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Extraction of antioxidative principles of *Achillea biserrata* M. Bieb. and chromatographic analyses

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Abstract: In this study, hexane, chloroform and water extracts were prepared by making consecutive extraction of *Achillea biserrata* from the family *Asteraceae.* Volatile oil of the same types was obtained by water distillation that was continued 4 hours in the Clevenger fixture and dried by adding anhydrous sodium sulfate. After GS/MS analysis, volatile oil of the same types was kept at 4 ºC in the refrigerator and dark until the work activity was performed. Volatile oil yield that was obtained from mixture of all dried flower and plant leaves were determined for *Achillea biserrata* 0.4%(v/w) The antioxidant activities of extracts, total phenol, flavonoid, proanthocyanidin and anthocyanin amounts were determined. Two different tests were performed for measurements of antioxidant activities.[1] Especially, for the purpose of meeting the general properties of good additives' free radical cleaning activity was done by DPPH method and the inhibition of oxidation activity was done by with β-carotene method. The 50% inhibition (IC_{50}) values of the water, methanol:water and chloroform extracts were 19.6, 37.9 and 114 µg /mL, respectively ın DPPH test. Besıdes, the nonpolar extracts were active in β-carotene/linoleic acid test system. Total phenolics and total flavonoid contents were highest in the water extract possessing 3.39% and 1.12%, respectively. Methanolic extract showed the presence of antioxidant phenolic compounds such as protocatecuic acid (177.83 g/g) and chlorogenic acid (164.78 g/g) ın HPLC analsıs. Automated extraction system obtained better separation of the active principles from plant tissues than conventional extraction procedures.

Keywords: *Achillea biserrata* M. Bieb, antioxidant activity, plant phenolics, HPLC and GC-MS analysis

1. Introduction

Asteraceae (Compositae) family can be considered as a cosmopolitan, but more likely grown in temperate and subtropical regions (Takhtajan, 1997). As much as 133 species and 1156 types are found in Turkey. The genus *Achillea* (Asteraceae, santolinoidea) is represented by about 85 species mostly found in Europe and Asia and a handful in North America (Könemann, 1999). Forty species of *Achillea* are widely distributed in Turkey (Davis, 1982) and locally known as 'Çivanperçemi' (Baytop, 1999). Chemical studies show that volatile oil of *Achillea* contain monoterpene (Vieira et. al., 2004) sesquiterpene (Palic et. al., 2000; Glasl, et. al., 1999), alkanes (Palic et. al., 2000), germakren and flavonoids (Palic et. al., 2000; Vieira et. al., 1997) Yarrow (*Achillea*) species has also been used for thousands of years all over the world etnopharmacology (Nemeth et. al., 2008).

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A lot of species of the genus *Achillea* ınclude flavonoids, flavonols, flavones and their derivatives. With regard to the literature principally pharmacological effects of volatile oil are due to dicaffeoylquinic acid and flavonoids of other sesquiterpene lactones (Nemeth et. al., 2008). Aerial parts of different species of the genus are found with many medicinal properties. The specie is still being used in the treatment of wound healing(Könemann, 1999). Ground aerial parts and essential oil of *Achillea biserrata* were widely used in pharmacology (Magiatis et. al., 2002). Different types of *Achillea* are utilized for the treatment of chronic indigestion, gastric and duodenal ulcer, weight loss, dearrangements of the digestive tract and mucosa of the digestive system. It is also effective for lowering blood pressure, the regulation period of mensurasyon, preventing infections occurring urinary tract, kidney and bladder, as well as being antiseptic in the treatment of hemorrhoids, upper respiratory tract infections such as colds and flu, cough, fever, and removing excess mucus secretion (Glasl et. al., 2001; Acartürk et. al. 1997).

A further detailed studies and varied extraction procedures would be beneficial to appraise the basic active constituents with varied polarities. Consequantly, this study was intended to (ı) acquire extracts with diverse polarities, e.g. starting with a non polar solvent, then utilizing more polar solvents; all being exerted in an automatic extraction system; (ıı) to arrange methanol extract and (ııı) the water distillation of the essential oil, (ıv) to apraise antioxidative capacities of the extracts as well the essential oil by measuring their ability to scavenge free radicals and inhibition of the oxidation of linoleic acid *in vitro* test systems, and finally (v) to analyse total phenolic, flavonoid, proanthocyanidine and anthocyanin ingredients of the extracts by HPLC analysis and chemical composition of the essential oil by GC/MS.

