ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 3:2 (2009) 104-109

records of natural products

Antiproliferative Effects of the Methanolic Extracts of *Sideritis libanotica* Labill. subsp. *linearis*

Ibrahim Demirtas[1*], Ayse Sahin[1,2], Bulent Ayhan,[1] Saban Tekin[2] and Isa Telci[3]

[1] *Laboratory of Plant Research, Department of Chemistry, Faculty of Science and Art, Gaziosmanpaşa University, Taşlıçiftlik Campus, 60240 Tokat, Türkiye*

[2] *Laboratory of Molecular Biology, Department of Biology, Faculty of Science and Art, Gaziosmanpaşa University, Taşlıçiftlik Campus, 60240 Tokat, Türkiye*

[3] *Department of Field Crops, Agricultural Faculty of Gaziosmanpasa University, Tokat, Türkiye*

(Received December 31, 2008; Revised March 6, 2009; Accepted March 7, 2009)

Abstract: Methanol extract from aerial parts of *Sideritis libanotica* Labill. subsp. *linearis* (Bentham) Borm. were investigated for its *in vitro* antiproliferative activities against Vero, HeLa and C6 cells. The tests were carried out as dose-dependent assay starting from 25 µg/mL to 250 µg/mL. The extract found to be active against African green monkey kidney (Vero), human uterus carcinoma (HeLa) and Rat Brain tumor cells (C6) cancer cell lines with IC_{50} values. The methanol extract of *S. libanotica* showed the highest activity against the Vero, HeLa and C6 cell lines at 250 µg/mL as dose-dependent assay starting from 25 µg/mL.

Keywords: Antiproliferation; *Sideritis libanotica*; Vero cells; plant extracts.

1. Introduction

Flora Cancer is one of the major causes of death in developed countries, together with cardiac and cerebrovascular diseases [1]. Cancer is clinically treated by surgery, radiotherapy and chemotherapy. After surgical ablation of progressive cancer, however, metastasized tumor cells continue to progress and this is one of the causes making cancer treatment difficult [2]. Anticancer drugs and radioactive rays mostly damage DNA or suppress DNA duplication to kill tumor cells growing rapidly. They also affect normal cells to cause serious adverse effects (bone marrow function inhibition, nausea, vomiting and alopecia etc.) [3]. More effective anticancer drugs with high selectivity against only malignant cells and with ability to repress tumor metastasis are desired. As

* Corresponding author: E-Mail: idemirtas@gop.edu.tr; Phone: +90-356-252-1616; Fax: +90-356-252-1585.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03/ 08/2009 EISSN:1307-6167

candidates for such drugs, cytotoxic, antitumor or anticancer natural products have been often sought, and plant components such as Vinca alkaloids, taxoids, etoposide and irinotecan are now used in clinical treatments [4].

The genus *Sideritis* is represented in Turkish flora by 46 species, of which 31 are endemic [5,6]. Some species are used in the treatment of gastrointestinal ailments, common colds as well as a herbal tea in Turkish folk medicine [7,8]. *Sideritis* species growing in Turkey are known to be rich in essential oils [9,10], diterpenes [11,12,13], flavonoids and phenylethanoid glycosides [12,13,14,15].

There is no report on the antiproliferative activity of *S. libanotica*. The present study aimed to investigate the antiproliferative effects on Vero, C6 and HeLa cells of the methanol extract from *Sideritis libanotica* Labill. subsp. *linearis* (Bentham) Borm. which is endemic to Turkish flora.

2. Materials and Methods

2.1. Materials

Three human cell lines were used for cytotoxicity screening: Vero cells (African green monkey kidney), C6 cells (Rat Brain tumor cells) and HeLa cells (human uterus carcinoma) were kindly provided by Assist. Prof. Dr. Ali Karagoz and Prof.Dr. Nazlı Arda (Department of Molecular Biology, Istanbul University, Turkey). *Sideritis libanotica* ssp. *linearis* was collected from Ballica area of Tokat, Turkey during June-July 2005 and was identified by Assist. Prof. Dr. H. Askin Akpulat from Cumhuriyet University, faculty of Science and Literature, Department of Biology. A voucher specimen has been deposited at the department of Botany, Faculty of Science and Art, Cumhuriyet University. The aerial part of the plant was collected and for the preparation of extracts the collected plant materials was air-dried at room temperature. All chemicals used were of reagent or higher grade.

