REVIEW ARTICLE

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records of natural products

Screening Some Plants for their Antiproliferative Compounds

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Abstract: This paper covers the screening of the secondary plant products to find a cure against cancer which were piled up during the years. In early stages of these studies highly active antitumor glycoproteins were obtained from native Arizona (USA) plants. Later smaller molecules were isolated showing antitumor activity in different test systems. Among these compounds sesquiterpene lactones with an exo-methylene group in the lactone ring, unsaturated diterpenoids and some triterpenoids exhibited activity *in vivo* and *in vitro* test systems. A few *Colchicum* alkaloids showed high activity against murine lymphocytic leukemia (P388). Activity also established in some flavonoidal compounds. Today all around the world research on Natural Products is still going on.

Keywords: Antitumor activity; glycoproteins; alkaloids; terpenoids.

A plant Screening Program has started by NIH (USA) some years ago to find a cure to cancer. As a part of this program we have screened quite a number of desert plants. Among them, three plants from different families have yielded proteinaceous substances. The aqueous extracts of the roots of the plants demonstrated activity toward the Sarcoma 180 test system in mice. Screening of the fractions was carried out by the Cancer Chemotherapy National Service Center (CCNSC) [1].

The dried root material of *Gutierrezia sarothrae* (Compositae) (3 kg) was extracted with petroleum ether, than with water at room temperature [2]. The aqueous fraction was washed with benzene and chloroform, the aqueous fraction was lyophilized in a Repp Industries model 15 sublimator. From 3 kg dried roots 200 g of a residue was obtained, this was dissolved in water and extracted with ether and chloroform until extinction, the remaining aqueous part was lyophilized, washed with EtOH, the powder dissolved in a 0.1 M phosphate buffer system of pH 8.04 and dialyzed against distilled water. After a week of dialysis, a precipitate occured in the dialysis tube, when

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centrifuged a precipitate (I) and solution (II) were separated, both were lyophilized and tested against Sarcoma 180 test system in mice (Table 1).

Table 1. Sarcoma 180 results of precipitate (I) and solution (II) in mice

	Dose (mg/kg)	% T/C [a]
I	60	18
	40	37
II	90	37
	50	28

[a]The criteria for activity is defined as being a %T/C (test/control) value of less than 42 in a satisfactory dose response test (2).

These active fractions were further separated and cleaned on Celite 565 (cellulose), Cellex-D and Sephadex columns using 0.005 M phosphate buffer solution of pH 7, and a gradient of buffers has passed from the column, thus six ninhydrin positive fractions were obtained, one fraction showed T/C of 22% at 50 mg/kg dose which was considered good. This fraction upon hydrolysis and 2D paper chromatography showed the presence of 30 amino acids and 3 sugar spots which were glucose, glucuronic acid and acetyl glucoseamine.

The second plant *Mirabilis multiflora* (Nyctaginaceae) has also yielded glycoproteins [3]. Its crude aqueous extract demonstrated activity against Sarcoma 180. After getting the final residue, the purification was attempted including columns substrates of DEAE Sephadex A-50, CM Sephadex C-50, G-50, G-100 and G-200. Fractions obtained from column separation were tested against *in vivo* tests using four different systems (Table 2).

From this plant, a protein and a glycoprotein were separated after further cleaning and lyophilization. The glycoprotein was hydrolyzed with 6 N HCl, the resulting solution was applied on 2D paper chromatography using phenol:water (3:1), in the first and BuOH:formic A.:water (7:1:3) in the second dimention. Also a Beckman model 120B amino acid analyser was used to find the percentage of the amino acids as seen in Table 3.

Table 2. *In vivo* tumor inhibition

	Dose (mg/kg)	% T/C[a]
Lewis Lung Carcinoma	12	38
P-1798 Lymphosarcoma	12	22
	8	55
	5.3	72
	3.5	53
Sarcoma 180	12	33
	10	8
	10	11
	4.4	44
	2.9	63
Walker Carcinosarcoma 256 (intramuscular)	45	39
	12	58
	8	79
	5.3	73
	3.5	69

[a]The criteria for activity is defined as being a %T/C (test/control) value of less than 42 in a satisfactory dose-response test.

Table 3. Preliminary amino acid analysis of *Mirabilis multiflora* proteins

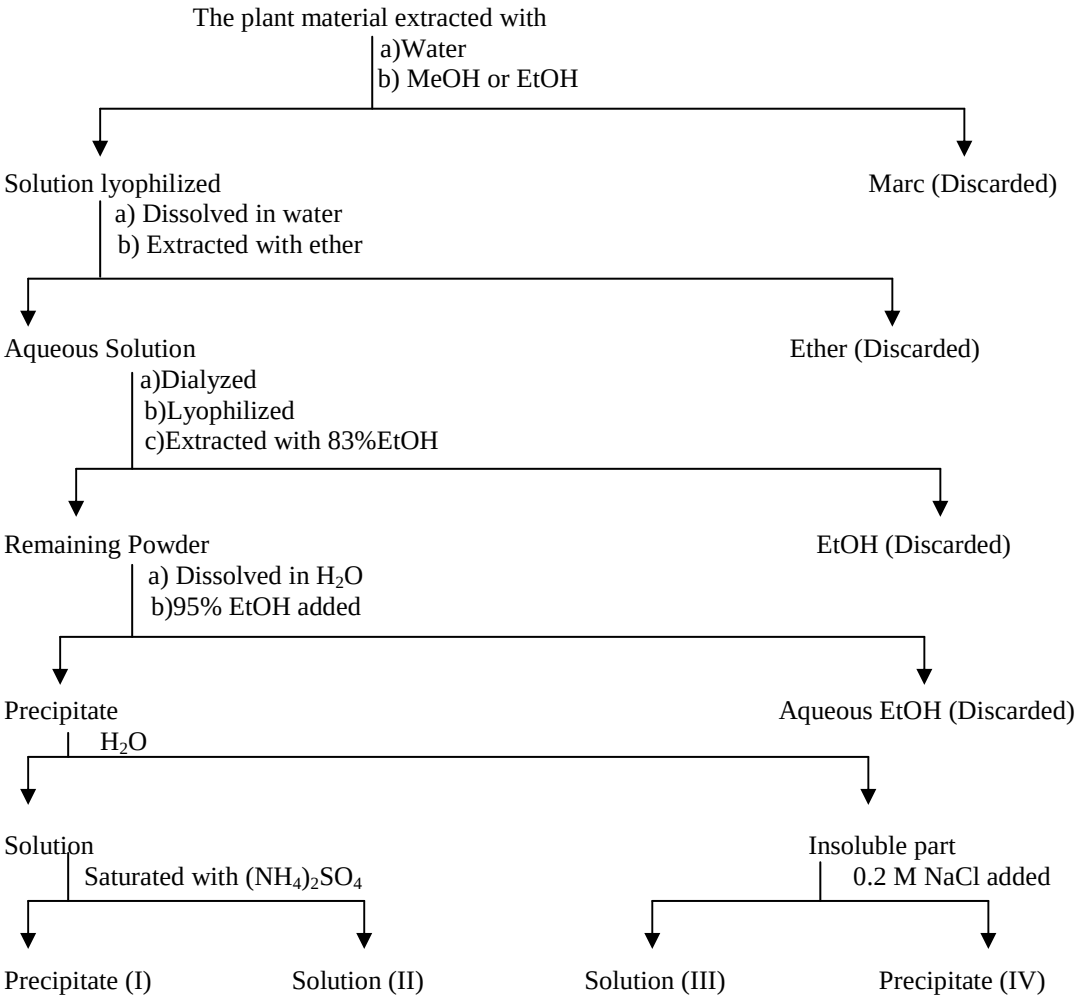
Amino acids	%
Lysine	5.74
Histidine	1.09
Arginine	4.32
Aspartic acid	12.49
Threonine	6.44
Serine	4.95
Glutamic acid	8.00
Proline	3.45
Glycine	6.10
Alanine	2.95
Half-cystine	6.54
Valine	5.24
Methionine	0.89
Isoleucine	6.91
Leucine	4.89
Tyrosine	6.54
Phenylalanine	4.62