2. Materials and Methods

2.1. Plant material

Achillea biserrata M. Bieb. specimens were picked in June 2009 in Trabzon, Turkey (Voucher no: A8: Trabzon, Tonya, Kadırga region, 1600m 20.VI.2009) at the full flowering stage. The taxonomic identification of the plant material was verified by Dr. Aşkın Akpulat, a senior plant taxonomist in Cumhuriyet University, Turkey.

2.2. Isolation of the essential oil (EO)

The plant (50 g) gathered were submitted to water distillation for 3 h using a Clevenger type apparatus and essential oil of plant was dried over anhydrous sodium sulphate. After filtering, kept at 4 °C until tested and analyzed.

2.3. Preparation of the extracts

Dried aerial parts of the plant were utilized for extraction. The extraction procedure is shown in Scheme 1 (Hatipoğlu et. al., 2013).

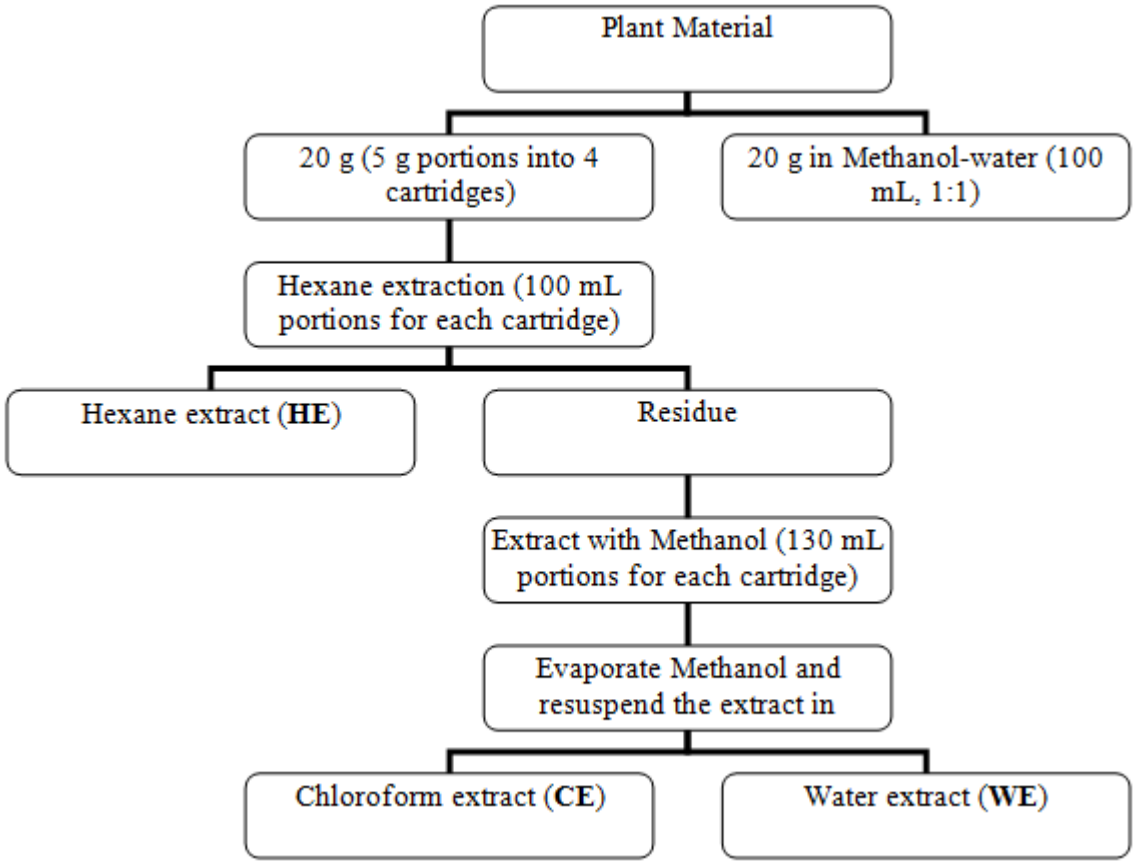
2.3.1. Hexane (HE), chloroform (CE) and water extract (WE):

Dried plant material was extracted with hexane (HE) in an automatic Soxtherm apparatus (Gerhardt Soxtherm Manager SX PC). Residual plant material was then extracted with 130 mL of methanol after hexane phase was evaporated. Optimum conditions were given in Table 1.

