

## Cell viability, anti-proliferation and antioxidant activities of *Sideritis syriaca*, *Tanacetum argenteum* sub sp. *argenteum* and *Achillea aleppica* subsp. *zederbaueri* on human breast cancer cell line (MCF-7)

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### ABSTRACT

The breast cancer is one of the most common types of cancer. Many scientists have focused on the treatment of this disease for a long time by studying anti-cancer activities of plant extracts as well as synthetics. Lamiaceae and Asteraceae have been used in anticancer studies due to their phytochemical content. The genus *Sideritis*, *Achillea* and *Tanacetum* are the members of these families. *Sideritis*, *Achillea* and *Tanacetum* are used as herbal medication for the treatment of variety of diseases. In present study, we demonstrated the biological activity of *Sideritis syriaca* (SS), *Achillea aleppica* (AAZ) and *Tanacetum argenteum* (TAA) methanol extracts on cell viability of the breast cancer line MCF7. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to determine the cell viability and proliferation of MCF7 cells. In a dose dependent manner, methanol extracts (0, 1, 5, 25, 100 and 250 µg/ml) of SS, AAZ and TAA were examined on MCF7, and viability of cells were determined with MTT staining. Especially, concentrations in 100 and 250 µg/ml of extracts decreased the cell viability ( $p < 0.001$ ). The results of the current study showed that methanol extracts of SS, AAZ and TAA effectively inhibited the cell proliferation by decreasing the cell viability of MCF7 cells. Suggesting that SS, AAZ and TAA can be considered as natural herbal-based anti-cancerous agents.

### INTRODUCTION

In archaeological studies, it has been reported that Neanderthals used medical plants including “Yarrow, Cornflower, and Hollyhock etc.” due to their medical features at approximately 60.000 years ago (Lietava, 1992). The number of plants used for the treatment of various diseases has increased continuously during the era of antiquity. From past to present, a

lot of medical plant species have been still used to treat numerous diseases from the flu to cancer. Moreover, the extracts of plants have been extensively studied by many scientists due to their possible medical effects (Kitdamrongtham *et al.*, 2013; Manfo *et al.*, 2014; Ulasli *et al.*, 2014). It is well-known that the natural compounds of plant extracts plays an important role in the treatment (Brusotti *et al.*, 2014). Hence, these compounds, directly or indirectly, have become used as daily food supplements for decades. As described in the studies including antioxidant (Yumrutas *et al.* 2012), anticancer (Yuet *et al.*, 2013), antimicrobial (Shen *et al.*, 2014), antiviral (Danaher *et al.*, 2011), anti-inflammatory (Ilboudo *et al.*, 2013) and analgesic (Dewan *et al.*,

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2013) etc., many researchers have focused on their excellent biological activities. Especially, researchers have focused on natural compounds showing antioxidant and anticancer activities. Previous studies have provided strong evidence that fruits and vegetables reduce the incidence of cancer (Adebamowo *et al.*, 2005; La Vecchia *et al.*, 2001). It is widely believed that the phytochemicals in medicinal plants are associated with the anticancer effects by affecting the molecular events in the initiation, promotion, and progression stages of cancer (Yang *et al.* 2001). The one of the most important classes of these phytochemicals is phenolic compounds that have an increasing attention due to their potential antioxidant activity, and are present in high amounts in medicinal plants. Some studies have been reported (Xu and Chang, 2012) that these compounds have significant levels of anti-proliferation and anticancer activities. It is believed that biological effects of multiple phytochemicals, rather than a single compound, contribute to cancer prevention (Birt *et al.*, 2001).

Although many of the medicinal plants, which belong to Lamiaceae and Asteraceae families, have been used for antioxidant studies, there are only limited numbers of studies considering their anticancer potentials. In our previous studies, we have determined the antioxidant and DNA protection activities of some species naturally growing in our country (reference). In the present study, our aim was to examine the anti-proliferation and antioxidant activities of *Sideritis syriaca* (Lamiaceae), *Tanacetum argenteum* subsp. *argenteum* (Asteraceae) and *Achillea aleppica* subsp. *zederbaueri* (Asteraceae) and to detect their phenolic acid contents.

