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Volatile and Phenolic Compositions of the Leaves of Two *Vinca* L. Species from Turkey

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Abstract

The genus *Vinca* (Apocynaceae) comprises seven species in Turkey. In this study, volatile oil and phenolic compositions is determined in Vinca herbacea and Vinca soneri. While volatile content is determined via GC-MS/MS and the phenolic composition of leaves is revealed by HPLC technique. In result of the GC-MS/MS analyses, tetrapentacontane (77.84%) for VH extract and 6- octadecanoic acid (28.85%) for VS were detected as major volatile components. The major phenolic compound is routine trihydrate (1280, 25 mg/100g) in VH extract and chlorogenic acid (401, 23 mg/100g) in VS extract. Finally, it has been concluded that leaf extracts contain very valuable natural compounds in terms of anticancer and antiproliferative effects.

Key Words: Periwinkle, endemic, Turkey.

1. Introduction

The genus *Vinca* (Apocynaceae) comprises the periwinkles of the temperate zone native to Europe, northwest Africa and southwest and central Asia. It contains seven species: *V. difformis, V. erecta*, *V. herbacea*, *V. soneri*, *V. ispartensis, V. major* and *V. minor*, the last four being the species occurring in Turkey (Güner, 2012; Koyuncu, 2012; Koyuncu et al., 2015; Stearn, 1978; Stearn, 1973).

Vinca alkaloids are an item of a class of organic compounds made up of, oxygen, nitrogen, hydrogen, and carbon also derived from several plants is called alkaloid. Many alkaloids have toxic characteristics and physiological effects too that make them useful to as a drug. (Sahelian, 2011). The *Vinca* alkaloids are the earliest group of the plant alkaloids that used to treatment of cancer disease (Brogan, 2010). Although the alkaloids derived from the genus are well known, there is not enough information about the phenolics.

Phenolic compounds are secondary metabolites and are present everywhere in plants and plant-derived viands. They display a large variety of structures, including simple molecules (e.g. vanillin, gallic acid, caffeic acid), and polyphenols such as stilbenes, flavonoids, and polymers derived from these various groups (Cheynier, 2012). Phenolic compounds have a wide range of biological activity such as anti-allergic, anti-carcinogenic, anti-inflammatory, anti-microbial, antioxidant, anti-proliferative effects (Benavente-Garcıa et al., 2000; Middleton et al., 2000; Puupponen-Pimiä et al., 2001).

In this study we preferred to study two *Vinca* species; *Vinca herbacea* and *Vinca soneri*. *V. herbacea*, known as herbaceous periwinkle, is a widespread species and grows mainly in steppes. *Vinca soneri* is an endemic species for Turkey and grows in stony dry slopes. Both of them are herbaceouse, but they are different mainly in point of stem form. *V. herbacea* is shorter and its stem is also creeping unlike of *V. sonerii*. Additionally, *V. herbacea* is a cosmopolitian species and it is known that there is no any special habitat prefer for its, in contrast of *V. sonerii* which grows on the serpentine slopes. The main aim of this study is to characterize and compare the volatile and phenolic compounds in the leaf content of two *Vinca* species which spreads in different habitats.

2. Material and Method

2.1. Plant Material

The *Vinca* species were collected from natural habitats. Their locality information was given in Table 1.

Table 1. The locality information's of collected samples

Species name	Locality	Collection date	Collector number
Vinca herbacea Waldst. et Kit.	C4 Konya; Kulu, Akın village, 6 km to south-eastern direction.	16.06.2015	TU-3260A
Vinca soneri Koyuncu	B6 Sivas; Zara-Divriği way, around Bolucan village,1500 m.	23.07.2015	TU- 3261

2.2. Extract preparation

The plant materials were dried at dark (without sunlight) and crumbled to small pieces under sterile conditions. Following, the prepared leaf samples of 15g of *V. herbacea* (VH) and *V.soneri* (VS) were extracted with methanol for 6-8 h via Soxhlet. Then the extracts were evaporated at 40°C by rotary evaporator to obtain total methanolic extracts. To avoid the loss of activity, extracts were kept at -20ºC until use.

