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RESEARCH ARTICLE

Secondary Metabolites *of Achillea sintenisii* HUB. MOR.

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SUMMARY

Achillea species (Asteraceae) are represented by approximately140 species in the world. In folk medicine, these species are used as herbal remedies due to their anti-inflammatory, analgesic, antispasmodic, digestive, wound healing, hemostatic and cholagogue effects. Flavonoids represent an important group of bioactive components in Achillea species. It has been reported that flavonoids, such as apigenin, luteolin, quercetin, and their glycosides as well as methyl derivatives have been isolated from different species of Achillea. Achillea sintenisii Hub. Mor. is an endemic species and distributed in central Anatolia. Phenolic composition of the aerial part extracts of A. sintenisii were investigated in current study by HPLC analysis on a SUPELCOSILTM ABZ+PLUS, 15 cm x 4.6 mm column using standard compounds.

Key Words: *Achillea, Achillea sintenisii, phenolic compounds, HPLC*

Achillea sintenisii HUB. MOR. Türünün Sekonder Metabolitleri

ÖZET

Achillea cinsi Dünya üzerinde yaklaşık 140 tür ile temsil edilmektedir. Bu türlerin anti-enflamatuvar, analjezik, antispazmodik, dijestif, yara iyi edici, hemostatik ve kolagog etkilerinden dolayı halk tıbbında kullanımı mevcuttur. Flavonoitler, Achillea türlerinin (Asteraceae) taşıdığı önemli bir biyoaktif bileşik grubudur. Farklı Achillea türlerinden, apigenin, luteolin, kersetin ve bunların glikozitleri ile metil türevleri gibi flavonoitlerin izole edildiği bilinmektedir. Achillea sintenisii Hub. Mor. Orta Anadolu'da yayılış gösteren endemik bir türdür. Bu çalışmada, SUPELCOSILTM ABZ+PLUS, 15 cm x 4.6 mm kolon üzerinde YBSK metodu ve standart maddeler kullanılarak A. sintenisii'nin toprak üstü kısımlarından hazırlanan ekstrelerin fenolik madde içeriği araştırılmıştır.

Anahtar Kelimeler: *Achillea, Achillea sintenisii, fenolik bileşikler, YBSK*

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INTRODUCTION

Flavonoids represent a large group of polyphenolic compounds with more than 10.000 structures (Skibola and Smith, 2000; Agati et al., 2012). They exert wide range of biochemical and pharmacological effects including antibacterial, antiviral, anti-inflammatory, antiallergic and vasodilatory activities (Cook and Samman, 1996; Skibola and Smith, 2000). Additionally, lipid peroxidation, platelet aggregation, capiller permeability and fragility as well as the activity of enzyme systems including cyclo-oxygenase and lipooxygenase inhibitory effects of flavonoids have been reported (Cook and Samman, 1996). These naturally occurring compounds widely spread in nature and they are consumed as a part of human diet in significant amounts (Gonzales et al., 2011).

Most of the species belonging to the *Achillea* genus have been reported to contain flavonoids including flavonols, flavones and their derivatives. Apigenin, luteolin, quercetin as aglycons, monoglycosides mainly *O*-glucosides, *C*-glucosides, and *O*-glucuronides, diglycosides, *O*-diglucosides, *C*-diglucosides, *O*-rutinosides, 6-*C*-glucosyl-8-*C*-arabinosyl, 6-*C*-arabinosyl-8-*C*-glucosyl, luteolin-6-*C*-apiofuranosyl-(1 → 2)-glucoside, 3-*O*-arabinosyl-(1 → 6)-glucoside, and methyl derivatives of flavonoids have been determined previously in different species of *Achillea* together with terpenes, lignans, phenolic acids and amino acid derivatives (Tuberoso et al., 2009).

Achillea species (Asteraceae) are distributed widely throughout the world and about 140 species were recorded (Goli et al., 2008; Nemeth and Bernath, 2008; Rahimmalek et al., 2009). Traditional indications of their use include digestive problems, liver and gall-bladder conditions, menstrual irregularities, cramps, fever, wound healing. Internal use for loss of appetite and dyspeptic ailments (gastric catarrh, spastic discomfort), externally usage in form of sitz bath or as a compress against skin inflammation, slow healing wounds, bacterial or fungal infections are approved by The Commission E (Nemeth and Bernath, 2008). The infusions of *Achillea* species are traditionally consumed for their diuretic, emmenagogue, wound healing analgesic activities and against abdominal pain and flatulence. The antioxidant activity of the infusions prepared from *Achillea* species on human erythrocytes and leucocytes has been reported (Konyalioglu and Karamenderes, 2005).