A third plant *Caesalpinia gilliesii* also showed potential antitumor activity [4]. The dried pods (10 kg) were extracted with water at room temperature, filtered and lyophilized to obtain 760 g of a residue, 100 g of this residue was treated as given in schematic diagram (Scheme 1). This separation procedure was also used in the other two plants namely *G. sarothrae* and *M. multiflora*. The proteinaceous part of *C. gilliesii* exhibited a T/C value of 3% at 33 mg/kg, another fraction 28% at 100 mg/kg. The UV, IR and amino acid data of the proteins were recorded.

The four fractions (I, II, III, IV) of *C. gilliesii* were further separated on Sephadex columns. Fractions I and II yielded two fractions, Ia and Ib as well as IIa and IIb. Paper electrophoresis of these six fractions were carried out, utilizing a barbital buffer solution pH 8.6, 0.05 ionic strenght, in a Spinco model Beckman instrument, and after 14 hr of electrophoresis using 2 ma. Current, the papers were developed using periodic acid and Schiff reagent. Mobilities of the six fractions sugar moieties, UV maxima and nitrogen and ash contents were given in Table 4. Amino acid analysis of four fractions were shown in Table 5.

Table 4. Physical and chemical characteristics of six protein fractions

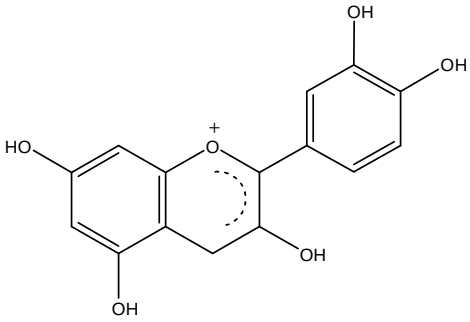
Fractions	Mobilities $cm^2/sec.v.$	Sugars	UV max (nm)	N %	Ash content %
Ia	$1.07x10^{-6}$	Mannose, fructose	204, 208	12.8	1.5
Ib	$6.4x10^{-7}$	Mannose, rhamnose	204, 265	11.6	1.5
IIa	$2.2x10^{-6}$	Mannose, glucosamine	204, 280	5.7	3.9
IIb	$1.2x10^{-6}$	Mannose, glucosamine	204, 280	6.2	0.0
III	$1.2x10^{-6}$	Mannose, glucosamine	204, 265	13.6	6.0
IV	$1.6x10^{-6}$	Mannose, rhamnose, glucosamine	204, 240, 280	14.08	6.0



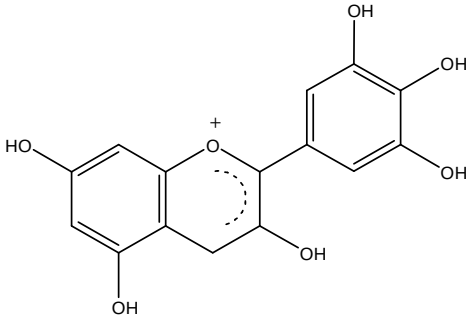
Scheme 1. Extraction procedure of *G. sarothrae, M. multiflora, C. gilleisii*

A survey in the literature has shown a number of publications concerning the relation between malignant tumors and the increase of glycoprotein concentration in the blood of test animals and humans. There are, however, several varied opinions as to the cause of this increase, Seibert and co-workers reported that the source of glycoproteins was the breakdown of the products of tissue necrosis [5]. There are several other suggestions on how the glycoproteins are found in the blood stream. Macbeth et al. suggested the liver is the most active agent in the synthesis of these glycoproteins [6]. By using isotopic techniques they have shown that the tumor system is capable of synthesizing and subsequently liberating glycoproteins into the blood stream. This work was performed on intact and hepatectomized rats.

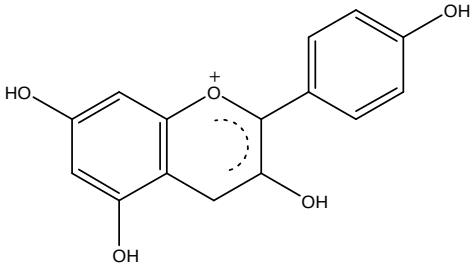
Other plants collected from the desert area of Arizona were *Abies concolor*, its hexane extract and especially alcohol soluble part showed activity against adenocarcinoma of the duodenum (7D1) test system (Table 6) [7]. The plant known as white fir, is an evergreen tree found in 900 - 3000 m elevations of Central and South USA. Polymeric phenolic compounds were present in the brown powder. Hydrolysis has yielded anthocyanidins, such as cyanidin (**1**) and delphinidin (**2**) (Figure. 1).