2.3. HPLC Analyses

After the extraction process, the solvent was dissolved in methanol 5mg/ml concentration and filtered through a 0.22µ sterile filter, then taken to Selçuk University Research and Development Center for HPLC analysis. Analyses were carried out via Shimadzu device using the INERTSIL ODS-3V (5um; 4.6 x 250 mm) column. The study was carried out at a temperature of 30 ° C, a flow rate of 1.0 ml / min and a wavelength of 280-330 nm. It was studied with 20 µl injection volume. In our study, 17 phenolic substances were scanned and quantified in our extracts. The used standards are; phenolic acids (gallic, protocathechuic, 4-hydroxybenzoic, caffeic, syringic, chlorogenic, trans-p-coumaric, trans-ferulic, and trans-cinnamic), flavonoids (catechin, rutin trihydrate, myricetin, quercetin, naringenin kaempferol, trans-resveratrol and isorhamnethin). Standards are prepared by dissolving in HPLC grade methanol. The total analysis time is approximately 45 minutes.

2.4. GC- MS/ MS Method

Extracts derived from leaves were dissolved in methanol 5mg / ml concentration and filtered through a 0.22µ sterile filter. Extracts were analysed using a Shimadzu brand GC-MS / MS instrument (QP-2010 ULTRA) containing an FID detector. Analyses were performed with RTX-5MS (0.25µm; 30m x 0.25mm) column. The work was carried out in GC at 40 ° C, at a flow rate of 40, 8 ml / min and at a pressure of 500-900 bar using Helium gas. MS was operated with argon gas at a voltage of 0.3 kV at 200 ° C. The total analysis time lasted approximately 58 minutes.

3. Results and Discussion

3.1. Volatile constituents of studied *Vinca* species

Extracts were analysed for volatile components by GC-MS / MS analysis and the most common components were taken into consideration. As a result of the analysis, obtained chromatograms are given Fig.1 and the volatile components of the extracts are given in Table 2 and 3.

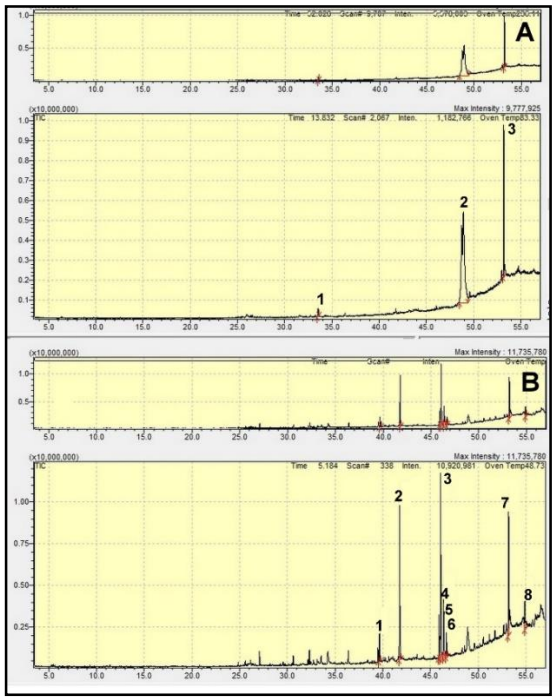

Fig.1. GC/MS chromatograms of studied samples (A: VH; B: VS)

Table 2. GC-MS/MS results of VH extract

Peak	Retention time	Area	Area %	Height	Height %	A/H	Name
1	33.534	1719770	1.36	394279	3.14	4.36	1,4-Metanobenzencyclodecane
2	48.934	98670595	77.84	4550635	36.22	21.68	Tetrapentacontane
3	53.201	26377727	20.81	7618350	60.64	3.46	Phenol, 2.2'-metylenebis

126768092	100.0	12563264	100.0	

When GC-MS / MS results are evaluated, it is determined that there are certain differences in the volatile content of two extracts. There were three component identified in VH extract; 1, 4-Metanobenzenecyclodecane, Tetrapentacontane ve Fenol, 2.2'- metilenebis. In the extract, the most noticeable compound is tetrapentacontane, which is present in about 78%. When we looked at the GC-MS / MS results of the VS extract, It was detected in order of 2-pentadecanone, hexadecanoic acid, 9, 12-octadecadioenic acid, 6-octadecanoic acid, phytol isomer and methyl stearate, as different from VH.

