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Full Length Research Paper

Determination of saikosaponin, phenolic and podophyllotoxin contents of five endemic *Bupleurum* root extracts and their effects on MCF-7 cells

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Bupleurum **species are among the plants used in Chinese medicine for phytotherapy. Various compounds obtained from plants have been found to exhibit anticancer activity. In this study, phenolic compounds, saikosaponins and podohyllotoxin contents of total root extracts from endemic *Bupleurum* species (*Bupleurum sulphureum, Bupleurum lycaonicum, Bupleurum turcicum, Bupleurum heldreichii, Bupleurum pauciradiatum*) were determined by high-performance liquid chromatography (HPLC). Total phenol content and free radical scavenging activities of total extracts were also identified. Finally, the effects of plant root extracts on viability of P-gp overexpressing paclitaxel resistant (MCF-7/Pac) and vincristine resistant (MCF-7/Vinc) MCF-7 mammary carcinoma cell lines and their parental line (MCF-7/S) were evaluated by cytotoxicity tests. Results showed that saikosaponin A, saikosaponin D and isoquercitrin contents of the root extracts were higher compared to podophyllotoxin, catechin and quercetin. While, *B. lycaonicum* root extract has about 1.5 fold more total phenol content with respect to others, *B. turcium* root extract has the highest free radical scavenging activity. According to cytotoxicity tests, *B. turcicum and B. pauciradiatum* root extracts were more toxic to the MCF-7/Pac cell line than the root extracts of other three species. In addition, *B. heldrechii* extract has been found to be the most toxic to the MCF-7/Vinc cell line among the others.**

Key words: *Bupleurum* species, plant phenolics, saikosaponin, podophyllotoxin, high-performance liquid chromatography (HPLC), MCF-7.

INTRODUCTION

Bupleurum species is among the plants used in traditional Chinese medicine (Yano et al., 1994) and major bioactive compounds have been found to be saikosaponins, phenolics, volatile oils and less polar substances (Zhang et al., 2010). *Bupleurum scorzonerifolium* is known as anti-inflammatory and anti-hepatotoxic plant and it was shown that saikosaponins found in plant root had anti-inflammatory and anti-carcinogenic activities (Mahato et al., 1988; Hsu et al., 2004). In addition, total root extract of *B. scorzonerifolium* has been proven to decrease the telomerase activity in lung carcinoma cell line by activating apoptosis (Cheng et al., 2003). Plant chemicals (phytochemicals) like polyphenols, organosulfurs, carotenoids, saponins, phytosterols, alkaloids, terpenes and more than thirty thousand types are the potential active ingredients exerting their effects on variety of the cells. Quercetin, rutin, isoquercitrin, catechin, robinin, tannin are examples for plant phenolic compounds which have antioxidant and chemopreventive activities (Cai et al., 2004). Saponins are glycosides with aglycan and glycan parts (Sparg et al., 2004). Saponins have been recently reported to possess anticancer activities by making complex with

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Table 1. Specific locations of endemic *Bupleurum* species collected in Konya, Turkey.

Species	Location
Bupleurum sulphureum	Beyşehir yolu
Bupleurum lycaonicum	Beyşehir yolu
Bupleurum heldreichii	Karapinar
Bupleurum turcicum	Tuz gölü
Bupleurum pauciradiatum	Karaman-Başkişla

cholesterols in cell membrane and producing pores that induce apoptosis (Sezgin and Artik, 2010). Plant lignans which are pinoresinol, steganacin and podophyllotoxin (PPT) are also polyphenolic compounds (Konuklugil, 1994). PPT and its derivatives like etoposide and teniposide display a wide selection in medical applications such as purgative, vesicant, antirheumatic, antiviral, and antitumor agents. Their anticancer activities have been heavily under study and they were used in various chemotherapies, including lung cancer, lymphomas, and genital tumors (Gordaliza et al., 2004).