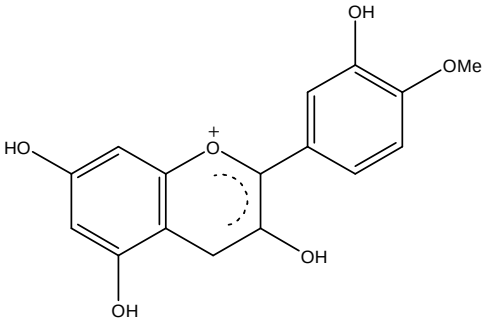
Cyanidin (**1**)



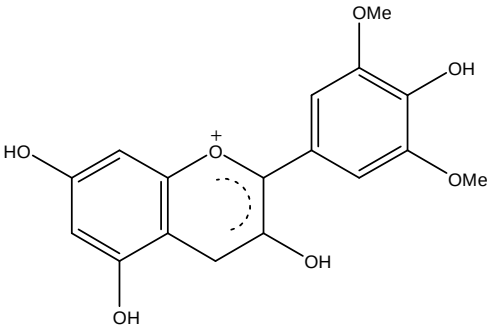
Delphinidin (**2**)



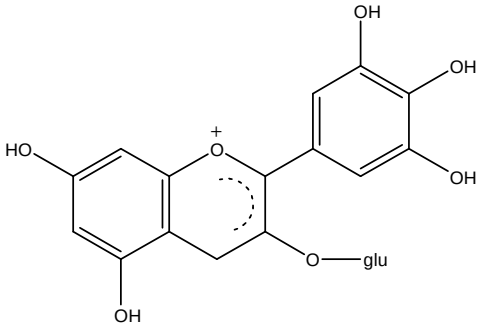
Pelargonidin (**3**)



Peonidin (**4**)



Malvidin (**5**)



Delphinidin-3-glucoside (**6**)

Figure 1. Anthocyanidins

Table 5. Preliminary amino acid analysis of *Caesalpinia* proteins

	% µm of Amino acids			
Amino Acids	I	II	III	IV
Aspartic acid	10.50	6.01	10.70	10.50
Threonine	6.04	7.66	5.81	5.08
Serine	6.66	13.00	5.88	6.01
Glutamic acid	16.20	7.18	13.60	15.80
Proline	5.02	3.32	4.90	4.95
Glycine	11.30	7.62	10.10	9.73
Alanine	8.89	13.30	10.90	8.90
Cystine	2.82	0.522	0.783	1.02
Valine	5.07	3.72	6.57	6.05
Methionine	1.04	0.572	1.52	1.27
Isoleucine	3.22	1.62	4.28	4.23
Leucine	6.02	3.53	7.75	7.87
Tyrosine	2.23	0.731	2.01	2.25
Phenylalanine	3.19	1.25	3.75	3.64
Lysine	3.99	2.41	3.21	3.57
Histidine	1.51	0.731	1.59	1.78
Arginine	6.24	1.51	5.95	6.73
Glucosamine	-	1.50[a]	0.644	0.454
Hydroxypyroline	-	23.80[a]	-	-

[a]Estimated values

Table 6. *In vivo* tumor inhibition of *Abies concolor*

	Dose (mg/kg)	% T/C
n-Hexane extract	175	32
	200	32
Brown powder (EtOH extract)	100	26
	200	11

Plant Antitumor Research Program has continued by screening about 100 Turkish plants (Table 7) [8]. Since *Merendera caucasica* was found to be highly active plants, they were further studied. The aqueous extract of the bulbus of *M. caucasica* was found active up to 240% in 150 mg/kg doses [9]. While the alkaloidal extract was also active even in smaller doses, 22 mg/kg 222% and 14 mg/kg doses 190% active in 3PS, P388 (murine lymphocytic leukemia) *in vivo* test system. Both parts were separately studied. The proteinaceous part was cleaned and separated on Sephadex G-200 column, the collected fractions were checked on UV at 236 and 280 nm. Two main fractions which were separately analysed in an amino acid analyser to give the polypeptide behavior (Table 8). The alkaloidal part was also studied, and single alkaloids were obtained [10].

Table 7. *In vivo* and *in vitro* test results of some plants collected in Turkey

Plant names	9 KB (*In vitro*)	*In vivo*
Symphytum orientale	$9.3x10^{-1}$	47 WM (-)
Paeonia decora	$2.7x10^{-1}$	-
Clematis vitalba	-	37 WM (+)
Genista tinctoria	$9.3x10^{-1}$	130 3LE (+)
Cystoceira barbata	$9.3x10^{-1}$	154 3PS (++)
Ceramium rubrum	-	147 3PS (++)
Verbascum lasianthum	-	141 3PS (+)
Merendera caucasica	$2.8x10^{-1}$	170 3PS (+++)
		210 3PS (+++)
Cynicus benedictus	-	138 3PS (+)
Arbutus andrachne	-	130 3PS (+)
Marsdenia erecta	-	40 WM (+)
Ceratonia siliqua	$2.7x10^{-1}$	-
Styrax officinale	-	141 3PS (+)
Campanula ephesia	-	120 3PS (-)
Genista lydia	-	122 3PS (-)
Plantago major	-	133 3PS (+)
Taxus baccata	-	136 3PS (+)
Pancratium maritimum	-	125 3PS (-)
Papaver rhoeas	-	126 3PS (-)
Typha angustifolia	-	125 3PS (-)
Merendera atticum	-	175 3PS (++)
		190 3PS (+++)
		200 3PS (+++)

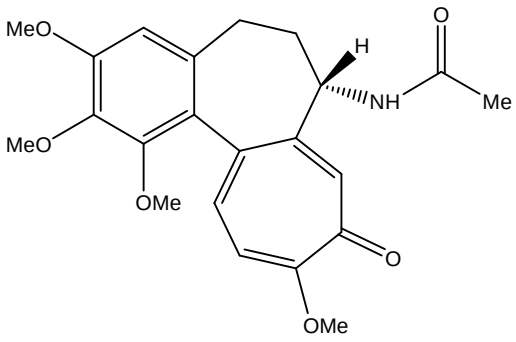
The criteria for activity the value over 130% is considered active

Table 8. Amino acids of polypeptides I and II

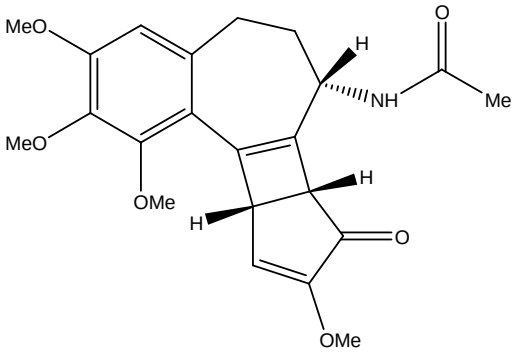
Amino acids µmol/100 mg	Polypeptide I	Polypeptide II	Free Amino Acids
Lysine	0.301	0.109	1.00
Histidine	-	-	-
Ammonia	3.396	56.96	65.33
Arginine	-	0.02	1.00
Aspartic Acid	0.434	65.85	4.00
Threonine	0.035	56.25	0.22
Serine	-	0.03	5.00
Glutamic Acid	0.648	137.88	5.00
Proline	0.489	3.80	65.00
Glycine	0.352	34.98	-
Alanine	-	26.92	21.00
Half Cystine	-	2.55	-
Valine	-	-	23.00
Methionine	-	-	-
Isoleucine	-	3.92	6.00
Leucine	-	4.04	13.00
Tyrosine	-	0.53	1.00
Phenylalanine	-	0.44	1.00

Single amino acids, such as *D*-threonine, and *D*-lysine were found active against Ehrlich tumor cells [11]. While the polypeptides were only moderately active against 3PS system (400 mg/kg doses 169%). When the aqueous alcoholic extract of *Merendera caucasica* acidified with dilute sulphuric acid