Table 3. GC-MS/MS results of VS extract

Peak	Retention time	Area	Area %	Height	Height %	A/H	Name
1	39.665	5705684	4.01	1701594	4.38	3.35	2- Pentadecanone
2	41.773	28879622	20.29	9274711	23.88	3.11	Hekzadecanoic acid
3	45.912	8625021	6.06	2607430	6.71	3.31	9,12-Octadecadioenic acid
4	46.064	41069063	28.85	11100585	28.59	3.70	6- octadecanoic acid
5	46.378	16585337	11.65	3525045	9.08	4.71	Phytol izomer
6	46.681	5624973	3.95	1472724	3.79	3.82	Metyl stearate
7	53.201	27669470	19.44	7442334	19.17	3.72	Phenol, 2.2'-metylenebis
8	54.895	8174202	5.74	1706478	4.39	4.79	Tetrapentacontane
		142333372	100.0	38830901	100.0		

Although there are few study about the *Vinca* species, it is not reached to any literature about the volatile content of our studied species. One of the rarely seen study is about the volatile content of *Catharanthus roseus* (formerly known as *Vinca rosea*) that is characterised by GC-MS technique. In the referred study, 15 constituents were identified and in which are described 9, 12, 15-octadecatrienoic acid, n-hexadecanoic acid, oleic acid, and ethyl ester (Doshi et al., 2018). In another study, volatile components of *Catharanthus roseus* leaves and plant residues were analysed by GC–MS. 76 components were identified such as; aldehydes, alkanes, fatty acids, terpenoids (Brun et al., 2001). Based on the literature, there is a study on the phytochemical structure of Vinca minor, which is cultured in Iran, but only alkaloid content data were given (Farahanikia et al., 2011).

It is remarkable that tetrapentacontane, which is common in varying proportions in both extracts, is common compound in the content of many plants such as *Sida rhombifolia* L., *Plectranthus amboinicus* (Lour.), and *Mallotus tetracoccus* (Roxb.) Kurz Spreng. In addition to these, it is known that these plants have cytotoxic and apoptotic effects on cancer cells (Brandao et al., 2013; Mah et al., 2017; Ramalakshmi and Muthuchelian, 2011; Swamy et al., 2017; Yulianto et al., 2016). From all, it is concluded that the *Vinca* taxa would be having a strong potential and a focusing point for the prevention and inhibition of cancer cells in future and the extracts obtaining from them may be scanned for chemotherapic and active agents in the next studies.

3.2. Characterization of phenolic compounds by HPLC

HPLC chromatograms of methanolic extracts of *V.herbacea* and *V.soneri* species and comparative analyse results of the phenolic constituent Figure 2 and Table 4.

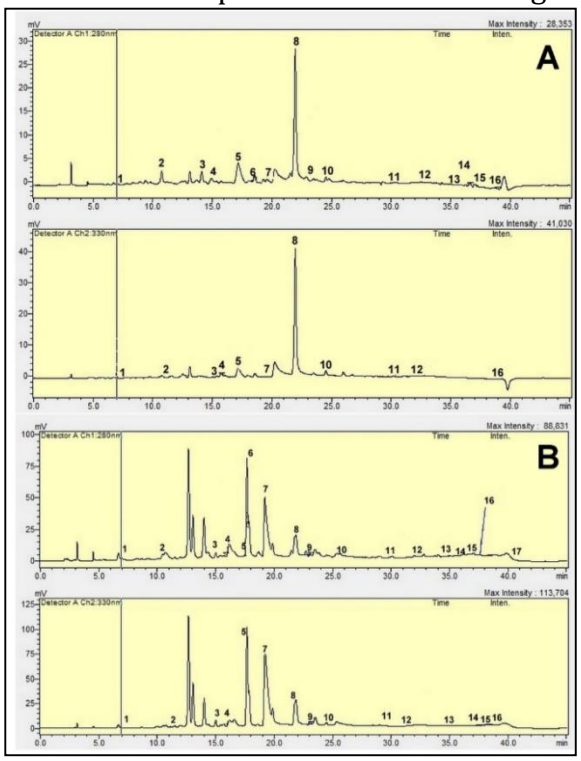

Fig.2. HPLC chromatograms of studied samples (A: VH; B: VS)

HPLC analysis revealed 16 phenolic substances in VH extract and 17 phenolic substances in VS extract. Kaempferol flavonoid was not detected in the VH. When the phenolic content of the VH is scanned, the most abundant components were, routine trihydrate (1280, 25 mg/100g), after caffeic acid (245, 31 mg/100g), catechin (107, 75 mg/100g), and protocatechuic acid (66, 30 mg/100g) respectively. In VS extract, chlorogenic acid (401, 23 mg/100g), routine trihydrate (277, 89 mg/100g), catechin (246, 28 mg/100g) and protocatechuic acid (128, 14 mg/100g) were found to be abundant in the content.