In addition to antiproliferative effects, many natural compounds obtained from plants and their modified forms have been investigated in terms of their drug resistance reversal activity (Ugocsai et al., 2005; Engi et al., 2006; Molnar et al., 2004). Multiple drug resistance (MDR) acquired against chemotherapy by cancer cells causes most of the chemotherapeutic agents not to show expected impact on the patients and leads to the progression of the disease. Therefore, a natural substance which has low toxicity may potentially be used as a drug resistance reversal agent. In this study, five endemic *Bupleurum* species have been collected at different locations in Konya (Turkey) between May to August and their root extracts have been investigated in terms of their antioxidant activities, total phenolic contents and antiproliferative effects on MCF-7 cells. Phenolic compounds, saikosaponins and podophyllotoxin contents were further determined by high-performance liquid chromatography (HPLC) in detail. MCF-7 cell line is a model cell line for breast cancer. Paclitaxel (MCF-7/Pac) and vincristine (MCF-7/Vinc) resistant cell lines which were previously developed from sensitive MCF-7 cells (MCF-7/S) were used in this study (Kars et al., 2011b; İşeri et al., 2010). The resistant cell lines overexpress *MDR1* gene and developed various resistance mechanisms as previously reported (Kars et al., 2011a). Not only the total root extracts but also the compounds which were found in high amount in the extracts of five endemic *Bupleurum* species have been tested on viability of sensitive and resistant MCF-7 cells.

MATERIALS AND METHODS

Plants and preparation of extracts

Endemic *Bupleurum sulphureum*, *Bupleurum lycaonicum, Bupleurum turcicum, Bupleurum heldreichii, Bupleurum pauciradiatum* were collected from Central Anatolia, Konya province in Turkey between May and August (voucher specimens are deposited at the Herbarium in Selçuk University, Turkey: HP 1001-1005 KNYA).

Specific locations from where endemic *Bupleurum* species were collected are given in Table 1. The roots of plants were separated from the main plant bodies. Plant roots were grinded in a blender as powder form. 10 g of root powder from each species was extracted in 60 ml 70% ethanol by ultrasonication (Transsonic digitals) at 100% power, 25 to 37°C, for 60 min. In order to protect heat-labile substances in the plant roots, ultrasonication has been chosen as an extraction method as it is done at relatively low temperatures. The remaining plant material was separated from the extract by filtration (Watman No. 1). The solvent of each extract was evaporated by rotary evaporator (Heidolph Laborota 4000), at room temperature for 40 to 45 min. The extracts were collected by distilled water to the vials and were frozen overnight at -20°C. Finally they were freeze dried in lyophilizer (Edwards Modulyo 4K Freezer), at -45°C. Dried extracts were stored at 8 to 10°C, in dark vials until use.

Total phenol content determination

The Folin-Ciocalteu method was used to assay total phenolics in the extracts (Lowry et al., 1951). A sample of 20 µl was mixed with 6.5 ml of deionised water. 0.5 ml of non-diluted Folin's Reagent (Sigma) and 3 ml of 10% anhydrous sodium carbonate solution were then added to the mixture. The mixture was kept in shaking incubator for 30 min at 40°C for color development. The absorbance was measured at 765 nm. Results were reported as milligram of gallic acid equivalents per gram of extract (mg GAE/g extract). Assay was repeated for two times independently.

DPPH antioxidant activity determination

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable highly colored free radical that can abstract labile hydrogen atoms from phenolic antioxidants with concomitant formation of a colorless hydrazine (DPPH-H) (Diouf et al., 2009). Extracts from each species were dissolved in methanol as 10 mg/ml concentration. The samples were diluted in range of 10 to 0.078 mg/ml. Several dilutions of Butylated hydroxytoluene (BHT, Sigma) and L-Ascorbic acid (L-AsA, Sigma) with high free radical scavenging activity were used as positive controls (200 to 1.56 µg/ml). 3 ml of DPPH solution (20 mg/L) was added to the sample solution and vortexed vigorously for 30 s. The mixture was kept at room temperature in dark for 30 min before the absorbance at 517 nm was measured. The scavenging activity was determined by comparing the absorbance with that of the blank (100%) containing only DPPH solution and solvent. The total free radical scavenging activity of each extract was expressed as the concentration of extract that reduce 50% of DPPH (IC_{50}). The Assay was repeated for two times independently.

Table 2. Total phenolic contents of the root extracts in terms of gallic acid equivalents.

Extract source	mg GAE/g extract ± SD
B. sulphureum	34.68 ± 0.02
B. lycaonicum	61.48 ± 0.01
B. turcicum	34.48 ± 0.00
B. heldrechii	31.48 ± 0.00
B. pauciradiatum	33.71 ± 0.00

SD: Standard deviation ($p < 0.05$).