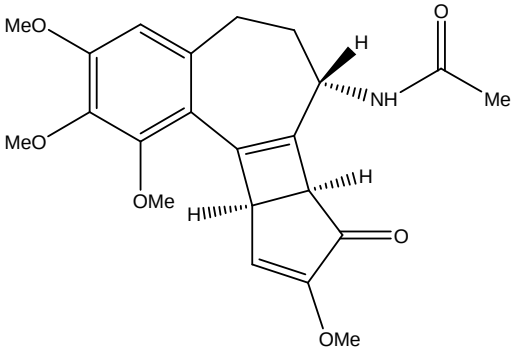
and extracted with chloroform a crude alkaloidal mixture was obtained, the mixture was tested *in vitro* 3PS test system which showed a high antitumor activity 222% in 22 mg/kg and 190% in14 mg/kg doses. By column chromatographic and preparative TLC separations of the alkaloidal mixture, colchicine (**7**), *β* and *γ*-lumicolchicine (**8**, **9**) were isolated and their structures were determined (Figure 2). Since they are highly toxic compounds no further study was performed.



Colchicine (**7**)



β-Lumicolchicine (**8**)



γ-Lumicolchicine (**9**)

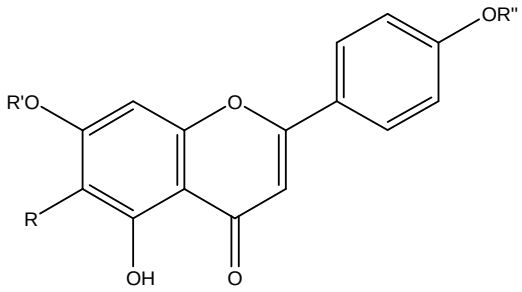
Figure 2. *Merendera* alkaloids

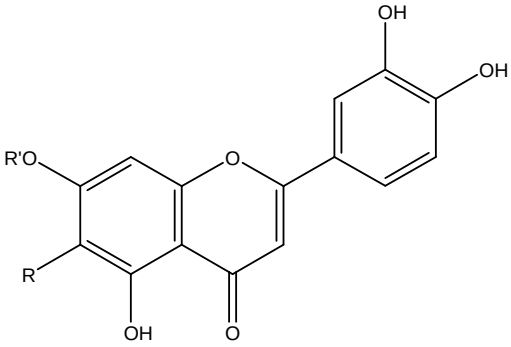
Another plant *Paeonia decora* which was collected from Hadimkoy (near Istanbul) has shown activity against cell culture 9KB test system ($3.0x10^{-1}$) [12]. The crude extract was also tested against lymphoid leukemia L1210 (LE) test system, no activity was found. The analysis of the crude extract has yielded a group of phenolic acids, such as protocatechuic acid, gallic acid, p-hydroxybenzoic acid, syringic acid, 3-methoxy-4-hydroxybenzoic acid and phloroglucin. From the flowers anthocyanins, cyanidin (**1**), pelargonidin (**3**), peonidin (**4**), malvidin (**5**) and delphinidin-3-glucoside (**6**) were obtained (Figure 1).

Crataegus monogyna (Rosaceae) alcoholic extract showed *in vitro* activity against 9KB test system in ($2.5x5.10^{0}$) [13]. Ursolic and oleanolic acids, crataegolic acid, caffeic acid, a group of fatty compounds and *β*-sitosterol were obtained.

Cytotoxic flavonoids were isolated from the chloroform and ethyl acetate fractions of *Centaurea urvillei* [14]. The flavonoids were hispidulin (6-methoxyapigenin) (**10**), apigenin (**11**), cirsimaritin (6-methoxyapigenin-7-methyl ether) (**12**), genkwanin (apigenin-7-methyl ether) (**13**), salvigenin (6-

methoxyapigenin 7,4'-dimethyl ether) (**14**), apigenin-7*β-D*-glucoside (**15**), cirsiliol (6-methoxyluteolin-7-methyl ether) (**16**), luteolin (**17**) and nepetin (6-methoxyluteolin) (**18**) (Figure 3). The crude extract and hispidulin (**10**) (main flavonoid, 380 mg) were tested while other compounds were between 5-15 mg and not tested. The activity test was against *L*-strain fibroblast in tissue culture. The crude flavonoid extract was found to be active and hispidulin was highly active in 0.05 mg/mL doses.



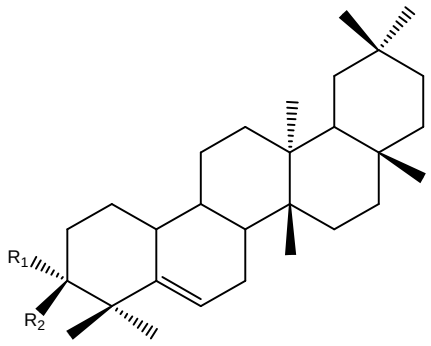


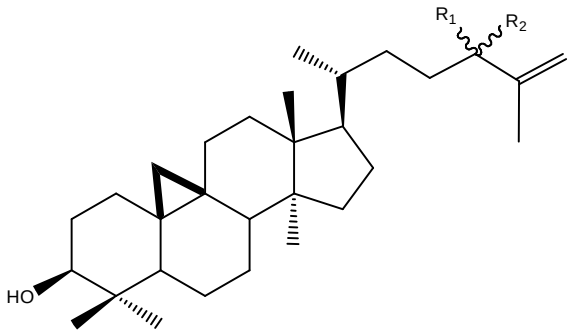
R= OMe; R' = R" =H Hispidulin (**10**)
R= R' =R" =H Apigenin (**11**)
R=OMe; R'=Me; R"=H Cirsimaritin (**12**)
R=H; R' =Me; R" =H Genkwanin (**13**)
R= OMe; R' = R" =Me Salvigenin (**14**)
R=H; R' =glu; R" =H Apigenin-7b-D-glucoside (**15**)

R=OMe; R'=Me Cirsiliol (**16**)
R= R' =H Luteolin (**17**)
R=OMe ; R'=H Nepetin (**18**)

Figure 3. *Centaurea* flavonoids

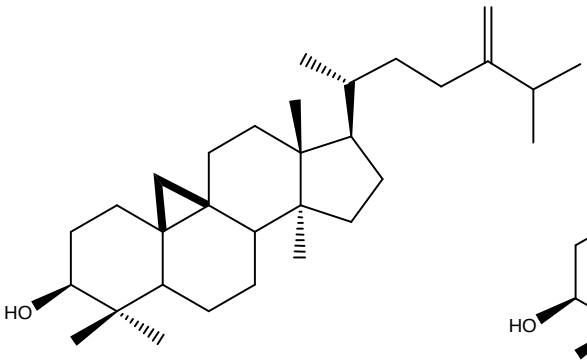
Euphorbia cyparissias latex is known as irritant. Two new and several known triterpenoids were isolated [15]. The new compounds were glut-5-ene-3*α*-methylbutyrate (**19**) and the other 3*β*-hydroxycycloart-25-ene-24-hydroperoxide (**22**), other compounds are as shown in Figure 4. The crude extract as well as the known compounds have shown *in vitro* cytotoxic potential with cultured P-388 (murine lymphocytic leukemia) and KB cells (Table 9).



