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# Determination of Phenolic Profile of *Cirsium arvense* (L.) Scop. Subsp. *vestitum* (Wimmer et Grab.) Petrak Plant

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**Abstract:** The chemical composition and fatty acid contents of *Cirsium arvense* subsp. *vestitum* aerial parts (flowers (CaF) and stem-leaf (CaSL)) was examined in this study. Aerial parts of this plant were extracted with various solvents such as hexane (CaFH and CaSLH), chloroform (CaFC and CaSLC) and methanol / chloroform (CaFMC and CaSLMC). Fatty acid analysis of the hexane extracts was carried out by GC-MS and the phenolic content of other extracts were determined by HPLC-TOF/MS. Palmitic acid methyl ester composition in extract of CaFH (9.99%) and α-amyrenyl acetate compound in extract of CaSLH (23.13%) were founded as main components. Compared with other extracts, it was determined that the number and amount of components in the chloroform extract was very low. Content analysis of CaFMC and CaSLMC extracts revealed some differences and different components. In these extracts, apigenin and apigenin-7-*O*-β-D-glucuronide were determined as the major compounds. In addition, phenolic component analysis was performed for the first time on this plant species.

**Keywords:** *Cirsium arvense* subsp. *vestitum*, GC-MS, HPLC-TOF/MS, phenolic compounds

## *Cirsium arvense (L.) Scop. Subsp. vestitum (Wimmer et Grab.) Petrak bitkisinin Fenolik Profolinin Belirlenmesi*

**Özet:** Bu çalışmada, *Cirsium arvense* subsp. *vestitum* bitkisinin toprak üstü kısımlarının (çiçek (CaF) ve gövde-yaprak (CaSL)) kimyasal bileşenleri ve yağ asidi içeriği incelenmiştir. Bu bitkinin toprak üstü kısımları hekzan (CaFH ve CaSLH), kloroform (CaFC ve CaSLC) ve metanol/kloroform (CaFMC ve CaSLMC) gibi değişik çözücüler ile ektrakte edildi. Hekzan ekstrelerinin yağ asidi analizi GC-MS cihazı ile gerçekleştirildi ve diğer ekstraktların fenolik içeriği HPLC-TOF / MS cihazı ile belirlendi. CaFH ekstresinde palmitic acid metil ester (9.99%) bileşiği ve CaSLH ekstresinde α-amyrenyl acetate (23.13%) bileşiği ana bileşenler olarak bulundu. Diğer ekstreler ile karşılaştırıldığında, kloroform ekstraktındaki bileşenlerin sayısının ve miktarının çok düşük olduğu belirlenmiştir. CaFMC ve CaSLMC ekstrelerinin içerik analizleri faklı bileşenleri ve bileşenler arasında bazı farklılıkların olduğunu ortaya çıkardı. Bu ekstreler içerinde, apigenin ve apigenin-7-*O*-β-D-glucuronide bileşikleri ana bileşen olarak belirlendi. İlave olarak bu bitki türü üzerinde ilk kez fenolik bileşen analizi gerçekleştirilmiştir.

**Anahtar Kelimeler:** *Cirsium arvense* subsp. *vestitum*, GC-MS, HPLC-TOF/MS, phenolic compounds



## 1. Introduction

*Cirsium* is belong to Asteracea family (Chabani et al. 2013). *Cirsium* that known as thistle in widespread throughout the world grows in Central Europe, Balkans, South Russia, Central Asia, East Anatolia, in subtropical and tropical regions. Generally it is perennial and inhabits humid meadows, near the roadside field, orchard and clay soils.

According to recent studies, there are more species than about 300 species (Güner et al. 2000). This genus is represented by 78 taxa at the species, subspecies and variety level, in Turkey (Yıldız et al. 2013).

*Cirsium* species is rich in secondary metabolites (Noh et al. 2013) and has biological activities like for healing of wounds (Raven and Edwards, 2001), antibacterial, antifungal (Khan et al. 2011), antioxidant, antidiabetic, anti-inflammatory, vasorelaxant, astringent, hepatoprotective and anticancer activities (Yildiz et al. 2013). In some countries, such as in Turkey, some *Cirsium* species are considered as edible plants.

*C. arvense* species are believed to be harmful in agricultural areas as they can reproduce and grow uncontrollably (Demirtaş et al. 2017). To our find out about the chemical content of this plant species. Therefore, in this study, we work on to investigate phytochemical contents and fatty acids composition of extracts from *C. arvense*.