HPLC analysis

HPLC analysis was performed using Varian Prostar HPLC system with pursuit 5u C18 (150 × 4.6 mm) column and PDA detector. The following conditions were applied during separation of saikosaponins, phenolics and podophyllotoxin in the extracts. Saikosaponin A and saikosaponin D (Sigma) were used as standards and they were detected and separated in the samples at 25°C, with PDA detector at 203 nm, in acetonitril: water mobile phase. The flow rate was 0.8 ml/min and flow condition was at t = 0 (30:70), t = 10 min (30:70), t = 18 min (40:60), t = 28 min (45:55), t = 35 min (45:55), t = 49 min (30:70). Cathechin, quercetin and isoquercitrin (Sigma) were used as standards for phenolic compounds. Standards and samples were detected and separated at 25°C, with PDA detector at 280, 360 and 360 nm for cathechin, quercetin, isoquercitrin respectively. Mobile phase was methanol: 2.5% formic acid in water, flow rate was 1 ml/min, flow condition was t = 0 (0:100), t = 7 min (0:100), t = 42 min (20:80), t = 57 min (60:40), t = 58 min (0:100), t = 63 min (0:100). Podophyllotoxin (Sigma) was used as standard and the samples were detected and separated at 25°C, with PDA detector at 285 nm, in ((methanol:acetonitril):water) mobile phase. The flow rate was 0.8 ml/min and flow condition was at t = 0 ((20:30):50), t = 15 min ((20:30):50.

Cell lines

In order to test the antiproliferative effect of plant root extracts, the sensitive and drug resistant MCF-7 cell lines have been used. The features and growth conditions of the parental cell line (MCF-7/S) and sublines resistant to 400 nM paclitaxel (MCF-7/Pac) and 120 nM vincristine (MCF-7/Vinc) were described previously (Kars et al., 2006; Kars et al., 2008). Not only the total root extracts but also the compounds which were found in high amount in extracts have been tested on aforementioned cell lines.

Cytotoxicity assay

The effects of the total extracts and compounds on the proliferation of sensitive and resistant MCF-7 cell lines were evaluated by means of the Cell Proliferation Kit (Biological Industries) in 96 well flat bottomed microtiter plates (Eskiocak et al., 2008). Briefly, first well is filled with 150 µl and all the wells except the cell control column (second) were filled with 100 µl medium. 200 µl of concentrated anticancer agent (prepared in medium) was added in to the third column and the compound was diluted horizontally by taking 100 µl portion of extract solution (3 mg/ml) or compound (100 µM) from the third column and putting in to the next column. Finally, the cells were seeded in to 96-well microtiter plates (5×10^3cells/well) and incubated for 72 h in medium containing horizontal dilutions of compound (except for medium control wells). Then, XTT reagent was applied to form a soluble dye. After incubation at 37°C for 4 h, the dissolution of formazan crystals that were produced by mitochondrial enzymes of the living cells occurred and then the optical density of chromogenic product was measured at 500 nm with a 96-well plate reader (BioTek microplate reader). The inhibition of cell proliferation and IC_{50} (inhibitory concentration 50) values were determined for each cell line.

Statistics

All the assays were repeated for two times independently. The results of assays were subjected to two-tailed t-test by using SPSS Software (SPSS Inc., Illinois, USA) to determine significant difference between means of groups ($p < 0.05$). The results were expressed as mean ± standard deviation.

RESULTS

Total phenol contents of plant extracts

Natural phenols are very diverse group of compounds carrying one or more phenolic group, but some well-known representatives of them could easily be identified in plant extracts by HPLC (Cai et al., 2004). In addition, total phenolics could be assayed by Folin-Ciocalteu method and expressed as milligram of gallic acid equivalents per gram of extract (mg GAE/g extract). Total phenolic contents of root extracts of *B. sulphureum*, *B. lycaonicum, B. turcicum, B. heldrechii* and *B. pauciradiatum* were found to be 35, 62, 35, 34 and 34 mg GAE/g root extracts respectively (Table 2).

According to results, *B. lycaonicum* root extract has about 1.5 fold more total phenol content with respect to others ($p < 0.05$).