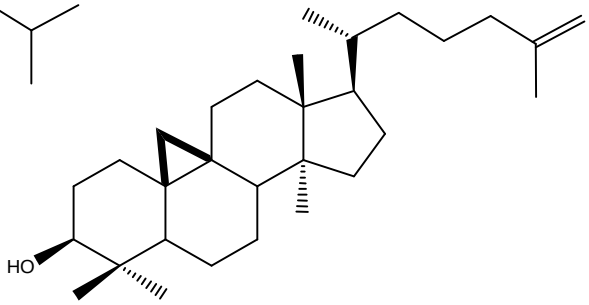


R_1=O-methylbutyl; R_2=H
Glut-5-ene-3*a*-methylbutyrate (**19**)
R_1=OH; R_2=H Glut-5-ene-3*a*-ol (**20**)
R_1-R_2=O Glut-5-ene-3-one (**21**)

R_1=OOH; R_2=H
3*b*-Hydroxycycloart-25-ene-24-hydroperoxide (**22**)
R_1-R_2=O 3*b*-Hydroxycycloart-25-ene-24-one (**23**)



24-Methylenecycloartanol (**24**)



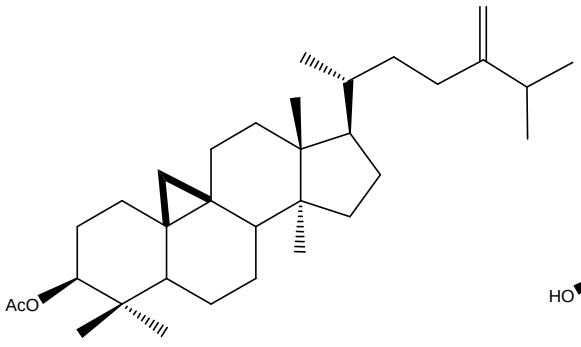
Cycloartanol (**25**)

Figure 4. *Euphorbia cyparissias* compounds

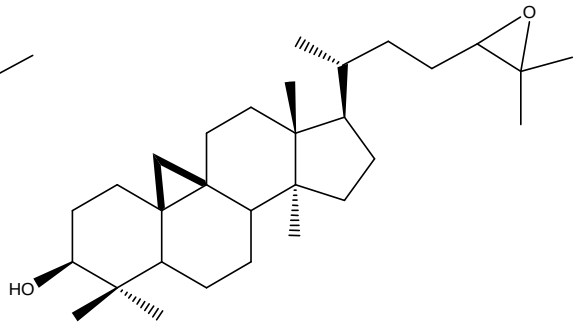
Table 9. Cytotoxicity of the isolated compounds (19-25) in the P-388 and KB systems.

	ED_{50} (µg/mL)	
Tested Compounds	KB	P-388
Glut-5-ene-3*α*-methylbutyrate (**19**)	>20	>5
Glut-5-ene-3*α*-ol (**20**)	>20	>5
Glut-5-ene-3-one (**21**)	>20	>5
3*β*-hydroxycycloart-25-ene-24-hydroperoxide (**22**)	16.4	>5
3*β*-hydroxycycloart-25-ene-24-one (**23**)	7.6	0.9
24-methylenecycloartanol (**24**)	>20	2.5
Cycloartanol (**25**)	8.9	11.1

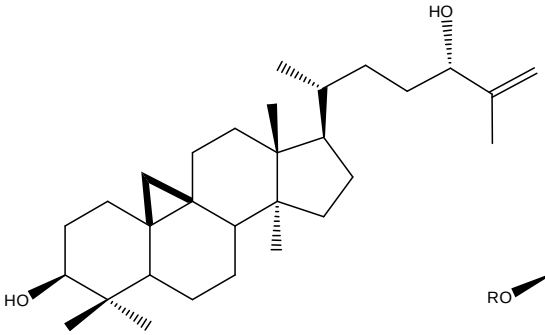
Another species *E. nicaeensis* subsp. *glareosa* both PE and EtOH extracts as well as the compounds (Figure 5) [16] were tested against P-388 (murine lymphocytic leukemia) test system, the results are given in Table 10. Except the acetyl derivatives, the compounds exhibited significant activities.



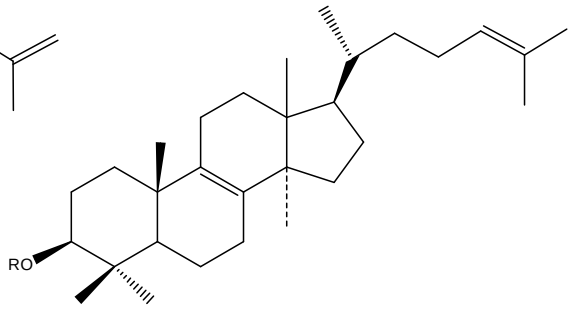
24-Methylenecycloartenyl acetate (**26**)



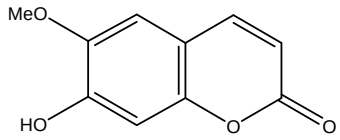
Cycloart-24,25-oxido-3*β*-ol (**27**)



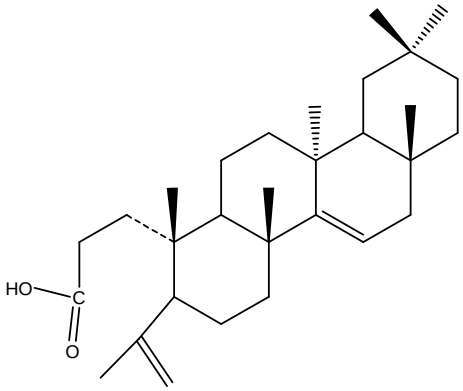
Cycloart-25-ene-3*β*,24-diol (**28**)



R=H Euphol (**29**)
R=Ac Euphol acetate (**30**)



Scopoletin (**31**)



3,4-Seco-4(23), 14-taraxeradien-3-oic acid (**32**)

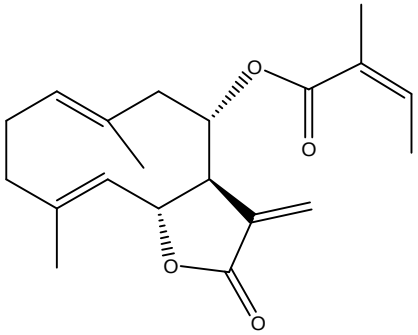
Figure 5. *Euphorbia nicaeensis* subsp. *glareosa* compounds

Table 10. Cytotoxicity of the extracts and isolated compounds from *E. nicaeensis* subsp. *glareosa* in the P-388 test system

Tested Compounds	ED_{50} (µg/mL)[a]
PE Extract	0.2
EtOH Extract	1.0
24-Methylenecycloartanol (**24**)	2.5
24-Methylenecycloartenyl acetate (**26**)	5
Cycloart-24,25-oxido-3*β*-ol (**27**)	1.3
Cycloart-25-ene-3*β*-24-diol (**28**)	2.4
Euphol (**29**)	2.4
Euphol acetate (**30**)	5
Scopoletin (**31**)	2.6
3,4-Seco-4(23),14-taraxeradien-3-oic acid (**32**)	3.8

[a]Pure compounds demonstrating ED_{50} value of < 4.0 µg/mL are considered active.