## 2. Materials and Method

### *2.1. Plant materials*

*Cirsium arvense* subsp. *vestitum* (*C. arvense*) was collected from Sivas, Karaçayır, on sixteenth miles of (GPS data: 39 51 29 North, 36 58 48 East 1568 m), in 2008 June, Turkey and identified by Prof. Dr. Neriman Özhatay. A voucher specimen (ISTE 85428) has been deposited at the Herbarium of Faculty of Pharmacy, Istanbul University.

### *2.2. Preparation of Extracts Using Different Solvents*

The areal parts of *C. arvense* [flowers (CaF) and steams-leaves (CaSL)] were cut into small pieces with liquid nitrogen. Firstly, CaF and CaSL parts were extracted with hexane, after were extracted chloroform and the last were used methanol-chloroform (1:1, v/v) for three times at room temperature. Then extracts were filtered through Whatman No. 2 filter paper and concentrated to dryness under vacuum. The crude extracts were stored under adequate conditions until the time of analysis.

### *2.3. GC-MS Analysis*

Fatty acid and volatile components analysis of CaFH and CaSLH extracts were carried by GC-MS performed on an Agilent Technologies model 7890 gas chromatograph equipped with a 5975 Triple Axis Detector Mass spectrometer. Analyzes were carried out using HP-5 ms capillary column (30 m x 250 m x 0.25 µm film thickness, 5%-phenylmethylpolysiloxane). Ultra-pure helium was used as a carrier gas at a flow rate of 1 ml/min, splitless 2 µL injections were used. Electron impact (EI) ion source were used at 70 eV. Injector, ion source and interface temperatures were 250, 250 and 270 °C. Oven temperature programme was arranged as follow: starting temperature was 100 °C. The temperature was kept at 100 °C for 10 min, then increased to 200 °C at a 10 °C/min rate, and held for 10 min, then 25 °C/min to 270 °C for 36 min, held for 20 min. Total run time is 84 min. Compounds in samples were identified comparing with those in the NIST and WİLEY search database. Mass spectra were recorded in the m/z 50–550 mass range.

For GC-MS analysis, approximately 40 mg of extracts was weighed. The extracts were dissolved by the addition of 3 mL of KOH solution prepared in 2 M methanol. 3 ml of hexane was added to the solution and vortexed for 2 minutes. After a few minutes of waiting, two phases were observed. The esterified and hexane-phase supernatant was carefully separated from the lower phase. Hexane parts placed in vials.

knowledge, there is only one report on biological activity that so far has not been able to

### *2.4. HPLC-TOF/MS Analysis*

Phenolic components analysis of CaFC, CaFMC, CaSLC and CaSLMC extracts were determined by Agilent Technology of 1260 Infinity HPLC System coupled with 6210 Time of Flight (TOF) LC/MS detector and ZORBAX SB-C18 (4.6 x100mm, 3.5µm) column. The mobile phases consisted of the A (ultra pure water with 0.1% formic acid) and B (acetonitrile-HPLC grade). Flow rate was 0.6 mL $min^{-1}$ and column temperature was 35°C. Injection volume was 10 µL. The solvent program was as follow: 0.min 10% B; 0-1.min 10% B; 1-20.min 50% B; 20-23.min 80% B; 23-25.min 10% B; 25-30. min 10% B. Ionization mode of HPLC-TOF/MS instrument was negative and operated with a nitrogen gas temperature of 325 °C, nitrogen gas flow of 10.0 L $min^{-1}$, nebulizer of 40 psi, capillary voltage of 4000 V and finally, fragmentor voltage of 175 V. For sample analysis, dried crude extracts (250 ppm) were dissolved in methanol. Samples were filtered passing through a PTFE (0.22 µm) filter by an injector to remove particulates.