2.2. Preparation of methanolic extracts of Sideritis libanotica ssp. *linearis (MESLL)*

The aerial part of the *Sideritis libanotica linearis* was cut into small pieces and extracted successively with methanol (2.5 L) for three times at room temperature. The extracts were filtered through Whatman No: 2 filter paper, and then concentrated in vacuum to dryness. The yield of MESLL was 14%.

2.3. BrdU cell proliferation assay

Cells were plated in 96-well plates (COSTAR, Corning, USA) at a density of 30.000 cells/well and incubated at 37°C with 5% CO_2 overnight for attachment. In each experimental set, cells were plated in triplicates and were washed and incubated for 24 h prior to treatments methanol extract. Cellular proliferations were measured by colorimetric immunoassay based on BrdU incorporation into the cellular DNA by following the instructions recommended by the vendor (Cell Proliferation ELISA, BrdU Kit; Roche Molecular Biochemical, Germany). Briefly, cells were pulsed with BrdU labeling reagent for 4 h followed by fixation in FixDenat solution for 30 min at room temperature. Thereafter, cells were incubated with 1:100 dilution of anti- BrdU-POD for 1.30 h at room temperature. Finally, the immune reaction was detected by adding the substrate solution and the color developed was read at 450 and 650 nm with a microplate ELISA reader (Rayto, RT-2100C, Germany).

2.4. Cell culture and cell antiproliferation assay

2.4.1. In vitro Growth Inhibition Test

Vero cells were grown in tissue culture flasks (TPP) with Dulbecco's modified Eagle's medium-high glucose (DMEM; Nutricell), supplemented with 10% (v/v) fetal bovine serum. Cells were detached from the flasks with trypsin-EDTA and resuspended to *c*. 10^5 cells mL^{-1} in MEM. To the 96-well, volumes of 1 mL of the cell suspension were distributed, respectively, and after cell monolayer formation they were used for the cytotoxic characterization analysis assays.

2.5. Statistical analysis

Numerical data for treatments of cells were subject to analysis of variance (ANOVA). Significance of differences among treatments in the cells were determined using Least Significant Degree [16]. All statistical analysis was performed using MSTATC software (Version 2.1. Michigan State University).

3. Results and Discussion

3.1 Effect of MESLL on proliferation of Vero, C6 and HeLa Cells

We checked the antiproliferative activity of MESLL against Vero, C6 and HeLa cells at 25-250 µg/mL for 48 h. As shown in Fig 1, MESLL inhibited the proliferation of Vero, C6 and HeLa cells in a dose-dependent manner during incubation for 48 h. After incubation for 48 h, the IC_{50} of Vero, C6 and HeLa cells were approximately 0.127, 0.580 and 0.546 at 250 µg/mL, respectively. At the concentration of 250 µg/mL, MESLL almost completely suppressed the increase in cell numbers of Vero, C6 and HeLa cells. Activity-guided fractionation of MESLL of areal part has led to see the major dose-depended antiproliferative constituent.