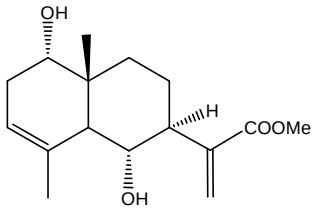
Some *Tanacetum* species exhibited cytotoxic activities. Among them *T. argenteum* is an endemic plant to Turkey which yielded a group of sesquiterpene lactones, one of them a germacranolide 8α-angeloyloxycostunolide (**33**) (Figure 6) [17] demonstrated general cytotoxic activity against human breast cancer (BC1), HT, human lung cancer (LU1), human colon cancer (COL-2), human epidermoidal carcinoma in mouth (KB), murine lymphocytic leukemia (P-388), A-431, hormon dependent human prostate cancer (LNCaP) ,ZR-75-1 and U373 cells in cultures. ED_{50} values 0.8, 0.9, 2.7, 1.9, 3.4, 0.6, 1.4, 1.7, 1.8, 1.0 µg/mL were observed, respectively.



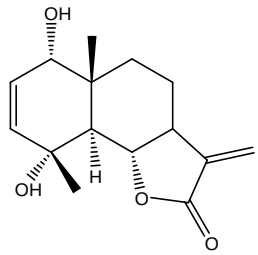
8α-Angeloyloxycostunolide (**33**)

Figure 6. Sesquiterpene lactone from *Tanacetum argentum*

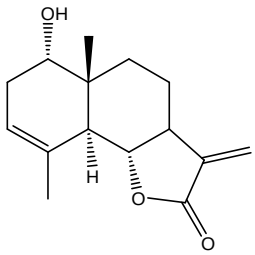
Another species of *Tanacetum*, *T. praeteritum* subsp. *praeteritum* has yielded ten sesquiterpene lactones and a sesquiterpene [18]. The compounds 1α,6α-dihydroxyisocostic acid methyl ester (**34**), 1α-hydroxy-1-deoxoarglanine (**35**), douglanin (**36**), santamarin (**37**), reynosin (**38**), 1-*epi*-tatridin B (**39**), ludovicin A (**40**), armexin (**41**), armefolin (**42**), armexifolin (**43**), 3α-hydroxyreynosin (**44**), tatridin A (**45**), and tamirin (**46**) were tested against human lung carcinoma cell line GLC_4, and colorectal cancer cell line COLO 320 (Figure 7). The results are given in Table 11, except arglanine derivative they are considered to have activity.



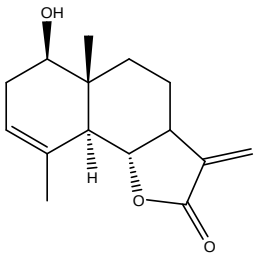
1α, 6α-Dihydroxyisocostic acid methyl ester (**34**)



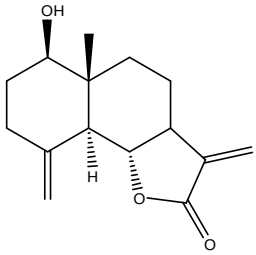
1α-Hydroxy-1-deoxoarglanine (**35**)



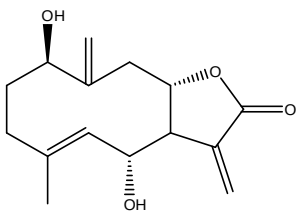
Douglanin (**36**)



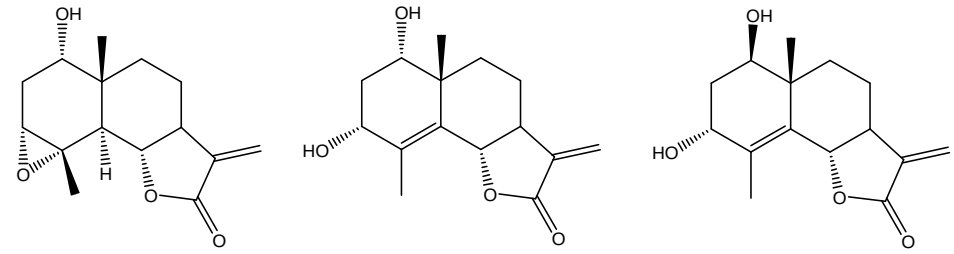
Santamarin (**37**)



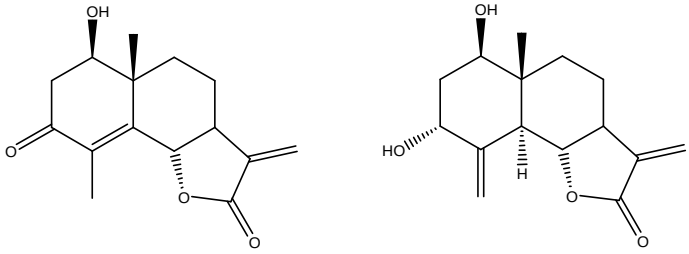
Reynosin (**38**)



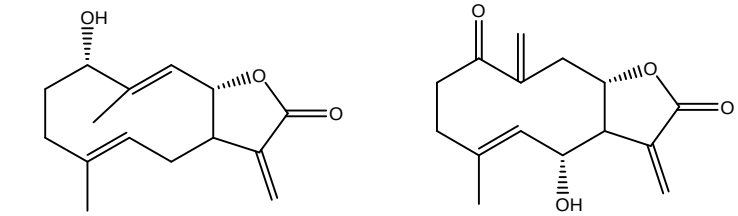
1-*Epi*-tatridin B (**39**)



Ludovicin A (**40**) Armexin (**41**) Armefolin (**42**)



Armexifolin (**43**) 3*α*-Hydroxyreynosin (**44**)



Tatridin A (**45**) Tamirin (**46**)

Figure 7. Sesquiterpenoids from *Tanacetum praeteritum* subsp. *praeteritum*

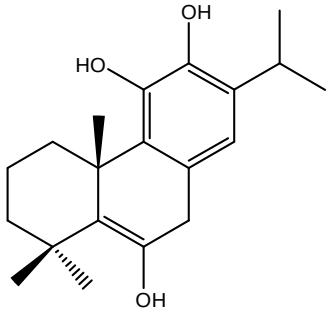
Table 11. Cytotoxic avtivity of the tested compounds (given in IC_{50} values; µM)