## MATERIAL AND METHODS

### Collection of plant materials

SS, AZZ and TAA were collected at flowering stage from Sof Mountain, Gaziantep-Turkey at 15<sup>th</sup> June 2012. The plant was identified by Dr. Mustafa PEHLIVAN.

### Preparation of the extracts

The air-dried and powdered leaves of plant (50 g) were extracted successively with 500 ml of MeOH by using Soxhlet extractor for 48 h at 30 °C. The extracts were then concentrated by using a rotary vacuum evaporator at 40 °C. Then, the extracts were kept in the dark at +4 °C until further experiments.

### Determination of total phenolic content (Folin-Ciocalteu method)

The amounts of total phenols were determined spectrophotometrically at 750nm, based on a colorimetric measurement for extracts as described in a previous method (Singleton & Rossi, 1965). This method gives a general measurement of phenolic content, as it is not completely specific for phenolic compounds and not all phenolic compounds exhibit the same level of activity in the assay.

Phenolic acids analysis was carried out using an HPLC equipped with ChemStation software. The separation was carried out with a Zorbax Eclipse XDB-C18 column (150 mm. 4.6 mm i.d. and 5 µm particle size) (Agilent, Waldbronn, Germany). Chromatographic separation was carried out using two solvents system: A) methanol: water: formic acid (10:88:2 v/v/v); B) methanol: water: formic acid (90:8:2 v/v/v) as reported elsewhere (Öztürk *et al.* 2007). The analyses were performed by using a linear gradient program. Initial condition was 100% A; 0-25 min. changed to 80% A; 25-54 min. to 50% A; 55-64 min. to 0%; 65-70 min. went back to 100% A. The flow-rate was 1ml/min and the injection volume was 10µl. Signals were detected at 280nm. Besides, IS (propyl paraben) technique was applied to the analysis to increase the repeatability. The relevant extracts were dissolved in a mixture of methanol and water (1:1 v/v) and injected into the HPLC.

### Antioxidant activities

DPPH assay was used as a rapid spectroscopic method to provide an evaluation of antioxidant activity due to scavenging free radicals. Antiradical activities of the extracts on DPPH· were estimated according to the method of Sanchez-Moreno *et al.* (1998) (Sánchez-Moreno *et al.*, 1998) with some modifications.

The oxidative losses of β-carotene in a β-carotene/linoleic acid emulsion were used to assess the antioxidation ability of the studied extracts (Miura *et al.*, 2002). Antioxidant activities (Inhibition percentage, I %) of the extracts were calculated using the following equation:

$$I\% = (A_{\beta\text{-carotene after 3h assay}} / A_{\text{initial } \beta\text{-carotene}}) \times 100$$

where  $A_{\beta\text{-carotene after 3 h assay}}$  is the absorbance of β-carotene after 3 h assay remaining in the samples and  $A_{\text{initial } \beta\text{-carotene}}$  is the absorbance of β-carotene at the beginning of the experiments. All tests were carried out in triplicate and inhibition percentages were reported as means ±SD of triplicates.

### Anti-proliferation and Cell Viability

Briefly, MCF7 cells (10,000 cells/well) were cultured in (50 µg/mL) coated 96-well culture plates, in a 200 µl total volume of DMEM with 1% FBS. Cells were treated with 0.2% DMSO (vehicle control) and extracts (0–250 µg/ml) for 24 h under the same conditions. The relative number of viable cells was then determined at 24 h after incubation. Cells were treated with 1 mg/mL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) and incubated for 45 minutes and subsequently analyzed spectrophotometrically. The absorption value of the solution at 595 nm directly represents relative cell numbers. The viability of cell percentages was then determined relative to the control group (Igarashi and Miyazawa, 2001). The percentage of cell viability was calculated according to the following equation;