DPPH antioxidant activity of plant extracts

Free radical scavenging activities (FRSA) of the plant root extracts have been determined using DPPH which is a stable highly colored free radical. *B*. *turcium* root extract showed the highest free radical scavenging activity with 57.37 µg/ml IC_{50} value ($p<0.05$, Table 3).

In contrast, *B*. *lycaonicum* and *B*. *sulphureum* extracts had the lowest free radical scavenging activities when compared to the other samples and the standards with high FRSA.

Table 3. Free radical scavenging activities (FRSA) of total extracts and known standards in terms of IC_{50}.

Extract source	FRSA(IC_{50}) (µg/ml) ± SD
B. sulphureum	403 ± 1.88
B. lycaonicum	443 ± 2.76
B. turcicum	57.37 ± 2.67
B. heldrechii	184±2.19
B. pauciradiatum	165 ± 1.82
L-AsA	2.26 ± 0.17
BHT	2.45 ± 0.30

FRSA: Free radical scavenging activity; SD: standard deviation ($p < 0.05$); L-AsA: L-ascorbic acid; BHT: butylated hydroxyltoluene.

Table 4. HPLC results presenting the amounts of compounds in the root extracts.

	Compound mg/g extract ± SD		
Extract source	**Saikosaponin A**	**Saikosaponin D**	**Podophyllotoxin**
B. sulphureum	ND	ND	ND
B. lycaonicum	6.842 ± 0.146	1.735 ± 0.031	0.132 **±** 0.003
B. turcicum	12.990 ± 2.065	17.958 ± 0.308	ND
B. heldrechii	3.608 ± 0.293	7.128 ± 0.400	0.496 **±** 0.006
B. pauciradiatum	8.200 ± 0.580	18.173 ± 0.223	ND

ND: not detected; SD: standard deviation ($p < 0.05$).

Table 5. HPLC results presenting the amounts of compounds in the root extracts.

	Compound mg/g extract ± SD		
Extract source	**Catechin**	**Isoquercitrin**	**Quercetin**
B. sulphureum	0.12 ± 0.03	5.78 ± 0.34	0.28 ± 0.03
B. turcicum	0.11 ± 0.03	1.85 ± 0.11	0.21 **±** 0.02
B. lycaonicum	ND	20.39 ± 1.23	0.51 ± 0.04
B. heldrechii	ND	2.27 ± 1.37	ND
B. pauciradiatum	ND	12.49 ± 0.75	0.39 ± 0.03

ND: not detected; SD: standard deviation ($p < 0.05$).

HPLC analysis of plant extracts

Major well-known groups of phenolic and saponin compounds have been elucidated by HPLC and the results are listed in Tables 4 and 5. Saikosaponin A, saikosaponin D and isoquercitrin were detected in higher amount in the *Bupleurum* root extracts when compared to others.

Antiproliferative activities of plant extracts and selected compounds

Root extracts affected sensitive and drug resistant MCF-7 cell viabilities. According to the IC_{50} values, *B. turcicum and B. pauciradiatum* root extracts are more toxic to the MCF-7/Pac cell line than the other three plant extracts. Regarding MCF-7/Vinc cell line, *B. heldrechii* root extract was found to be the most toxic among the others. IC_{50} values are listed in Table 6. In addition to total extracts, the effects of saikosaponin A, saikosaponin D and isoquercitrin which were detected in higher amounts in the root extracts were also tested for cytotoxicity. IC_{50} values for saikosaponin A and saikosaponin D are presented in Figure 1. The highest application dose of isoquercitrin for XTT test was 1200 µM however the compound did not exert any cytotoxicity and it was accounted as non-toxic with very high IC_{50}

Table 6. Antiproliferative effects of root extracts on MCF-7 cell lines.