Compounds	GLC_4	COLO 320
1*α*,6*α*-dihydroxyisocostic acid methyl ester (**34**)	15.4±0.5	20.6±1.2
1*α*-hydroxy-1-deoxoarglanine (**35**)	18.8±2.5	23.6±0.6
Douglanin (**36**)	7.8±0.3	8.1±0.3
Santamarin (**37**)	8.1±0.2	7.4±0.4
Reynosin (**38**)	10.7±0.4	8.9±0.3
1-*Epi*-tatridin B (**39**)	8.7±0.4	7.3±0.3
Ludovicin A (**40**)	7.6±0.7	11.0±1.3
Armexin (**41**)	8.7±0.4	11.3±0.7
Armefolin (**42**)	15.2±1.0	18.5±1.6
Armexifolin (**43**)	2.5±0.1	4.3±0.3
3α-Hydroxyreynosin (**44**)	16.0±0.4	18.2±2.6
Tatridin A (**45**)	4.7±0.5	5.1±0.3
Tamirin (**46**)	1.0±0.1	2.2±0.1
Cisplatin[a]	1.0±0.2	3.0±0.4

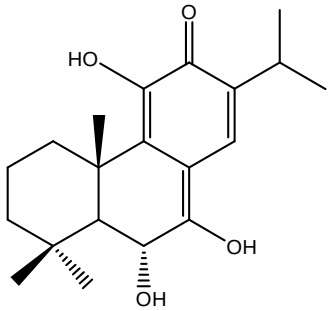
[a]Standard

The diterpenoids obtained from the extract of *Salvia hypargeia* were 6*α*-hydroxysalvinolone (**47**), 6*α*-hydroxytaxodone (**48**), ferruginol (**49**), saprorthoquinone (**50**), 11,12-dioxo-abieta-8,13-diene

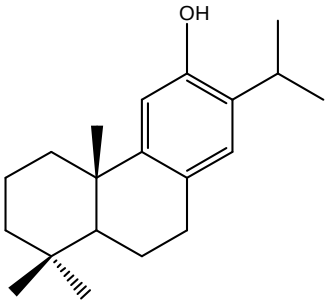
(**51**), taxodione (**52**), hypargenin A (**53**), hypargenin D (**54**) (Figure 8) [19]. These diterpenoids were tested against a panel of human cancer cell lines, human breast cancer (BC 1), human lung cancer (LU 2), human colon cancer (COL 2), human epidermoidal carcinoma in mouth (KB), vinblastine-resistant KB-VI, hormone-dependent human prostate cancer (LNCaP), as well as P388 and ASK cells in culture (Table 12). Taxodione (**52**), previously shown to mediate antitumor activity in the Walker intramuscular carcinosarcoma 256 model, was the most active substance tested. 6*α*-Hydroxytaxodone (**48**) and ferruginol (**49**) showed weak but selective activity against colon cancer cells (COL 2) and human prostate cancer cells (LNCaP). 6*α*-Hydroxysalvinolone (**47**) and saprorthoquinone (**50**) mediated generalized responses. Other compounds were either not active or mediated weak responses in one or two systems.



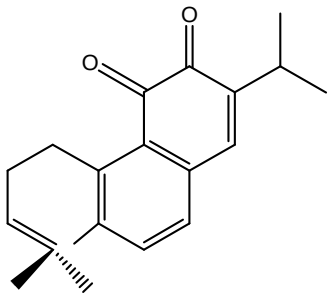
6*α*-Hydroxysalvinolone (**47**)



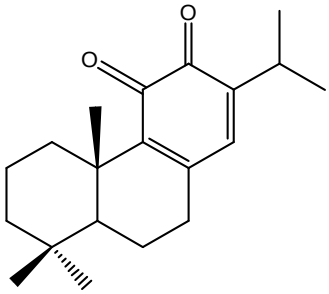
6*α*-Hydroxytaxodone (**48**)



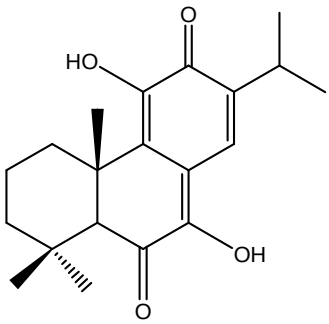
Ferruginol (**49**)



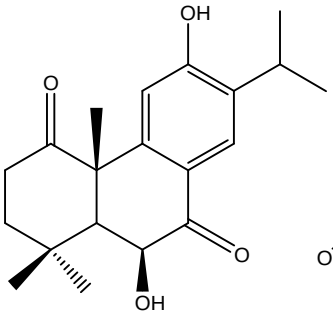
Saprorthoquinone (**50**)



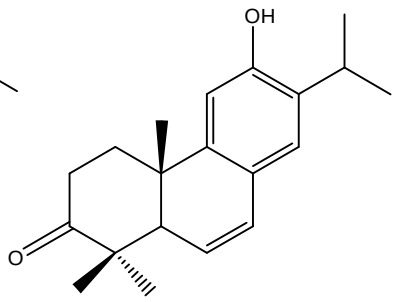
11,12-Dioxo-abieta-8,13-diene (**51**)



Taxodione (**52**)



Hypargenin A (**53**)



Hypargenin D (**54**)

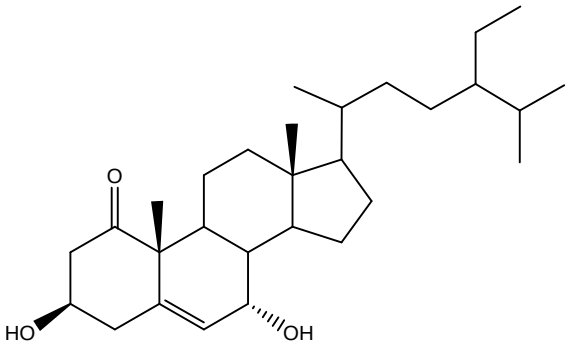
Figure 8. Diterpenoids from *Salvia hypargeia*

Table 12. Cytotoxic activity of the tested compounds and the standard ellipticine[a]

Tested Compounds	BC1	LU1	COL2	KB	KB-IV	LNCaP	P388
6α-Hydroxysalvinolone (**47**)	4.7	4.2	10.1	9.7	5.6	4.0	>5
6α-Hydroxytaxodone (**48**)	>20	>20	9.0	>20	>20	12.9	>5
Ferruginol (**49**)	>20	>20	9.7	>20	>20	>20	>5
Saprorthoquinone (**50**)	9.2	16.4	3.3	>20	9.1	>20	2.3
11,12-Dioxo-abieta-8,13-diene (**51**)	>20	>20	>20	>20	>20	>20	>5
Taxodione (**52**)	1.2	5.1	0.7	3.4	4.1	0.7	0.3
Hypargenin A (**53**)	>20	>20	>20	>20	>20	>20	>5
Hypargenin D (**54**)	12.6	>20	12.3	>20	>20	>20	>5
Ellipticine[b]	0.2	0.02	0.3	0.04	0.3	0.8	0.1

[a]Data are given as ED_{50} values in μg/mL, BC1, human breast cancer; COL2, human colon cancer; LU1, Human lung cancer; KB, originally derived from human nasopharyngeal cancer; KB-VI, multidrug-resistant KB; LNCaP, human prostate cancer; P388, mouse lymphocytic leukemia.
[b]Ellipticine was used as a positive control.