According to the HPLC data, there were some differences in the amounts of bioactive phytochemicals contained in the extracts. For example; the amount of caffeic acid and routine trihydrate were higher in the VH extract while the amounts of chlorogenic acid, myricetin, quercetin and campherol were found high amounts in the VS. Thus, it has been found that both extracts include the components which known to have anti-proliferative and apoptotic effects on cancer cells. It has been reported that caffeic acid and chlorogenic acid showed antioxidant properties and inhibited the formation of mutagenic and carcinogenic N-nitroso compounds in vitro (Han et al., 2007). Also, the fact that some seconder metabolites detected in our extracts like quercetin, kaempferol, myricetin and their glycosides (e.g., routine) is very important, already are reported in many medicinal plant examples of which have anticancer properties, too (Cai et al., 2004; Shan et al., 2005). More specifically, the anticancer and apoptosis-inducing effects of gallic acid, catechin, and quercetin have been previously

reported in diverse papers (Guo et al., 2015; He et al., 2016; Ma et al., 2014; Qıan et al., 2012; Verma et al., 2013).

Table 4. Comparative phenolic content of VH and VS extracts

Number	Compound name	VH (mg/100g plant)	Standard deviation	VS (mg/100g plant)	Standard deviation
1	Gallic acid	3,25	1,13	6,42	0,84
2	Protocathechuic acid	66,30	9,30	64,05	0,92
3	Cathechin	245,31	14,43	246,28	4,10
4	4-Hydroxybenzoic acid	15,33	5,35	29,41	2,30
5	Caffeic acid	107,75	10,66	18,27	1,12
6	Syringic acid	6,42	1,75	128,14	2,16
7	Chlorogenic acid	25,27	8,23	401,23	7,83
8	Routine Trihydrate	1280,25	105,94	277,89	66,82
9	Trans-P-coumaric acid	8,69	9,66	7,31	1,89
10	Trans-Ferulic acid	10,46	6,37	12,77	0,50
11	Myricetin	9,05	7,68	55,01	5,22
12	Trans-Resveratrol	4,66	3,65	8,31	1,30
13	Quercetin	2,85	0,91	18,36	4,87
14	Trans-Cinnamic acid	0,29	0,04	8,35	3,04
15	Naringenin	2,82	0,67	26,48	12,03
16	Kaempferol	-	-	33,35	4,45
17	Isorhamnetin	2,74	0,86	1,54	0,26

According to literaturally the scanning, there is very limited information on the phenolic content of Vinca species. As concerning with Vinca species, campherol, dihydroxybenzoic acid, and chlorogenic acid were detected in Vinca minor leaf content (Nishibe et al., 1996). In another study was reported that the presence of ferulic acid (1.1−280 mg/100 g), caffeic acid (1.2−60 mg/100 g) and gallic acid (1-21 mg/100g) was detected in Vinca rosea by RP-HPLC method (Proestos et al., 2005). Vinca major subsp. hirsuta, contains phenolic compounds such as, gallic acid, chlorogenic acid, vanillic acid, caffeic acid, and p-coumaric acid in leaf content (Saral et al., 2015). Therefore, it could be inferred from referenced studies that Vinca taxa have very important medicinal agents which are known the effects in the prevention and treatment of several cancer types. Moreover, this could be seen as too big opportunity and potential in cancer phytotherapy applications.

4.Conclusions

As far as we know, this is the first study to analyse the volatile and phenolic content of two *Vinca* species via GC-MS/MS and HPLC. It is very important that the leaf extracts contain very valuable natural compounds in terms of anticancer and anti-proliferative effects. Such studies, which are attempting to determine the active and active substances from extracts or droplets, and their pharmaceutical potentials, especially from wild plant sources, are of great importance in terms of more specific studies to be carried out in the future.

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