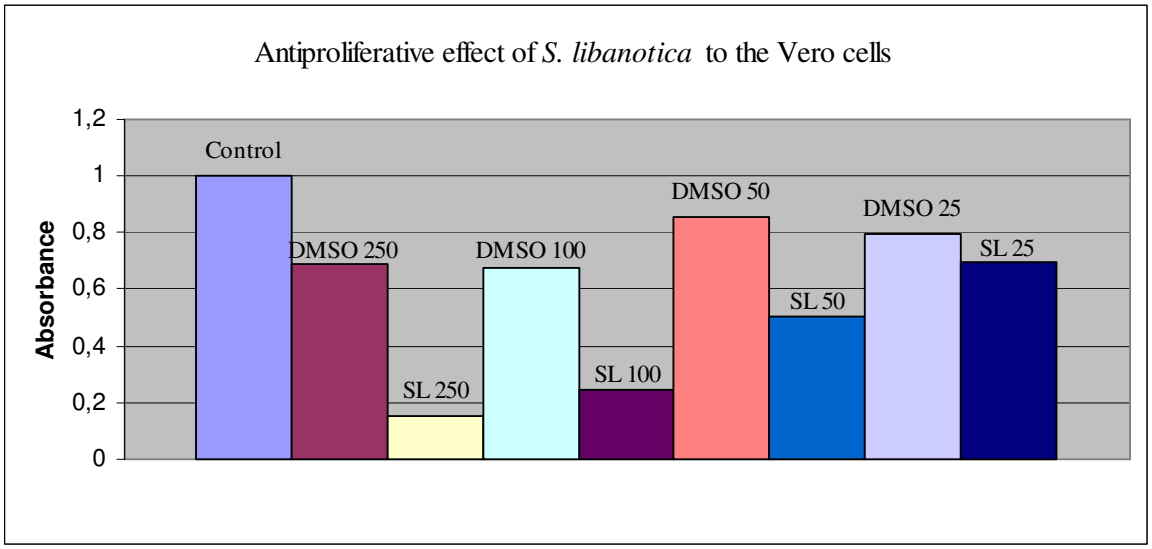

Figure 1. Antiproliferative effect of *S. libanotica* to the Vero cells**.**

Antiproliferative activity of *S. libanotica* (SL) crude extracts was investigated methanol extracts prepared from areal of *S. libanotica* (SL) were tested for cytotoxic activity on Vero (V) cells using the Brdu assay. Brdu assay was used to evaluate the reduction of viability of cell cultures in the presence and absence of the SL extracts. Cell viability was inhibited to different extents by the extracts as seen in Fig 1.

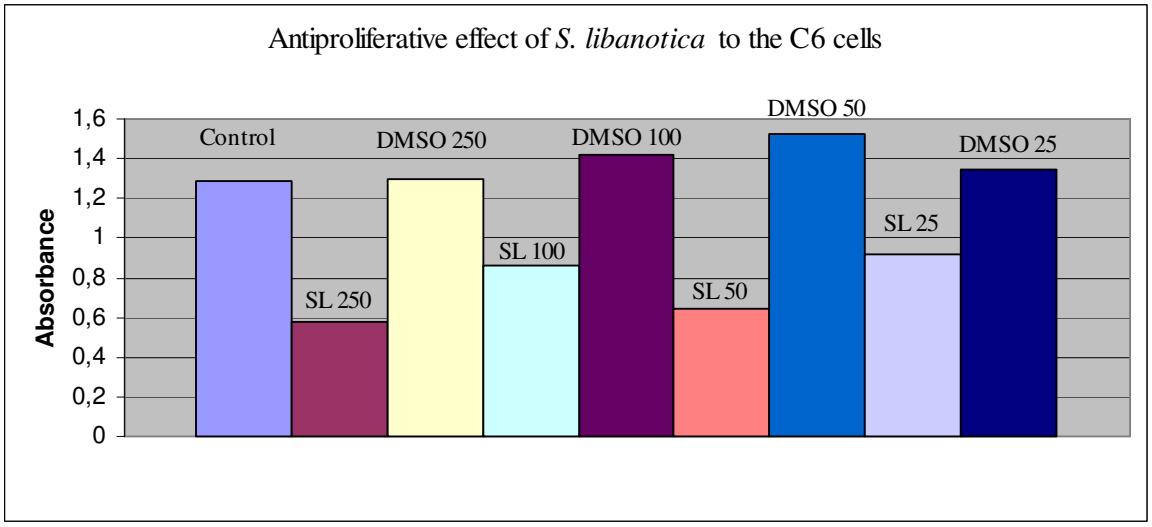

Figure 2. Antiproliferative effect of *S. libanotica* to the C6 cells.

The antiproliferative activity of the crude extract was evaluated in terms of capacity to cell viability. The crude extract of the more polar solvent fraction (methanol) showed considerable cytotoxic activity against C6 cells especially in higher doses as shown in Fig 2.