Cell line	Reagent/ Extract	IC_{50} (mg/ml) ± SD
MCF-7/S	Paclitaxel	$1.89 \times 10^{-3} \pm 0.28 \times 10^{-3}$
	Vincristine	0.05 ± 0.00
	B. sulphureum	0.45 ± 0.08
	B. lycaonicum	1.62 ± 0.11
	B. turcicum	0.22 ± 0.10
	B. heldrechii	0.79± 0.11
	B. pauciradiatum	0.80 ± 0.11
MCF-7/Pac	Paclitaxel	0.27 ± 0.00
	B. sulphureum	2.70 ± 0.38
	B. lycaonicum	3.71 ± 0.16
	B. turcicum	0.85 ± 0.00
	B. heldrechii	1.46 ± 0.32
	B. pauciradiatum	0.85 ± 0.00
MCF-7/Vinc	Vincristine	0.015 ± 0.003
	B. sulphureum	1.60 ± 0.11
	B. lycaonicum	2.70 ± 1.20
	B. turcicum	3.47 ± 0.66
	B. heldrechii	1.03 ± 0.08
	B. pauciradiatum	1.51 ± 0.78

SD: standard deviation ($p < 0.05$).

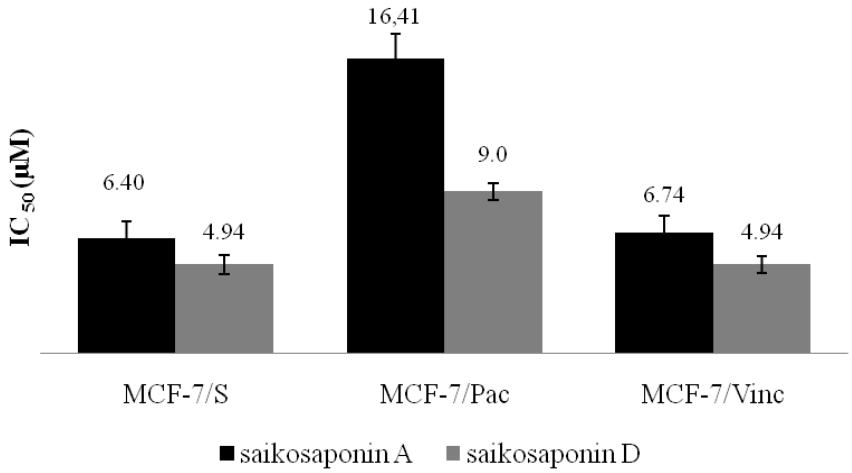


Figure 1. Effects of saikosaponin A and saikosaponin D on the proliferation of cell lines. Error bars represent mean ± SD ($p < 0.05$).

values for three cell lines.

DISCUSSION

Cancer is a serious clinical problem and has a significant impact on the human health. Although, there are significant efforts developing new therapeutic strategies, the disease still affects millions of patients worldwide. Natural products including plants and microorganisms provide rich sources for anticancer drug discovery (Schwartsmann et al., 2002). Based on ancient and modern herbal medicine sources and Pharmacopoeia, there are many anticancer plants for the identification of

new sources for cancer therapy, and receive scientific attention recently (Bonham et al., 2002; Hu et al., 2002; Kao et al., 2001; Yano et al., 1994). For the purpose of finding the potential anticancer agents and/or MDR modulating agents, from natural sources, five endemic *Bupleurum* species have been collected from various locations in Turkey between May and August. And, ethanol extracts of the plant roots have been prepared using ultrasonication for the first time to test the cytotoxicity on the MCF-7/S, MCF-7/Pac and MCF-7/Vinc cell lines. We also clarified the major chemical contents of the root extracts in terms of phenolics, saikosaponins and podophyllotoxin amounts.

The extracts were finally compared by their free radical scavenging activities. It was reported previously that ethanol extracted Chinese herbal mixture including *Bupleurum* species exerted FRSA about 430 µg/ml (Liu et al., 2005) which is very close to that of *B. sulphureum* and *B. lycaonicum* found in this study. However, *B. turcicum* has the highest FRSA activity with 57 µg/ml in our study. According to our findings, *B. lycaonicum* has the highest total phenolic content and also coherently it has the highest isoquercitrin amount among the other extracts. In a study done by Cai et al. (2004), it was reported that *B. scorzonerifolium* had 470 mg total phenolics/g extract, which is about 8 fold more than that of *B. lycaonicum* found in this study.

When we consider the HPLC analysis results, the total extracts were found to contain saikosaponin A, saikosaponin D and isoquercitrin in considerable amounts. On the contrary, cathechin, quercetin and podophyllotoxin contents of the root extracts were not enough to be considered in cytotoxicity tests. In paralel to our findings saikosaponins, quercetin, rutin and isoquercitrin were found to be the major compounds of several *Bupleurum* species reported by different authors (Barrero et al., 2000; Cai et al., 2004).

