkumar T, Raveendran Pillai K, Shiji R, Preethi G, Kusumakumary P, Sreelekha T (2017) Computational and mechanistic studies on the effect of galactoxyloglucan: imatinib nanoconjugate in imatinib resistant K562 cells. Tumor Biol 39:1010428317695946. https://doi.org/10.1177/1010428317695946

Jóźwiak M, Filipowska A, Fiorino F, Struga M (2020) Anticancer activities of fatty acids and their heterocyclic derivatives. Eur J Pharmacol 871:172937. https://doi.org/10.1016/j.ejphar.2020.172937

Kageyama M, Li K, Sun S, Xing G, Gao R, Lei Z, Zhang Z (2018) Antitumor and anti-metastasis activities of honey bee larvae powder by suppressing the expression of EZH2. Biomed Pharmacother 105:690–696. https://doi.org/10.1016/j.biopha.2018.06.034

Khajapeer KV, Baskaran R (2016) Natural products for treatment of chronic myeloid leukemia. In: Anti-cancer drugsnature, synthesis and cell, 1st edn. Intech Publications, Croatia, pp 1–48. https://doi.org/10.5772/66175

Larionova M, Spengler I, Nogueiras C, Quijano L, Ramírez-Gualito K, Cortés-Guzmán F, Cuevas G, Calderón JS (2010) A *C*-glycosylflavone from *Piper ossanum*, a compound conformationally controlled by CH/π and other weak intramolecular interactions. J Nat Prod 73:1623–1627. https://doi.org/10.1021/np100004v

Ligor M, Buszewski B (2012) Study of xanthophyll concentration in spinach leaves by means of HPLC coupled with UV–VIS and Corona CAD detectors. Food Anal Methods 5:388–395. https://doi.org/10.1007/s12161-011-9256-7

Liu WN, Leung KN (2014) Apoptosis-and differentiation-inducing activities of jacaric acid, a conjugated linolenic acid isomer, on human eosinophilic leukemia EoL-1 cells. Oncol Rep 32:1881–1888. https://doi.org/10.3892/or.2014.3446

Lomnitski L, Carbonatto M, Ben-Shaul V, Peano S, Conz A, Corradin L, Maronpot RR, Grossman S, Nyska A (2000) The prophylactic effects of natural water-soluble antioxidant from spinach and apocynin in a rabbit model of lipopolysaccharide-induced endotoxemia. Toxicol Pathol 28:588–600. https://doi.org/10.1177/019262330002800413

Lou SN, Lai YC, Huang JD, Ho CT, Ferng LH, Chang YC (2015) Drying effect on flavonoid composition and antioxidant activity of immature kumquat. Food Chem 171:356–363. https://doi.org/10.1016/j.foodchem.2014.08.119

Mainou-Fowler T, Proctor SJ, Dickinson AM (2001) γ-Linolenic acid induces apoptosis in B-chronic lymphocytic leukaemia cells *in vitro*. Leuk Lymphoma 40:393–403. https://doi.org/10.3109/10428190109057939

Metha D, Belemkar S (2014) Pharmacological activity of *Spinacia oleracea* Linn.-a complete overview. Asian J Pharm Res Dev 2:83–93

Mithöfer A, Jakupovic J, Weiler E (1999) A triterpenoid glycoside from *Spinacia oleracea*. Nat Prod Res 14:5–10. https://doi.org/10.1080/10575639908045427

Modlin RF, Alred PA, Tjerneld F (1994) Utilization of temperature-induced phase separation for the purification of ecdysone and 20-hydroxyecdysone from spinach. J Chromatogr A 668:229–236. https://doi.org/10.1016/0021-9673(94)80112-6

Moloudizargari M, Mortaz E, Asghari MH, Adcock IM, Redegeld FA, Garssen J (2018) Effects of the polyunsaturated fatty acids, EPA and DHA, on hematological malignancies: a systematic review. Oncotarget 9:11858. https://doi.org/10.18632/oncotarget.24405

Moustapha B, Marina GA, Raúl FO, Raquel CM, Mahinda M (2011) Chemical constituents of the Mexican mistletoe (*Psittacanthus calyculatus*). Molecules 16:9397–9403. https://doi.org/10.3390/molecules16119397

Nikolov N, Seligmann O, Wagner H, Horowitz R, Gentili B (1982) Neue flavonoid-glykoside aus *Crataegus monogyna* und *Crataegus pentagyna*. Planta Med 44:50–53. https://doi.org/10.1055/s-2007-971401

Nyska A, Lomnitski L, Spalding J, Dunson DB, Goldsworthy TL, Grossman S, Bergman M, Boorman G (2001) Topical and oral administration of the natural water-soluble antioxidant from spinach reduces the multiplicity of papillomas in the Tg. AC mouse model. Toxicol Lett 122:33–44. https://doi.org/10.1016/S0378-4274(01)00345-9

Nyska A, Suttie A, Bakshi S, Lomnitski L, Grossman S, Bergman M, Ben-Shaul V, Crocket P, Haseman JK, Moser G (2003) Slowing tumorigenic progression in TRAMP mice and prostatic carcinoma cell lines using natural anti-oxidant from spinach, NAO—a comparative study of three anti-oxidants. Toxicol Pathol 31:39–51. https://doi.org/10.1080/01926230390173833

Parekh J, Chanda S (2008) Antibacterial activities of aqueous and alcoholic extracts of 34 Indian medicinal plants against some Staphylococcus species. Turk J Biol 32:63–71. https://doi.org/10.3906/biy-0707-4

Parrinello M, Rahman A (1981) Polymorphic transitions in single crystals: a new molecular dynamics method. J Appl Phys 52:7182–7190. https://doi.org/10.1063/1.328693