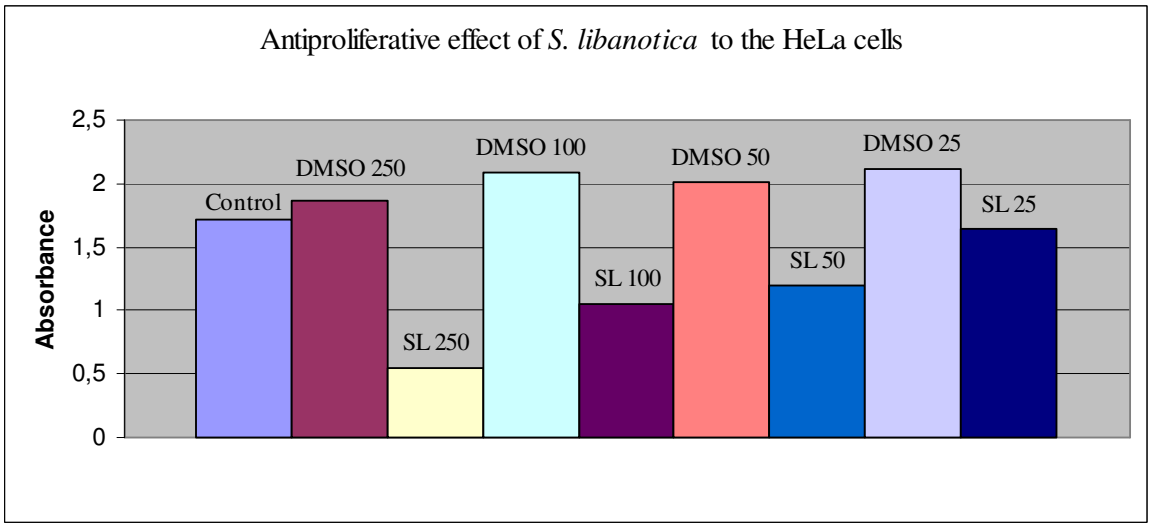

Figure 3. Antiproliferative effect of *S. libanotica* to the HeLa cells.

The antiproliferative properties *in vitro* for methanolic extract of *S. libanotica* were evaluated for Vero, C6 and HeLa cells. Significant viability decreases in all cells (Vero, C6 and HeLa) treated with different concentrations of extract were observed, suggesting that these derivatives has statistically significant antiproliferative activities (See Table 1).

The methanol-soluble portions of the plant (SL) were active against all tumour cell lines (Vero, C6 and HeLa). However, the methanol extract showed stronger dose-dependent cytotoxic activity against HeLa cells than the others (see Fig 3 and Table 1). The extract exhibited the most effective cytotoxic activity to Vero cells at 250 µg/mL (Table 1).

Table 1. The antiproliferative effect of methanol extract of *S. libanotica* ssp. *linearis* (MESLL) in Vero, C6 and HeLa cells. Cells were treated with MESLL (25-250 µg/mL) for 48 h.

Treatments	**Vero cells**$^{+}$	**C6 cells**$^{+}$	**HeLa cells**$^{+}$
Control	0.999 a	1.291 a	1.719 a
S. Libanotica (250 µg/mL)	0.127 d	0.580 b	0.546 c
S. Libanotica (100 µg/mL)	0.146 d	0.865 ab	1.050 bc
S. Libanotica (50 µg/mL)	0.532 c	0.641 b	1.197 ab
S. Libanotica (25 µg/mL)	0.675 b	0.915 ab	1.635 ab
LSD	0.122**	0.600**	0.615*

*p<0.05; **P<0.01

$^{+}$ *Vero, C6 and HeLa cells were incubated with 25, 50, 100 and 250* µg/mL of MESLL in combination with BrdU for 48 h.

Many polyphenols and flavonoids have been shown to inhibit carcinogenesis and tumorigenesis in animal experiments [17,18] and to inhibit proliferation and angiogenesis of tumor cells in vitro [19]. Flavonoids and the related compounds are found to be widely distributed in *S. libanotica* in our ongoing study.

Cell proliferation involves complex combinations of many biochemical processes, and different flavonoids might influence different biochemical processes or stages in different manners. The less polar (rather lipophilic) flavones such as 6- and 7-methoxyflavones and flavones with a "naked" B ring might inhibit cell growth by mechanisms different from those of the highly oxygenated flavones.

The results suggest strong antiproliferative properties and support the ethnomedical claims for the plant. In vivo studies are needed to confirm the pharmacological efficacy and safety of *S. libanotica* ssp. *linearis*.