Several studies aim to confirm the traditional uses of *Achillea* species. Antioxidant and anti-inflammatory effects are profoundly investigated and the results on the analgesic, anti-ulcer, choleretic, hepatoprotective and wound healing activities are remarkable, however some therapeutical uses such as antihypertensive, antidiabetic, antitumor, antispermatogenic activities still need further investigations (Hoşbaş et. al, 2011). Yarrow (*Achillea millefolium* L.) which is the most known species of this genus can be also used as an insect repellent. Contact dermatitis as an adverse effect may be connected to sesquiterpenes (Nemeth and Bernath, 2008). This species have also been reported to be used as choleretic, antiphlogistic and spasmolytic (Benedek and Koop, 2007). The diversity and complexity of the effective compounds of *Achillea* species explain the broad spectrum of their activity. According to the literature, the pharmacological effects are mainly due to the essential oil, proazulenes and other sesquiterpene lactones, dicaffeoylquinic acids and flavonoids. Synergistic actions of these and other compounds are also supposed. *Achillea* species have different chemical and therapeutical values. Despite of numerous data, correct evaluation of the results is difficult because of missing generally accepted taxonomical nomenclature. The used chemical-analytical methods and bio-assays are utmost diverse, making the comparison complicated. Further research on the activity, using exactly defined plant material, standardized methods and chemical analysis is needed.

Achillea genus is represented by 46 species and 52 taxa in Turkey, where *A. sintenisii* is endemic and distributed in central Anatolia (Ağar, 2010). The antimicrobial activity of the essential oil obtained from *A. sintenisii* on *Candida albicans* and *Clostridium perfringens* has been reported. This study demonstrated that the lipophilic fraction of methanol extract and especially essential oil whose major components were found to be camphor, eucalyptol, *β*- pinene, borneol and piperitone exhibited antimicrobial activity *in vitro* (Sökmen et al., 2003). According to the phytochemical analysis this species is found to contain flavonoids, terpenoids sesquiterpene lactones (Gören et al., 1988). In current study, phytochemical composition the extracts prepared with the aerial parts of *A. sintenisii* were investigated by HPLC using some phenolic acid and flavonoid standards.

MATERIAL AND METHODS

Plant Material

Plant material was collected from Sivas-Turkey. Identification of the plant was confirmed by Assist. Prof. Mehmet Tekin from Sivas Cumhuriyet University, Department of Pharmaceutical Botany. Voucher specimen was kept in the herbarium of Sivas Cumhuriyet University (Herbarium number: CUFH 1331), Department of Biological Sciences.

Preparation of Extracts

Aerial parts and roots of the plant were separated, than dried and powdered. Aerial parts of the powdered plant material were weighed as 497.44 g. Plant material was extracted three times with distilled water (2000 mL), using BANDELIN SONOREX DIGITEC ultrasonic bath for 30 min. The filtrate was combined, freeze-dried and refrigerated. The yield of this (WE) was 16.24 g (3.27% w/w) . The plant material was dried again and extracted with a water-ethanol mixture (75% ethanol in water) (2000 mL) three times. The filtrate was combined, concentrated firstly under reduced pressure and low temperature (40-50°C) on a rotary evaporator, than lyophilized to give 6.88 g (1.4% w/w) crude extract (WEtOHE).

Both lyophilized water and water-ethanol (25:75 v/v) extracts were subjected to further fractionation for immiscible liquid-liquid extraction. Water extract dissolved in water and then transferred into the separatory funnel and extracted three times with the same portion of ethyl acetate. Water-ethanol extract was dissolved in water-ethanol mixture and and then transferred into the separatory funnel and extracted three times with the same portion of chloroform. Ethyl acetate and chloroform fractions were concentrated to dryness under reduced pressure and low temperature on a rotary evaporator. After this process, remaining water and water-ethanol fractions were frozen and lyophilized to remove the water. Weight of dried residues as follow; water fraction residue: 8.14 g (RWP); ethyl acetate fraction residue: 0.64 g (EtOAcP); water-ethanol fraction residue: 4.01 g (RWEtOHP) and chloroform fraction residue: 0.46 g ($CHCl_3$P).