Antiproliferative effects of the total root extracts on drug resistant MCF-7 cell lines demontrate that the root extracts from the endemic *Bupleurum* species may be used in anticancer therapy strategies. According to the report of Cheng et al. (2005), acetone extracted *B. scorzonerifolium* root is about 10 fold more toxic to the different cancer cells including MCF-7 than the endemic species in our study.

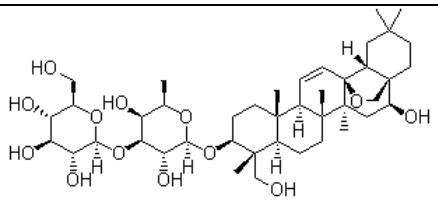
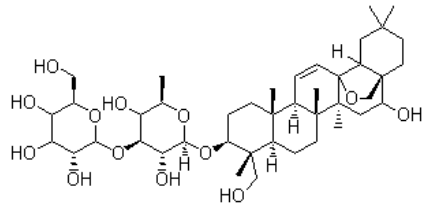
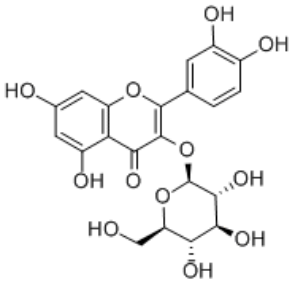
This may be due to the species differences or due to the extraction method. In addition to the total root extracts, we also tested the cytotoxicity of dominating molecules in the total extracts on the cell lines. Saikosaponin A and D exerted antiproliferative effects to some extend on MCF-7/S and MCF-7/Vinc that was about 1.5 to 2 fold more than that of on MCF-7/Pac. A study of Hsu et al. (2004) declares that saikosaponin D exerted antiprolifreative activity on A549 lung cancer cell line with IC_{50} value of 10 µM that is close to our findings with about 1 to 2 fold difference. When the antiproliferative effects of total extracts were compared to that of saikosaponin compounds, it is revealed that the saikosaponins are more cytotoxic to the cells when applied individually than root extracts which contain numerous compounds. Antiproliferative effects of saikosaponin A and D are dramatically high (50 to 150 fold) on the cells than root extracts.

Therefore, it can be deduced that saikosaponin A and saikosaponin D are important active ingredients of the endemic *Bupleurum* root extracts. Saponins make complex with cholesterol and produce pores on cell membrane and may induce apoptosis (Li et al., 2005). Saponins have amphipathic nature that may cause the absorption of macromolecules and polar drugs. It was reported that triterpen saponin obtained from *Phytolacca americana* reversed MDR in resistant ovarian cancer cells (Wang et al., 2008). In addition, Li et al. (2005) presented that saikosaponin A and D affected membrane fluidity. Most of the MDR reversing agents alter membrane fluidity and increase membrane permeability (Drori et al., 1995; Callaghan et al., 1993). The changes in the lipid membrane may modify the functional conformation of the P-gp. Alterations in the physical state of plasma membrane lipids can influence a number of important carrier-mediated processes and they appear to be important factors modulating efflux pump systems (Shin et al., 2006). Therefore, based on these facts, saikosaponins obtained from the endemic *Bupleurum* roots may be potential agents for MDR reversal.

According to results presented here, isoquercitrin was not cytotoxic to the cell lines. This may be due to chemical characteristics or physiological effects of the compound in the cells (Table 7). The root extracts were found to contain considerable amount of phenolic compound isoquercitrin. Plant phenolics are known to have antioxidant activities and chemopreventive features (Cai et al., 2004). Phenolic compounds produce phenolates by hydroxyl groups of proteins in physiological conditions. Then they make hydrogen bonds with electronegative atoms of peptides or ionic bonds with basic aminoacids. These interactions may alter the three dimensional structures or activities of proteins. Manthey and Guthrie (2002) reported that P-gp may be inhibited due to such interactions by phenolic compounds.