Acknowledgements

This work was supported by the grants from the State Planning Organization (DPT 2003K120510), Turkey and Gaziosmanpasa University (BAP 2008/12). We would like to thank Assist. Prof. Dr. H. Askin Akpulat for identifying the plant used in this study.

References

[1] World Health Organization (1998). World Health Statistics Annual 1996, World Health Organization, Geneva.

[2] I.J Fidler and M.L. Kripke (1977). Metastasis results from preexisting variant cells within a malignant tumor. *Science* 197, 893-895.

[3] M.M. Kligerman (1973). Cancer Medicine, ed. By Holland, J.F., Frei, E., III, Lea and Febiger, Philadelphia, 541-565.

[4] V. Srivastava, A.S. Negi, J.K. Kumar, M.M. Gupta and S.P.S. Khanuja (2005). Plant-based anticancer molecules: A chemical and biological profile of some important leads. *Bioorgan. Med. Chem.* **13**, 5892-5908.
[5] H. Duman (2000). *Sideritis* L. In: A. Güner, N. Özhatay, T. Ekim, K.H.C. Başer, (Eds.), Flora of Turkey and The East Eagean Islands, supplement II, vol. 11. University Press, Edinburgh, pp. 201-204.
[6] Z. Aytaç and A. Aksoy (2000). A new *Sideritis* species (Labiatae) from Turkey. *Flora Mediter.* **10**, 181-184.
[7] T. Baytop (1999). Therapy with Medicinal Plants in Turkey (Past and Present). Nobel Tip Publications, Istanbul, p 193.
[8] E. Yesilada, G. Honda, E. Sezik, M. Tabata, T. Fujita, T. Tanaka, Y. Takeda and Y. Takaishi (1995). Traditional medicine in Turkey V. Folk medicine in inner Taurus mountains. *J. Ethnopharmacol.* **46**, 133-152.
[9] N. Ezer, R. Vila, S. Canigueral and T. Adzet (1996). Essential oil composition of Turkish species of *Sideritis*. *Phytochemistry* **41**, 203-205.
[10] K.H.C. Baser (2002). Aromatic biodiversity among the flowering plant taxa of Turkey. *Pure and Appl. Chem*. **74**, 527-545.
[11] G. Topcu, A.C. Gören, T. Kılıç, Y.K. Yıldız and G. Tümen (2002). Diterpenes from *Sideritis trojana*. *Nat. Prod. Lett.* **16**, 33-37.
[12] F.P. Sahin, N. Ezer and I. Çalış (2004). Three new acylated flavon glycosides from *Sideritis ozturkii* Aytac and Aksoy. *Phytochemistry* **65**, 2095-2099.
[13] F.P. Sahin, D. Tasdemir, P. Rüedi, N. Ezer and I. Çalış (2005). Erratum to Three new acylated flavon glycosides from *Sideritis ozturkii* Aytac and Aksoy. *Phytochemistry* **66**, 125.
[14] N. Ezher, M.K. Sakar, B. Rodriguez and M.C. De la Torre (1992). Flavonoid glycosides and a phenylpropanoid glycoside from *Sideritis perfoliata*. *Int. J. Pharmacog*. **30**, 61-65.
[15] Y. Akcoş, N. Ezer, İ. Çalış, R. Demirdamar and B.C. Tel (1999). Polyphenolic compounds of *Sideritis lycia* and their anti-inflammatory activity. *Pharm. Biol.* **36**, 1-5.
[16] K.A. Gomez and A.A. Gomez (1984). Statistical procedures for agricultural research. John Wiley and Sons, Inc., New York.
[17] M.G. Hertog, P.C. Hollman, M.B. Katan and D. Kromhout (1993). Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. *Nutr. Cancer* **20**, 21-29.
[18] V. Elangovan, N. Sekar and S. Govindasamy (1994). Chemopreventive potential of dietary bioflavonoids against 20-methylcholanthrene-induced tumorigenesis. *Cancer Lett.* **87**, 107-113.
[19] T. Fotsis, M.S. Pepper, E. Aktas, S. Breit, S. Rasku, H. Adlercreutz, K. Wahala, R. Montesano and L. Schweigerer (1997). Flavonoids, dietary inhibitors of cell proliferation and in vitro angiogenesis. *Cancer Res.* **57**, 2916-2921.