**Table 1.** GC-MS results of *C. arvense* extracts (CaFH and CaSLH)

| No | RT (min) | Compounds name | % Area | |
|---|---|---|---|---|
| | | | CaFH | CaSLH |
| 1 | 17.16 | 1-Dodecene | 0.03 | - |
| 2 | 17.29 | Tetradecane | 0.03 | 0.06 |
| 3 | 19.29 | Lauric acid, methyl ester | 0.83 | - |
| 4 | 20.20 | Cetene | 0.04 | 0.05 |
| 5 | 20.30 | Hexadecane | 0.11 | 0.15 |
| 6 | 22.13 | Myristic acid, methyl ester | 0.21 | 0.53 |
| 7 | 23.25 | 1-Octadecene | 0.03 | 0.04 |
| 8 | 23.38 | Octadecane | 0.08 | 0.11 |
| 9 | 23.89 | Pentadecanoic acid, methyl ester | 0.08 | 0.14 |
| 10 | 24.19 | Phytol, acetate | 0.14 | 0.19 |
| 11 | 24.34 | Hexahydrofarnesyl acetone | 0.51 | 0.71 |
| 12 | 24.72 | Phytol, acetate | 0.02 | 0.03 |
| 13 | 25.14 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 0.05 | 0.06 |
| 14 | 26.23 | Palmitic acid, methyl ester | 9.99 | 20.51 |
| 15 | 28.20 | Palmitic acid, ethyl ester | 0.05 | 0.41 |
| 16 | 28.34 | Eicosane | 0.04 | - |
| 17 | 29.25 | Palmitic acid, isopropyl ester | 0.10 | 0.10 |
| 18 | 29.35 | Hexadecanoic acid, 15-methyl-,methyl ester | - | 0.24 |
| 19 | 31.36 | Linoleic acid, methyl ester | 2.16 | 0.91 |
| 20 | 31.48 | Linolenic acid, methyl ester | 1.01 | 1.14 |
| 21 | 31.68 | Phytol | 1.73 | 2.58 |
| 22 | 31.88 | Stearic acid, methyl ester | 2.32 | 3.19 |
| 23 | 32.15 | 10-Nonadecenoic acid | 0.06 | 0.19 |
| 24 | 32.76 | 1-Hexadecanol, 2-methyl- | 0.04 | 0.06 |
| 25 | 32.82 | Nonadecane | 0.11 | 0.10 |
| 26 | 33.14 | Nonadecanoic acid methyl ester | 0.03 | - |
| 27 | 33.75 | Octadecanoic acid, 2-methyl-,methyl ester | - | 0.10 |
| 28 | 33.89 | Heneicosane | 1.55 | 0.13 |
| 29 | 34.01 | 2-Nonadecanone | 0.18 | - |
| 30 | 34.21 | Arachidic acid methyl ester | 3.08 | 1.75 |
| 31 | 34.56 | 4,8,12,16-Tetramethylheptadecan-4-olide | - | 0.06 |
| 32 | 34.93 | Heptadecane, 9-hexyl- | 0.19 | 0.15 |
| 33 | 35.27 | Heneicosanoic acid, methyl ester | 0.11 | 0.08 |
| 34 | 35.99 | 1-Eicosanol | 0.17 | 0.24 |
| 35 | 36.04 | Tetracosane | 0.65 | 0.47 |
| 36 | 36.15 | Erucic acid methyl ester | - | 0.17 |
| 37 | 36.43 | Behenic acid, methyl ester | 2.67 | 2.24 |
| 38 | 37.29 | Eicosane, 10-methyl- | 0.11 | 0.25 |
| 39 | 37.74 | Tricosanoic acid, methyl ester | - | 0.25 |
| 40 | 38.75 | Hexacosane | 5.05 | 5.14 |
| 41 | 39.29 | Lignoceric acid methyl ester | 0.58 | 2.36 |
| 42 | 40.49 | Heptacosane | 0.48 | 0.32 |
| 43 | 42.71 | Octacosane | 9.62 | 5.87 |
| 44 | 43.54 | Cerotic acid methyl ester | - | 0.29 |
| 45 | 46.73 | Ethanol, 2-(9-octadecenyloxy)- | - | 0.16 |
| 46 | 48.70 | Hentriacontane | 2.57 | 1.34 |
| 47 | 59.14 | β -Sitosterol | 0.30 | 0.50 |
| 48 | 60.92 | β-Amyrin | 1.18 | 3.92 |
| 49 | 63.71 | α-Amyrin | 1.88 | 7.22 |
| 50 | 67.85 | 12-Oleanen-3-yl acetate | 4.43 | 0.48 |
| 51 | 69.76 | Lupeol acetate (Isomers) | 0.41 | 3.38 |
| 52 | 70.59 | Lupeol | 1.75 | - |
| 53 | 71.09 | α-Amyrenyl acetate | 7.71 | 23.13 |
| 54 | 71.43 | Lupeol acetate (Isomers) | 5.95 | 1.70 |
| 55 | 78.86 | Lupeol acetate (Isomers) | 5.82 | 0.79 |
| 56 | 80.15 | Lupeol acetate (Isomers) | 2.50 | 6.03 |

For HPLC-TOF/MS analysis, about 2 mg was weighed from each extracts. On the extracts were dissolved by adding 2 ml of methanol and 1000 ppm stock solutions were prepared. From the stock solutions, 200 ppm new solutions were prepared and transferred to the vials and analysed.