$$\% \text{ cell viability} = 1 - (\text{Sample/Control}) \times 100$$

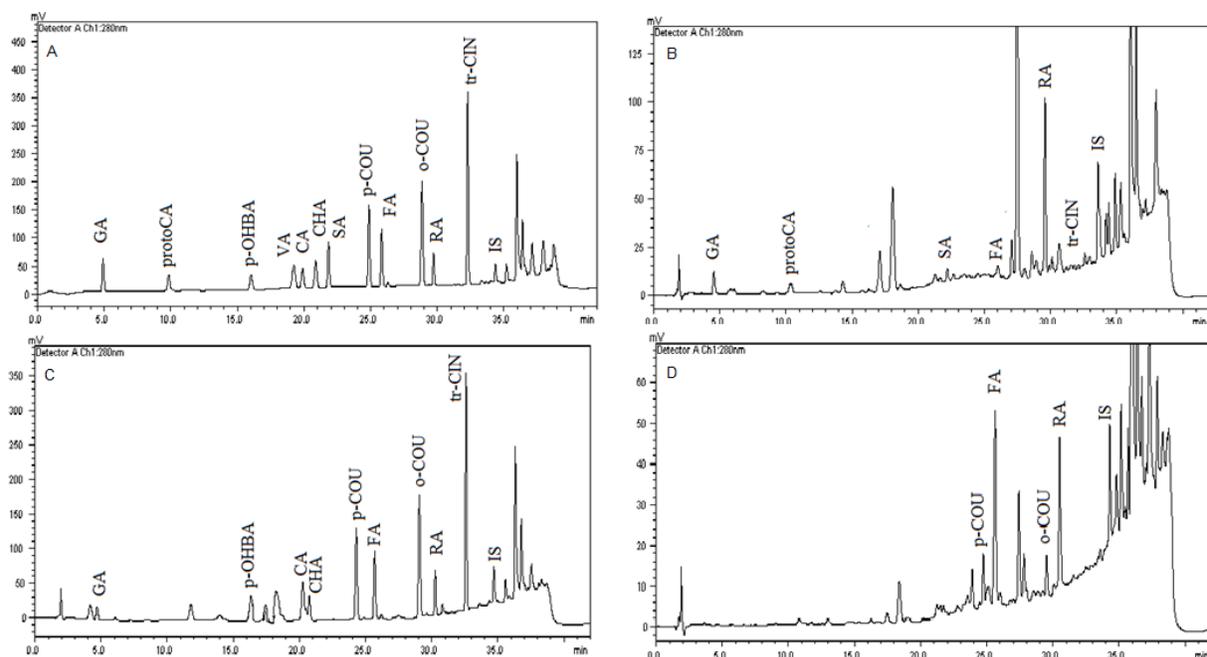


Fig. 1: The original chromatograms of phenolic acids by HPLC: A:Standard, B:*Sideritis syriaca*, C: *Tanacetum argenteum*, D: *Achillea aleppica*

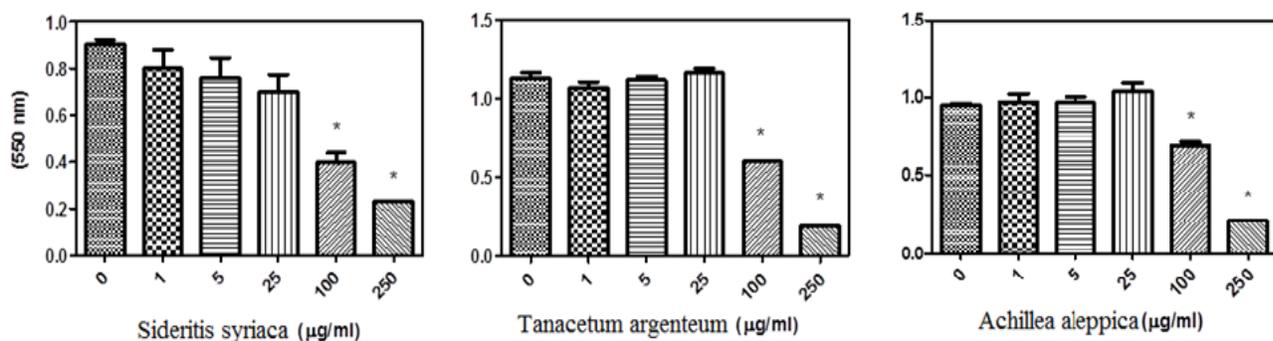


Fig. 2: Effect of the extracts on cell viability and proliferation.

### Statistical Analysis

Resulting data were statistically analyzed by SPSS package program for Windows. All values were reported as mean  $\pm$  SD. Statistical analysis was carried out by One-way analysis of variance (ANOVA) and LSD Multiple Comparison test. All statistical analysis was two-tailed. P values  $<$  0.05 were considered as significant changes.