To conclude, these novel findings indicate that root extracts of the endemic *Bupleurum* species contain valuable compounds like saponins and phenolics. In addition, they have both free radical scavenging and antiproliferative activities. As further studies in the light of these results, MDR reversal activities of saikosaponin A, saikosaponin D and isoquercitrin will be evaluated in P-gp overexpressing MCF-7 cells by fluorescent methods. Their interactions with anticancer agents will also be determined by combination drug therapy *in-vitro*. Accordingly, we will suggest endemic *B. turcicum, B. sulphureum, B. lycaonicum, B. pauciradiatum* and

Table 7. Chemical formula and structure of agents found high amount in root extracts. http://www.chemblink.com, http://www.chemicalbook.com.

Agents	Chemical structure	Chemical formula
Saikosaponin A		$C_{42}H_{68}O_{13}$
Saikosaponin D		$C_{42}H_{68}O_{13}$
Isoquercitrin		$C_{21}H_{20}O_{12}$

B. heldrechii for the use in anticancer therapy and/or as MDR reversal agents as natural sources in future.

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REFERENCES

Barrero AF, Haidour A, Sedqui A, Mansour AI, Rodriguez-Garcia I, Löpez A, Munoz-Dorado M (2000). Saikosaponins from roots of *Bupleurum gibraltaricum* and *Bupleurum spinosum*. Phytochemistry, 54: 741-745.

Bonham M, Arnold H, Montgomery B, Nelson PS (2002). Molecular effects of the herbal compound PC-SPES: identification of activity pathways in prostate carcinoma, Cancer Res., 62: 3920-3924.

Cai Y, Luo Q, Sun M, Corke H (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., 12: 2157-2184.

Callaghan R, Stafford A, Epand RM (1993). Increased accumulation of drugs in a multidrug resistant cell line by alteration of membrane biophysical properties. Biochim Biophys Acta, 1175: 277-282.

Cheng YL, Chang WL, Lee SC, Liu YG, Lin HC, Chen CJ, Yen CY, Yu DS, Lin SZ, Harn HJ (2003). Acetone extract of *Bupleurum scorzonerifolium* inhibits proliferation of A549 human lung cancer cells via inducing apoptosis and suppressing telomerase activity. Life Sci., 73: 2383-2394.

Cheng YL, Lee SC, Lin SZ, Chang WL, Chen YL, Tsai NM, Liu YC, Tzao C, Yu DS, Harn HJ (2005). Anti-proliferative activity of *Bupleurum scrozonerifolium* in A549 human lung cancer cells *in vitro* and *in vivo.* Cancer Lett., 26: 183-193.

Diouf PN, Stevanovic T, Cloutier A (2009). Antioxidant properties and polyphenol contents of trembling aspen bark extracts. Wood Sci. Tech., 43: 457-470.

Drori S, Eytan GD, Assaraf YG (1995). Potentiation of anticancer drug cytotoxicity by multidrug resistance chemosensitizers involves alterations in membrane fluidity leading to increased membrane permeability. Eur. J. Biochem., 228: 1020-1029.

Engi H, Gyemant N, Lorand T, Levai A, Ocsovszki I, Molnar J (2006). Cinnamylidene ketones as potential modulators of multidrug resistance in mouse lymphoma and human colon cancer cell lines. *In vivo*, 20: 119-124.

Eskiocak U, İşeri OD, Kars MD, Biçer A, Gündüz U (2008). Effect of doxorubicin on telomerase activity and apoptotic gene expression in doxorubicin-resistant and -sensitive MCF-7 cells: an experimental study. Chemother., 54: 209-216.

Gordaliza M, Garcia PA, del Corral JM, Castro MA, Gomez-Zurita MA (2004). Podophyllotoxin: distribution, sources, applications and new cytotoxic derivatives. Toxicon, 44: 441-459.

Hsu YL, Kuo PL, Lin CC (2004). The proliferative inhibition and apoptotic mechanism of saikosaponin D in human non-small cell lung cancer A549 cells. Life Sci., 75: 1231-1242.