## 3. Results

This study includes the phytochemical study on the aerial parts of the *Cirsium arvense* subsp. *vestitum* plant genus. The results of GC-MS analysis of the hexane fractions of the this plant are given in Table 1. In addition, the GC-MS chromatograms of both extracts are presented in Figure 1 and 2. Analysis of the phenolic components for other extracts were quantitatively performed on HPLC-TOF/MS and quantitative differences in the extracts are shown in Table 2. On the other hand, HPLC-TOF/MS chromatograms of these extracts are shown in Figure 3. 57 compounds were determined in the hexane extracts. Palmitic acid methyl ester and α-amyrenyl acetate were determined as main components in CaFH extract (9.99%) and CaSLH extract (23.13%), respectively.

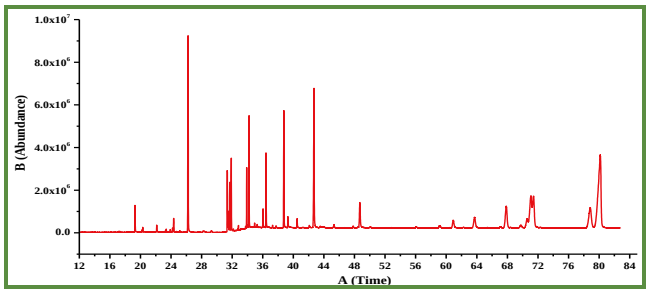


**Figure 1.** GC-MS chromatogram of CaFH

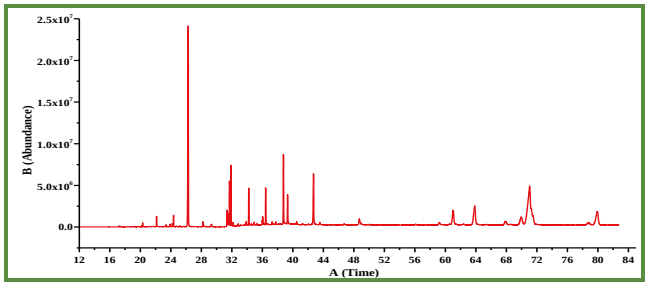


**Figure 2.** GC-MS chromatogram of CaSLH

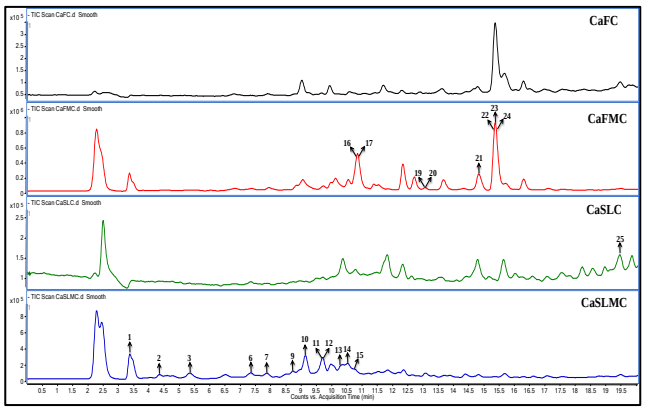


**Figure 3.** HPLC-TOF/MS TIC chromatogram of *C. arvense* extracts in negative ion mode

**Table 2.** Quantitative phenolic components of *C. arvense* plant extracts by HPLC-TOF/MS

| No | Compounds name | RT | Results (mg phenolic / kg plant) | | | |
|---|---|---|---|---|---|---|
| | | | CaFC | CaFMC | CaSLC | CaSLMC |
| 1 | Fumaric acid | 3.29 | | 16.36 | | 2.23 |
| 2 | Gentisic acid | 4.50 | | 15.46 | 0.93 | 10.06 |
| 3 | Chlorogenic acid | 5.47 | | 20.38 | 0.47 | 28.95 |
| 4 | 4-hidroxybenzoic acid | 6.96 | 2.74 | 7.47 | 1.25 | 5.94 |
| 5 | Protocatechuic acid | 7.09 | | 9.71 | | 2.30 |
| 6 | Caffeic acid | 7.45 | | 0.31 | | 0.15 |
| 7 | Vanillic acid | 7.87 | 3.28 | 9.62 | 1.15 | 2.45 |
| 8 | Syringic acid | 8.08 | 4.08 | 13.07 | 1.90 | 2.53 |
| 9 | Polydatine | 8.61 | | 1.12 | | |
| 10 | Rutin | 9.24 | | 21.93 | | 43.88 |
| 11 | Scutellarin | 9.73 | | 8.59 | | |
| 12 | Quercetin-3-β-D-glucoside | 9.77 | | 14.55 | | 37.29 |
| 13 | Naringin | 10.50 | 0.30 | 6.21 | 3.17 | 4.99 |
| 14 | Diosmin | 10.62 | | 31.95 | 3.56 | 6.37 |
| 15 | Hesperidin | 10.76 | | | | 3.01 |
| 16 | Apigetrin | 10.79 | | 65.10 | | |
| 17 | Apigenin-7-*O*-β-D-glucuronide | 10.88 | | 364.50 | | |
| 18 | Neohesperidin | 11.08 | | | | 3.31 |
| 19 | Morin | 13.01 | 1.91 | 7.95 | 1.15 | 2.45 |
| 20 | Salicylic acid | 13.11 | | 0.35 | | |
| 21 | Cinnamic acid | 15.16 | 1.42 | | 0.92 | |
| 22 | Diosmetin | 15.36 | 7.66 | | | |
| 23 | Naringenin | 15.38 | | 1.82 | | |
| 24 | Apigenin | 15.39 | 54.23 | 558.80 | | 1.48 |
| 25 | Wogonin | 19.46 | 9.91 | 17.17 | 8.21 | 7.80 |

