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ABSTRACT

Based on our previous biological screening of certain Egyptian edible leafy vegetables against leukemia K562 cell line, Spinacia oleracea (SO) leaves demonstrated a potential antileukemic activity. Thus, the aim of this study is to carry out detailed phytochemical and biological investigations of SO leaves cultivated in Egypt. Bio-guided study of the n-hexane fraction using GC/MS analysis (Table 1 and 2) resulted in the identification of twenty-five compounds in the saponifiable matter and the isolation of twelve compounds (1-12) from the unsaponifiable matter. Phytochemical study of SO ethyl acetate fraction resulted in the isolation of two new flavone C-glycosides: Isoswertisin -3''-O-xyloside (13) and Vitexin 3''-O-xyloside (14), alongside with three known compounds. The biological study revealed that compounds (1, 4, and 6) exhibited a remarkable antiproliferative activity against K562 cells invitro. In Silico mechanistic study showed that compounds (1, 4, and 6) exhibited a strong binding affinity towards Abl Kinase (docking score = -8.5523, -7.6724 and -9.6475 Kcal/Mole, respectively). Moreover, compound (1) showed a strong binding affinity towards topoisomerase (docking score = -8.4926 and Kcal/Mole). As a result of our findings, we recommend the incorporation of spinach leaves in the food regimen for chronic myeloid leukemia (CML) patients.

GC/MS ANALYSIS

Table 1 Results of GC/MS analysis of fatty acid methyl ester (FAME) of the n-hexane fraction of SO 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 n-hexane fraction of SO 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 %	

MOLECULAR DOCKING

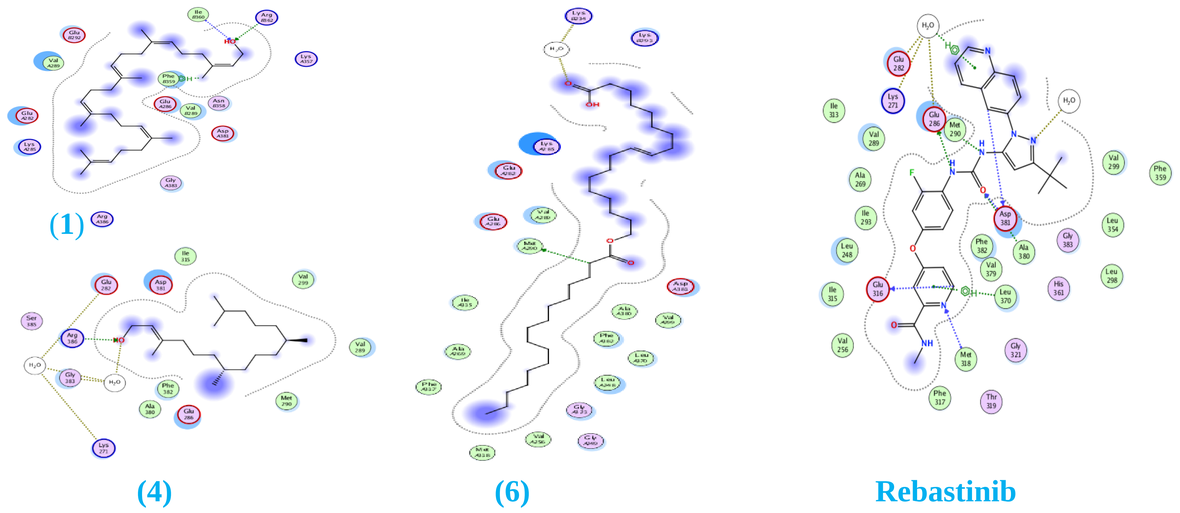


Fig.1.Binding affinity of compounds (**1**, **4**, and **6**) isolated from *SO* leaves compared to Rebastini against Abl kinase