Hu H, Ahn NS, Yang X, Lee YS, Kang KS (2002). *Ganoderma*

lucidum extract induces cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. Int. J. Cancer, 102: 250-253.
İşeri ÖD, Kars MD, Arpaci F, Gündüz U (2010). Gene expression analysis of drug resistant MCF-7 cells: implications for relation to extracellular matrix proteins. Cancer Chemother. Pharmacol., 65(3): 447-455.
Kao ST, Yeh CC, Hsieh CC, Yang MD, Lee MR, Liu HS (2001). The Chinese medicine Bu-Zhong-Yi-Qi-Tang inhibited proliferation of hepatoma cell lines by inducing apoptosis via G0/G1 arrest. Life Sci., 69: 1485-1496.
Kars MD, İşeri OD, Gündüz U (2011 a). A microarray based expression profiling of paclitaxel and vincristine resistant MCF-7 cells. Eur. J. Pharmacol., 657: 4-9.
Kars MD, İşeri OD, Gündüz U (2011 b). Drug resistant breast cancer cells overexpress ETS1 gene. Biomed. Pharmacother., 65: 40-45.
Kars MD, İşeri OD, Gündüz U, Molnar J (2008). Reversal of MDR by synthetic and natural compounds in drug resistant MCF-7 cell lines. Chemother., 54: 194-200.
Kars MD, İşeri OD, Gündüz U, Ural AU, Arpaci F, Molnar J (2006). Development of rational *in vitro* models for drug resistance in breast cancer and modulation of MDR by selected compounds. Anticancer Res., 26: 4559-4568.
Konuklugil B (1994). Lignans with anticancer activity. Ankara Ecz. Fak. Der., 23: 64-75.
Li XQ, Gao QT, Chen XH, Bi KS (2005). High performance liquid chromatographic assay of saikosaponins from radix Bupleuri in China. Biol. Pharm. Bull., 28: 1736-1742.
Liu CT, Wu CY, Weng YM, Tseng CY (2005). Ultrasound-assisted extraction methodology as a tool to improve the antioxidant properties of herbal drug Xiao-chia-hu-tang. J. Ethnopharmacol., 54: 448-454.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 65-275.
Mahato SB, Sarkar SK, Poddar G (1988). Triterpenoid saponins. Phytochemistry, 27: 3037-3067.
Manthey JA, Guthrie N (2002). Antiproliferative activities of citrus flavonoids against six human cancer cell lines. J. Agric. Food Chem., 50: 5837-5843.
Molnar J, Gyemant N, Mucsi I, Molnar A, Szabo M, Körtvelyesi T, Varga A, Molnar P, Toth G (2004). Modulation of multidrug resistance and apoptosis of cancer cells by selected carotenoids. *In vivo*, 18: 237-244.
Schwartsmann G, Ratain MJ, Cragg GM, Wong JE, Saijo N, Parkinson DR (2002). Anticancer drug discovery and development throughout the world. J. Clin. Oncol., 20: 47-59.
Sezgin AEC, Artik N (2010). Determination of saponin content in Turkish tahini halvah by using HPLC. Adv. J. Food Sci. Technol., 2: 109-115.
Shin SY, Choi BH, Kim JR, Kim JH, Lee YH (2006). Suppression of P-glycoprotein expression by antipsychotics trifluoperazine in adriamycin-resistant L1210 mouse leukemia cells. Eur. J. Pharm. Sci., 28: 300-306.
Sparg SG, Light ME, Staden J (2004). Biological activities and distribution of plant saponins. J. Ethnopharmacol., 94: 219-243.
Ugocsai K, Varga A, Molnar P, Antus S, Molnar J (2005). Effects of selected flavonoids and carotenoids on drug accumulation and apoptosis induction in multidrug-resistant colon cancer cells expressing MDR1/LRP. *In vivo*, 19: 433-438.
Wang L, Bai L, Nagasawa T, Hasegawa T, Yang X, Sakai J, Bai Y, Kataoka T, Oka S, Hirose K, Tomida A, Tsuruo T, Ando M (2008). Bioactive triterpene saponins from the roots of *Phytolacca americana*. J. Nat. Prod., 71: 35-40.
Yano H, Mizoguchi A, Fukuda K, Haramaki M, Ogasawara S, Momosaki S (1994). The herbal medicine sho-saiko-to inhibits proliferation of cancer cell lines by inducing apoptosis and arrest at the G0/G1 phase. Cancer Res., 54: 448-454.
Zhang TT, Zhou JS, Wang Q (2010). HPLC analysis of flavonoids from the aerial parts of *Bupleurum* species. Chin. J. Nat. Med., 8: 107-113.