HPLC Analysis

HPLC analyses were carried out using HP Agilent 1100 series chromatograph with a quaternary pump (G1311A), autosampler (G1313A), column (G1316A), DAD detector (G1315B) on SUPELCOSIL™ ABZ+PLUS, 15 cm x 4.6 mm, 3 µm column as stationary phase. Gradient system was used for elution with 1 mL/min flow rate. Mobile phase was made up of acetonitrile and water mixture, initially 10% acetonitrile (pump B) and 90% of water + 0.2% of formic acid (pump A), at 36th minute 100% acetonitrile. Column temperature was 40 °C. 215, 230, 254, 280 and 350 nm were used for detection of the compounds.

Samples for HPLC analysis were prepared as follow; 10 mg water extract dissolved in 1 mL of deionized water. 10 mg water/ethanol (25:75 v/v) extract was dissolved in 1 mL of DMSO. 5 mg/mL solution of the ethyl acetate and chloroform as well as remaining water and water/ethanol (25:75) fraction were prepared by dissolving 5 mg of each in 1 mL DMSO. Then the samples were filtered (0.45 µm) before HPLC analysis. Injection volume was 10 µL.

RESULTS AND DISCUSSION

A. setacea extracts and fractions, the water extract (WE), the water-ethanol extract (WEtOHE), the ethyl acetate fraction (EtOAcP), the chloroform fraction ($CHCl_3$P), the water fraction (RWP) and the water-ethanol fraction (RWEtOHP), were examined using high performance liquid chromatography (HPLC) with the aim to analyze their content of phenolic compounds. The compounds present were identified by their UV spectra and mass spectrometric ions through library search and comparison with the literature. The profile of the different phenolic compounds in the WE is shown in **Figure 1.** Two *C*-glycosides, vitexin and schaftoside, two *O*-glycosides of quercetin and luteolin are present. The WEtOHE chromatogram is shown in **Figure 2.** The same two *C*-glycosides and luteolin-*O*-glycoside are also present in WEtOHE. Besides them, three flavone compound, luteolin, apigenin and scutellarein dimethylether, and one more *C*-glycoside, vitexin rhamnoside, are also present in WEtOHE. *p*-coumaric acid is detected only EtOAcP. All results were given **Table 1** with their retention times. **Figure 3** shows the structures of the compounds identified qualitatively in *A. sintenisii*.