Table 2 shows the results of the phenolic compounds contained in *C. arvense* plant extracts. The quantitative analysis of 25 compounds in the extracts were defined with HPLC-TOF/MS. It was determined that the compound number increased in the methanol/chloroform extract while the number of components in the chloroform extracts were very low. It has been determined that the CaFMC and CaSLMC extracts are present in different components, as well as are similar molecules. Rutin and Quercetin-3-β-D-glucoside compounds were found to be the main components of the CaSLMC extract while Apigenin and Apigenin-7-O-β-D-glucuronide compounds were the main components of CaFMC extract.

## 4. Discussion

Plants are capable of producing different fatty acids in different organs. The content analysis of the hexane extracts of *Cirsium arvense* subsp. *vestitum* plant shows differences from each other. This result indicates that fatty acids may be a chemo-taxonomical marker for plant species (Stuessy, 2009; Zhang et al. 2015). Palmitic, linoleic, and linolenic acids can have protective effect against hormone-dependent breast cancer. The protective mechanism involves the inhibition of aromatase activity, an enzyme participating in estrogen synthesis (Chen et al. 2006). Many *cirsium* species have got many compounds from the terpene class like steroids. The hexane extracts of this subspecies are rich in steroids-derived compounds. Steroids have many biological activities especially asthma treatment. For example, Ergosterol exhibits anti-inflammatory and anticancer effects (Barros et al. 2008). It has been scientifically determined that many steroids have a completely destructive effect on cancer cells such as HL-6, SF-295,

MDA-MB-435 and HCT-8 (Martucciello et al., 2018). Apigenin compound in CaFC, Apigenin-7-O-β-D-glucuronide compound in CaFMC extract, Wogonin compound in CaSLC extract and Rutin compound in CaSLMC extract are the most abundant compounds. In Demirtas and his co-workers' study in 2017, it is due to these compounds that cirsium arvense extracts show high activity against different cancer cells (Demirtaş et al. 2017). Rutin has some activities such as antibacterial, antitumor, anti-inflammatory, antiallergic and antioxidant in literature (Calabro et al. 2005).

## 5. Conclusions

Although the *Cirsium arvense* subsp. *vestitum* plant is considered to be an agricultural pest and idle plant among the population, our results show that aerial parts of this plant species contain very rich primer and secondary compounds. Besides, this plant species can be used as an odor and flavor in food and perfumery industry, but also because of its medical properties.

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