Pool-Zobel B, Bub A, Müller H, Wollowski I, Rechkemmer G (1997) Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. Carcinogenesis 18:1847–1850. https://doi.org/10.1093/carcin/18.9.1847

Porrini M, Riso P, Oriani G (2002) Spinach and tomato consumption increases lymphocyte DNA resistance to oxidative stress but this is not related to cell carotenoid concentrations. Eur J Nutr 41:95–100. https://doi.org/10.1007/s003940200014

Prapalert W, Santiarworn D, Liawruangrath S, Liawruangrath B, Pyne SG (2016) The isolation of lutein and lutein 3′-methyl ether from *Peristrophe lanceolaria*. Nat Prod Commun 11:1793–1795. https://doi.org/10.1177/1934578X1601101205

Ragasa CY, Lim K (2005) Sterols from *Cucurbita maxima*. Philipp J Sci 134:83–87

Saab AM, Lampronti I, Grandini A, Borgatti M, Finotti A, Sacchetti G, Gambari R, Guerrini A (2011) Antiproliferative and erythroid differentiation activities of *Cedrus libani* seed extracts against K562 human chronic myelogenus leukemia cells. Int J Pharm Biol Arch 2:1744–1748

Sacha T (2014) Imatinib in chronic myeloid leukemia: an overview. Mediterr J Hematol Infect Dis 6:e2014007. https://doi.org/10.4084/mjhid.2014.007

Sakar MK, Engelshowe R, Friedrich H (1980) A new flavonol glycoside from *Papaver orientale* leaves. Planta Med 40:193–196. https://doi.org/10.1055/s-2008-1074958

Sattlermc M, Griffin JD (2003) Molecular mechanisms of transformation by the BCR-ABL oncogene. Semin Hematol 40:4–10. https://doi.org/10.1053/shem.2003.50034

Schulz S, Arsene C, Tauber M, McNeil JN (2000) Composition of lipids from sunflower pollen (*Helianthus annuus*). Phytochemistry 54:325–336. https://doi.org/10.1016/S0031-9422(00)00089-3

Schüttelkopf AW, Van Aalten DM (2004) PRODRG: a tool for high-throughput crystallography of protein–ligand complexes. Acta Crystallogr D 60:1355–1363. https://doi.org/10.1107/S0907444904011679

Shahrabi S, Azizidoost S, Shahjahani M, Rahim F, Ahmadzadeh A, Saki N (2014) New insights in cellular and molecular aspects of BM niche in chronic myelogenous leukemia. Tumor Biol 35:10627–10633. https://doi.org/10.1007/s13277-014-2610-9

Shamroe CL, Comeau JM (2013) Ponatinib: a new tyrosine kinase inhibitor for the treatment of chronic myeloid leukemia and Philadelphia chromosome–positive acute lymphoblastic leukemia. Ann

Pharmacother 47:1540–1546. https://doi.org/10.1177/1060028013501144

Skok Z, Zidar N, Kikelj D, Ilaš J (2019) Dual inhibitors of human DNA topoisomerase II and other cancer-related targets. J Med Chem 63: 884–904. https://doi.org/10.1021/acs.jmedchem.9b00726

Sparreboom A, Loos WJ (2004) Protein binding of anticancer drugs. In: Figg WD, McLeod H (eds) Handbook of anticancer pharmacokinetics and pharmacodynamics. Cancer drug discovery and development. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-59259-734-5_12

Sultana B, Anwar F (2008) Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. Food Chem 108:879–884. https://doi.org/10.1016/j.foodchem.2007.11.053

Toledo ME, Ueda Y, Imahori Y, Ayaki M (2003) L-Ascorbic acid metabolism in spinach (*Spinacia oleracea* L.) during postharvest storage in light and dark. Postharvest Biol Technol 28:47–57. https://doi.org/10.1016/S0925-5214(02)00121-7

Toyoda M, Asahina M, Fukawa H, Shimizu T (1969) Isolation of new acyclic C25-isoprenyl alcohol from potato leaves. Tetrahedron Lett 10:4879–4882. https://doi.org/10.1016/S0040-4039(01)88836-5

Valencia-Serna J, Gul-Uludağ H, Mahdipoor P, Jiang X, Uludağ H (2013) Investigating siRNA delivery to chronic myeloid leukemia K562 cells with lipophilic polymers for therapeutic BCR-ABL down-regulation. J Control Release 172:495–503. https://doi.org/10.1016/j.jconrel.2013.05.014

Wang R, Furumoto T, Motoyama K, Okazaki K, Kondo A, Fukui H (2002) Possible antitumor promoters in *Spinacia oleracea* (spinach) and comparison of their contents among cultivars. Biosci Biotechnol Biochem 66:248–254. https://doi.org/10.1271/bbb.66.248

Wei W, Ying X, Zhang W, Chen Y, Leng A, Jiang C, Liu J (2014) Effects of vitexin-2″-*O*-rhamnoside and vitexin-4″-*O*-glucoside on growth and oxidative stress-induced cell apoptosis of human adipose-derived stem cells. J Pharm Pharmacol 66:988–997. https://doi.org/10.1111/jphp.12225

Wolf FT, Coniglio JG, Davis JT (1962) Fatty acids of spinach chloroplasts. Plant Physiol 37:83–85. https://doi.org/10.1104/pp.37.1.83

Zamakshshari NH, Ee GC, Ismail IS, Ibrahim Z, Mah SH (2019) Cytotoxic xanthones isolated from *Calophyllum depressinervosum* and *Calophyllum buxifolium* with antioxidant and cytotoxic activities. Food Chem Toxicol 133:110800. https://doi.org/10.1016/j.fct.2019.110800