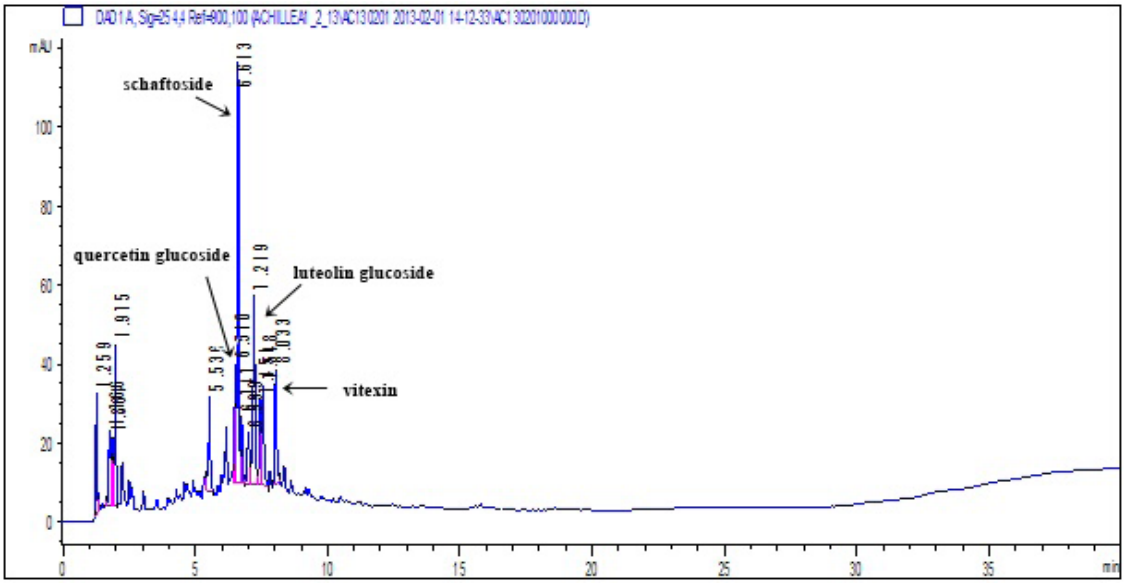


Figure 1. HPLC Chromatogram of *A. sintenisii* water extract

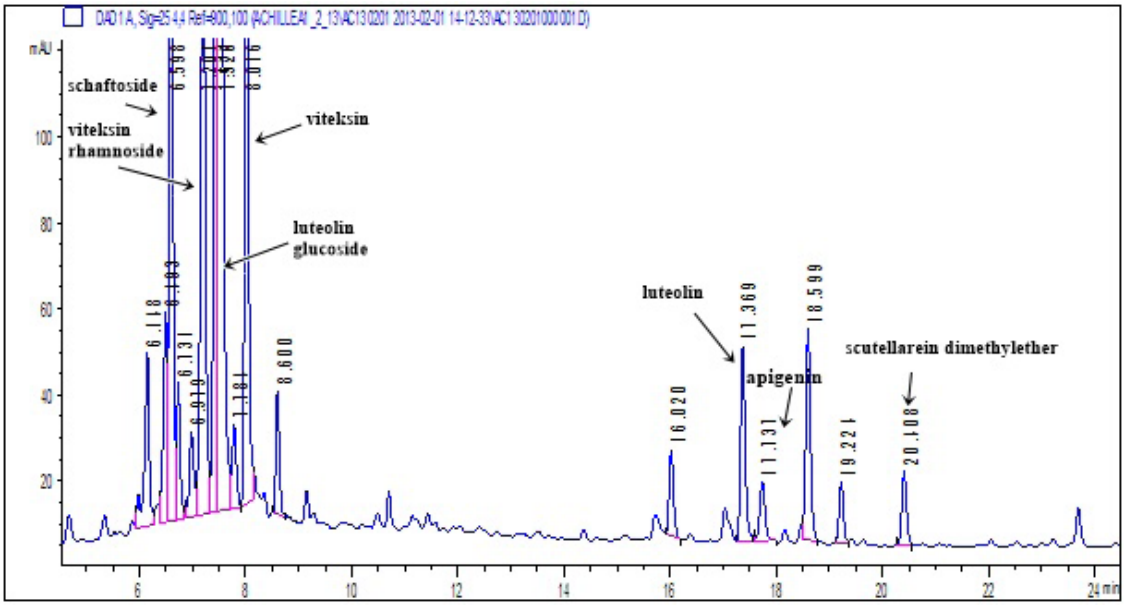
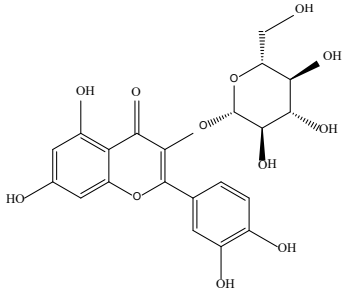
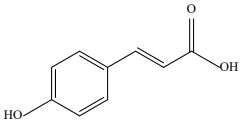


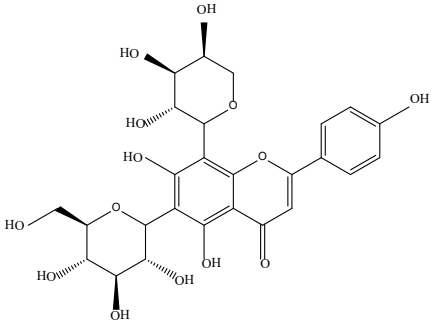
Figure 2. HPLC Chromatogram of *A. sintenisii* water-ethanol extract