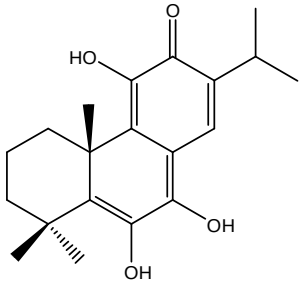
A new steroidal compound, isolated from *S. glutinosa* was tested against P-388 and KB systems, only marginal activity was found (Figure 9) [20].



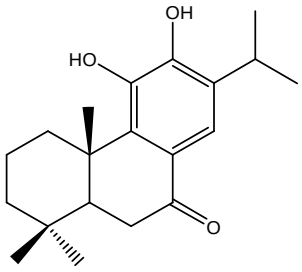
1-Oxo-7*α*-hydroxysitosterol (**55**)

Figure 9. Steroidal compound from *Salvia glutinosa*

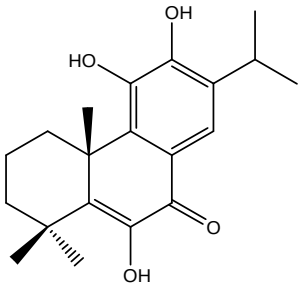
In a recent study with the extracts of about 15 *Salvia* species collected from various parts of Anatolia were tested against A2780 human ovarian cancer cell line (Table 13) [21]. Compounds 6α-hydroxysalvinolone (**47**) and demethylcryptojaponol (**57**) were found to be active against A2780 cell line with IC_{50} values of 3.9 and 1.2 μg/mL (Figure 10).



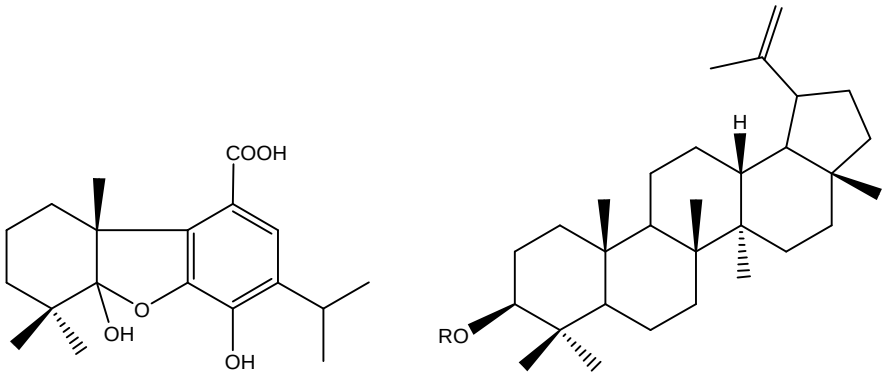
5,6-Didehydro-7-hydroxytaxodone (**56**)



Demethylcryptojaponol (**57**)



14-Deoxycoleon U (**58**)



Salvicanaric acid (**59**)

R=H Lupeol (**60**)
R=Ac Lupeol acetate (**61**)

Figure 10. Further compounds from *Salvia hypargeia*

Table 13. Cytotoxicity of some *Salvia* extracts against the A2780 human ovarian cancer cell line[a]

Plant extract	IC_{50} (µg/mL)
S. amplexicaulis Lam. (root-EtOH)	21.0
S. aucheri Benth. (root-EtOH)	39.6
S. bracteata Banks and Sol. (root-EtOH)	38.2
S. candidissima Vahl (root-EtOH)	31.9
S. cassia G. Samuelsson ex.Rech.f. (aerial parts-EtOH)	29.4
S. eriophora Boiss and Kotschy ex. Boiss. (aerial parts-EtOH)	NA
S. heldreichiana Boiss. Ex Benth. (root-EtOH)	36.0
S. hypargeia Fisch and May (root-acetone)	15.5
S. napifolia Jacq. (root-EtOH)	35.6
S. pilifera Mont. and Auch. Ex Benth.(aerial parts-EtOH)	33.3
S. recognita Fisch and Mey. (aerial parts-acetone)	29.7
S. staminea Mont. and Auch. Ex Benth. (whole plant-MeOH)	36.2
S. syriaca L. (whole plant-EtOH)	41.7
S. tomentosa Mill. (root-EtOH)	36.3
S. triloba. L. (whole plant-CH_2Cl_2)	17.2

[a]The tests were carried out as dose-dependent assay starting from 50 µg/mL doses.

The dried and grounded plant materials were exhausted by maceration in EtOH for their ovarian cytotoxic activity. The most active plant was *S. hypargeia*. Taxodione (**52**) was the most active compound against cancer in a number test system. Also compounds such as 14-deoxycoleon U (**58**) and its 14-hydroxylated derivative coleon U (6α-hydroxysalvinolone (**47**) were also found to be highly active against several cancer lines, Table 14 gives the results of the compounds against A2780 cell line.

Table 14. Cytotoxicity of the isolated abietane diterpenoids and fatty acid mixture against the A 2780 cell line[a]

Tested Compounds	IC_{50} (µg/mL)
S. hypargeia [frac (125-211)]	13.4
5,6-Didehydro-7- hydroxytaxodone (**56**)	18.8
Demethylcryptojaponol (**57**)	3.9
14-Deoxycoleon U (**58**)	1.2
Salvicanaric acid (**59**)	15.0
Lupeol (**60**)	34.0
Lupeol 3-acetate (**61**)	9.0
Fatty acid mixture	0.6

[a]The test was carried out as dose-dependent assay starting from 50µg/mL doses

The cause of cancer is still unknown, however there are a number of suggestions about its occurence, among them cigarette smoking, air pollution, radioactivity, viruses, family history could be mentioned. Scientists in many countries are trying hard to find the cause of cancer. As more research is done and more knowledge is collected about its etiology, the complexity of the problem appears. Nevertheless the difficulty does not stop the scientists they will work until the problem is solved. As a group we humbly tried to add our effort to find a way for the treatment of cancer. We have screened the plants first in Arizona (USA), then in Istanbul (Turkey). We have wored with hundreds of plants and obtained rather good results and published them so they could be used by pharmaceutical institutions.

We have isolated and described the properties of active glycoproteins, sesquiterpene lactones, diterpenoids, flavonoids, alkaloids. We established the structures and described the isolation techniques and biological test systems.

Acknowledgment

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