## RESULTS

### Determination of total phenolic and phenolic acid contents

Total phenolic contents of methanol extracts of *Tanacetum*, *Achillea* and *Sideritis* were determined as  $156.41 \pm 1.71$ ,  $140.06 \pm 1.14$  and  $111.20 \pm 0.33$  GAE mg/g. In addition to these results, phenolic acid contents of extracts were detected and were given in Table 1. As can be seen in Table, the most of the phenolic acids, which are gallic acid (GA), Protocatechuic acid (PCA), p-hydroxybenzoic acid (p-HA), caffeic acid (CA), chlorogenic acid (CHA), p-coumaric acid (p-Cou), ferulic acid (FA), o-coumaric acid (o-Cou), rosmarinic acid (RA) and trans-

cinnamic acid (tr-CIN), were determined in *Sideritis*, whereas a few of them were determined in *Achillea* (Table 1). The original chromatogram of HPLC results was given in Figure 1.

Table 1: Phenolic acid contents of methanol extracts obtained from the plants (mg/g).

Phenolic acids	<i>Sideritis</i>	<i>Tanacetum</i>	<i>Achillea</i>
GA	0,62	0,52	-
PCA	-	0,57	-
p-HA	0,02	-	-
VA	-	-	-
CA	2,34	-	-
CHA	2,24	-	-
SA	-	0,25	-
p-Cou	2,78	-	1,21
FA	3,74	0,33	7,79
o-Cou	3,26	-	0,99
RA	3,45	4,37	6,46
tr-CIN	2,82	0,31	-

### Determination of Antioxidant Activities

To determine the antioxidant activities of extracts, two separate methods were employed; DPPH and  $\beta$ -carotene linoleic acid tests. In DPPH test, the highest activity was observed in

Achillea in a dose dependent manner, whereas the lowest activity was in Sideritis. Activities of BHT and RA were determined in parallel tests (Table 2). Activity of AZ was similar to the activity of synthetic antioxidant BHT. The lowest antioxidant inhibition activity was observed in Sideritis. Activities of BHT and RA were determined in parallel tests (Table 2). Activity of Achillea extract was similar to the activity of synthetic antioxidant BHT. The lowest antioxidant inhibition activity was observed in Sideritis extract, whereas the highest activity was in RA. In  $\beta$ -carotene linoleic acid test system, the highest activity was observed in *Sideritis syriaca* among extracts. Contrary to DPPH, in this assay, Achillea exhibited the lowest activity. *Sideritis syriaca* showed more strong activity than synthetic antioxidant BHT. As parallel to DPPH assay, the highest activity was determined in RA.

**Table 2:** DPPH inhibition activity of plant extracts.

Extracts	Inhibition %		
	$9.6 \times 10^{-4}$ *	$1.8 \times 10^{-3}$ *	$3.6 \times 10^{-3}$ *
Sideritis	31.42 ± 1.25	35.41 ± 0.81	42.68 ± 0.90
Tanacetum	42.24 ± 0.34	45.41 ± 0.71	48.63 ± 0.68
Achillea	41.91±0.74	47.92±0.82	52.51±0.50
BHT	41.53±0.84	51.42±0.09	57.92±0.34
RA	41.91±0.73	73.01±0.18	92.95±1.63

**Table 3:**Antioxidant activities of plant extracts in Beta-carotene linoleic acid test system

Extracts	Inhibition % <sup>1</sup>
Sideritis	63.58 ± 0.35
Tanacetum	56.17 ± 0.64
Achillea	42.67 ± 0.78
BHT	44.40 ± 0.56
RA	76.30 ± 0.50
Control	1.84 ± 0.05

$$^1\% \text{ Inhibition} = 100 \times [1 - (A_s^0 - A_s^{180}) / (A_k^0 - A_k^{180})]$$

### Determination of Cell viability and Anti-proliferation Activities

As presented in Figure 1, in doses of 100 and 250  $\mu\text{g/ml}$  of all of the extracts, anti-proliferation activity on human breast cancer cell line MCF7 was observed. Especially, the first activity of Sideritis was observed in concentration of 25  $\mu\text{g/ml}$ , whereas the activities of other extracts were only determined in doses of 100 and 250  $\mu\text{g/ml}$ . The cell viability inhibition values for 100 and 250  $\mu\text{g/ml}$  concentrations of extracts were determined as 45.08 and 25.76 % for Sideritis (81.78 % in concentration of 25 $\mu\text{g/ml}$ ), 52.58 and 16.71 % for Tanacetum and 71.89 and 21.86 % for Achillea.