Quercetin glucoside



p-coumaric acid



Schaftoside

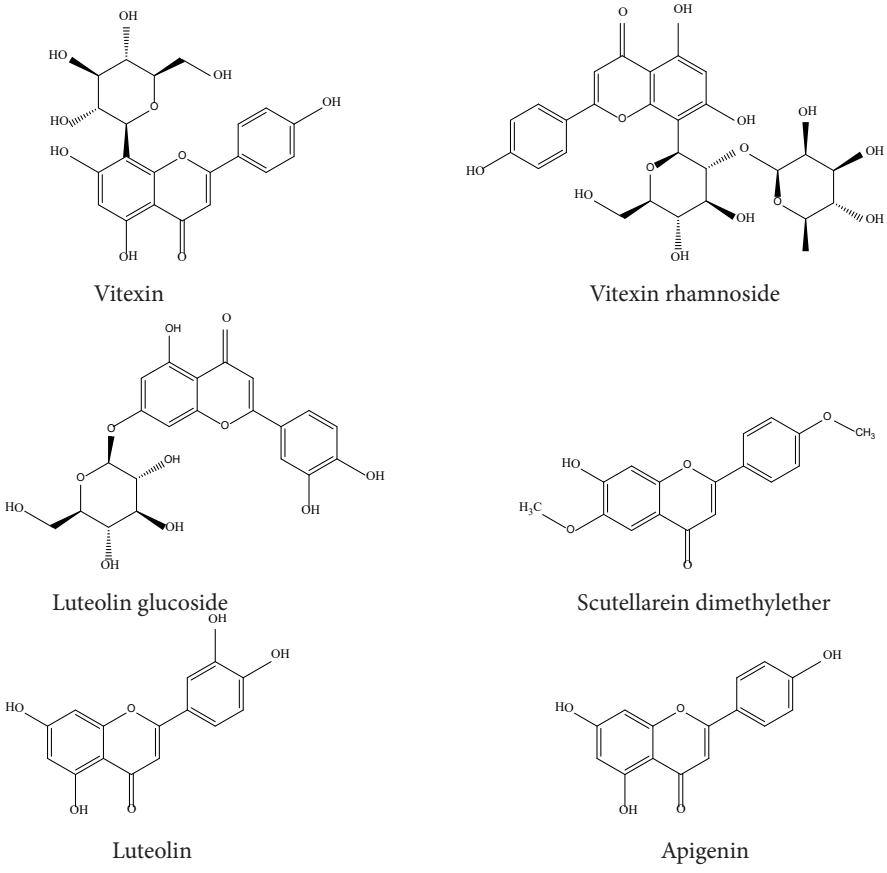


Figure 3. The structures of the compounds identified qualitatively in *A. sintenisii*

Table 1. Phenolic composition of *A. sintenisii* extracts

	WE	EtOAcP	RWP	WEtOHE	$CHCl_3$P	RWEtOHP
1 (Rt:6.50)	+					
2 (Rt:6.61)	+		+	+		+
3 (Rt:7.19)				+		+
4 (Rt:7.22)	+		+	+		+
5 (Rt:8.03)	+	+		+		+
6 (Rt:9.28)		+				
7 (Rt:17.36)		+		+	+	+
8 (Rt:17.73)		+		+	+	
9 (Rt:20.40)				+	+	+

1: Quercetin glucoside; **2**: Schaftoside; **3**: Vitexin rhamnoside; **4**: Luteolin glucoside; **5**: Vitexin; **6**: *p*-coumaric acid; **7**: Luteolin; **8**: Apigenin; **9**: Scutellarein dimethylether (WE: Water Extract, EtOAcP: Ethyl Acetate Fraction, RWP: Water Fraction, WEtOHE: Water:Ethanol Extract, $CHCl_3$P: Chloroform Fraction, RWEtOHP: Water:Ethanol Fraction)

C-glycosides and *O*-glycosides, especially apigenin *C*-glycosides and luteolin *O*-glycosides, were previously reported to occur in the genus *Achillea* (Valant-Vetschera, 1987; Tuberoso et al., 2009). Apigenin, luteolin, luteolin glucoside, quercetin glucoside, vitexin and schaftoside have all been reported from *A. millefolium* and *A. biebersteinii* (Ivancheva et al., 2002; Tuberoso et al., 2009; Hammad et al., 2013). Scutellarein 6,4'-dimethylether have been reported from *A. colina* and *A. asplenifolia*. (Nikolova et al., 2013). In a previous study, one of the major phenolic acids of *A. biebersteinii* and was reported as *p*-coumaric acid (Bashi et al., 2012). However, this is the first time the content compounds of *A. sintenisii* have been analyzed in detail. Besides the already known substances, vitexin-2"-rhamnoside was identified for the first time in the genus *Achillea*.

Previous reports show that, water is not an efficient solvent for extracting phenolic compounds. Because they are most soluble in organic solvents less polar than water. According to do some authors, by using a mixture of water with organic solvents such as ethanol, methanol and acetone, the extraction efficiency of phenolic compunds can increased (Munhoz et al., 2014).

The aerial parts of *Achillea* species are widely used in folk medicine throughout the world. The traditional uses of this genus for the treatment of skin diseases, cold, diarrhea, gastrointestinal spasms and other disorders and migraine were formerly reported. It is also known that *Achillea* species were used to heal the wounds in wars (Gören et al., 1988).

This genus contains phenolic compounds which possess several biological activities. In the current study, the phenolic content of an endemic species, *A. sintenisii* was confirmed. The activity tests have revealed an important potential of *Achillea* species for various therapeutical uses due to antispasmodic, anti-ulcer, immunosuppressive, antioxidant, antidiabetic, antimicrobial, antiviral, ostrogenic and antispermatogenic activities which are evaluated by *in vitro* assays (Peirce, 1999). Nevertheless, further researches on biological activities of this genus are needed.

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