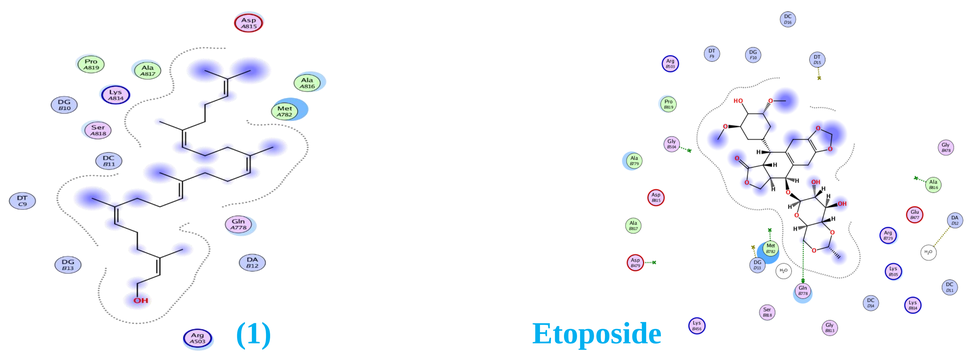
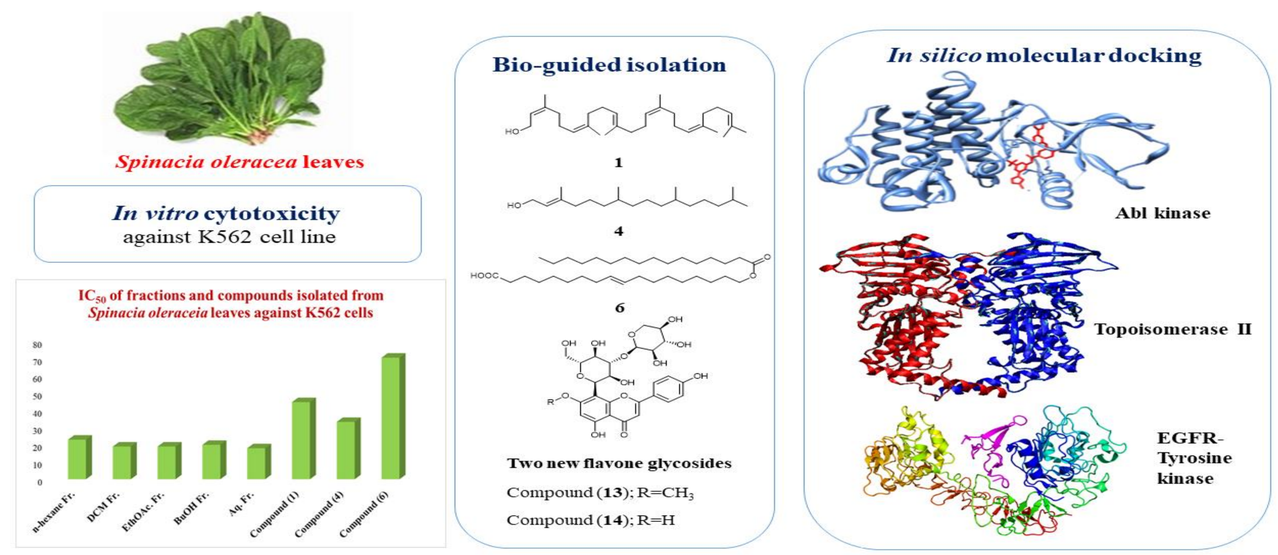


Fig.2. Binding affinity of compound (**1**) isolated from *SO* leaves compared to Etoposide against topoisomerase

GENERAL SCHEME



CONCLUSIONS

The n-hexane fraction of Egyptian Spinach leaves as well as its isolated compounds; Hexaprenol (1), Phytol (4) and 18-[(1-Oxohexadecyl) oxy]-9-octadecenoic acid (6) showed 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 the incorporation of Spinach leaves in the food regimen for the CML patients

ACKNOWLEDGMENTS

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