### DISCUSSION

Today, numerous research have been designed to show the possible effects of natural or synthetic compounds on human cancer cells (Borsato *et al.*, 2014; Sreelatha *et al.*, 2011; Zhang *et al.*, 2012). In some of these studies, plant species especially naturally growing ones have been used against the proliferation and cell viability of cancer cells (Tai *et al.*, 2012; Tayarani-Najaran *et al.*, 2010; Xu & Chang, 2012). In our study, the possible antiproliferation and antioxidant activities of polar methanol extracts of Achillea, Sideritis and Tanacetum species which grow naturally in Turkey were determined. Firstly, total phenolic

contents of the methanol extracts were determined spectrophotometrically by Folin-Ciocalteu method, then phenolic acid contents were determined by HPLC analyses. According to the obtained results, it was determined that the methanol extracts of these plants have significant amounts of total phenolic content. According to results, Tanacetum had the highest phenolic content, whereas that of Sideritis had the lowest phenolic content. In addition to this, types and amounts of the phenolic acids in extracts were also determined, and the most types of phenolic acids were detected in Sideritis, whereas the least of phenolic acid types were in Achillea. As shown in Table 2, it was seen that especially ferulic and rosmarinic acids were found in all of the species. Although ferulic and rosmarinic acids were common in each plants, especially, the rosmarinic acid amount of Achillea was higher than other plants, and this amount was related with its DPPH scavenging activity value ( $p < 0.05$ ). When assessed for other species, a similar relationship was found between rosmarinic acid and antioxidant activity. As a result, it can be concluded that antioxidant activities can be effected by type and amount of specific phenolic compounds rather than the total amount of phenolics, because in the total phenolic assay, Folin-Ciocalteu reacted with another compounds excepting the phenolics (Que *et al.*, 2006; Zielinski and Kozłowska, 2000). There are many studies showing the biological activities including antioxidant effect of rosmarinic acid (De Oliveira *et al.*, 2012). Contrary to DPPH assay, Siderites showed the highest activity in the  $\beta$ -carotene assay among extracts. While the extract of Siderites was exhibited the highest activity, Achillea was exhibited the lowest activity. It can be explained that the mechanism of action of the phenolic compounds may vary in the different test systems (Gomes *et al.*, 2003; Ohnishi *et al.*, 2004; Proestos *et al.*, 2006). Also, it should be noted that the methanol extracts are complex mixtures, and the many available different chemicals rather than phenolics may play a role in the processing of various activities (Rice-Evans *et al.*, 1996). The MTT assay was performed to assess cell viability and cytotoxicity. Anti-proliferation and cytotoxicity activities of many plant extracts were performed by using this method (Sreelatha *et al.*, 2011; Tayarani-Najaran *et al.*, 2010). In the present study, effects of the plant extracts on proliferation and cell viability of MCF7 cells were determined. It was observed that the methanol extracts significantly affected MCF7 breast cancer cells in a dose dependent manner. In doses 100  $\mu\text{g/ml}$ , the all of extracts exhibited strong anti-proliferative effect. The highest effect on cell viability was observed in extract of *Sideritis syriaca*, and the cells decreased in value of 54.91 %, whereas the lowest effect was in *Achillea aleppica* (28.10 %). It has been seen that the 100  $\mu\text{g/ml}$  of extracts of Sideritis and Tanacetum exhibited strong effects in this test, whereas that of Achillea exhibited lower effect.

### CONCLUSION

Consequently, plant extracts can be regarded as natural substances that can be used in the fields of medicine and pharmacology. However, this study is the basic working stage for

complex bioactivity studies including the cancer. To be able to fully understand the mechanism of action of these plants, some specific methods should be evaluated and these tests should be supported by the animal experiments. We believe that this study is important in terms of being a source for plant-based anti-cancer compound studies.

## CONFLICT OF INTEREST

All authors disclose that there is no actual or potential conflict of interest.

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