Table 1. Extraction conditions providing highest extract yields

Parameter	Hexane	Methanol
T- Classification	200 ^{0}C	200 ^{0}C
Extraction Temperature	120 ^{0}C	140 ^{0}C
Reduction Time	1 min. 30 sec.	1 min. 30 sec.
Reduction Interval	3 sec.	4 sec.
Hot Extraction	0 h 30 min.	2 h 30 min.
Evaporation A	9xinterval	8xinterval
Extraction Time	0 h 40 min.	2 h 30 min.
Evaporation B	3xinterval	5xinterval
Evaporation C	10 min.	10 min.
Total Extraction Time	1 h 43 min.	5 h 25 min.

Methanol extract was partitioned with chloroform and water mixture (1:1). Two fractions were separated and chloroform was evaporated. Water phase was frozen at -80 ^{o}C and lyophilized (Christ Alpha 1-4 LD plus).



Scheme 1. Extraction procedure

2.3.2. Metanol-water extract (MWE):

A 20 g portion of plant material was extracted with 100 mL of methanol-water mixture (1:1) by maceration method shaking the mixture for 24 h at room temperature. Afterwards filtration the residue was frozen at -80 ^{o}C and lyophilized.

2.4. Antioxidant activity tests

2.4.1. DPPH assay

The experiment was exerted by following a protocol given elsewhere (Kartal et.al., 2007). Briefly, the stock solutions (10-20 mg/mL) of the each extract were prepared in methanol. A 50 µL portion of the extract solution was mixed with 5 mL of a freshly prepared 0.004 % (w/v) DPPH radical methanol solution. After these solutions were kept 30 minute in the dark at room temperature, their absorbance values were measured at 517 nm, methanol used as a blank. Each measure was carried out in triplicate. The inhibitions of DPPH radical in percent (I%) were computed as follows:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where;

A_{blank} = the absorbance value of the control reaction

A_{sample} = the absorbance value of the extract.

2.4.2. β-Carotene/linoleic acid bleaching assay

In the *β*-carotene/linoleic acid mixture, the antioxidant competes with *β*-carotene for transferring hydrogen atom(s) to the peroxyl radicals ($R_1R_2HCOO•$) formed from the oxidation of linoleic acid in the presence of molecular oxygen (O_2) and converts them to hydroperoxides (R_1R_2HCOOH) leaving the *β*-carotene molecules intact (Huang, et. al., 2005). A mixture of *β*-carotene and linoleic acid was prepared by adding together 0.5 mg *β*-carotene in 1 mL chloroform (HPLC grade), 25 µL linoleic acid and 200 mg Tween 20. Both extract and BHT (positive control) were dissolved in ethanol (2 mg/ mL), 350 µL portion was placed into a test tube and added 2.5 mL of *β*-carotene and linoleic acid mixture and then the test tubes were incubated in room temperature for 24 h (each measure in triplicate). The absorbance values were measured at 490 nm with an ultraviolet and visible (UV-VIS) spectrometer and the antioxidant activities of the samples was calculated by using the following equation:

$$RAA\% = (A_{samle}/A_{BHT}) \times 100$$

2.5. GC-FID and GC-MS analysis conditions

The essential oil was analyzed utilizing Hewlett-Packard 5890 II GC performed with FID detector and HP-5 MS capillary column (30 m x 0.25 mm; film thickness, 0.25 μm). The oven temperature was set from 50 °C for 3 min, increased to 150 °C at a rate of 3 °C/min, held isothermal for 10 min, and eventually increased to 250 °C at 10 °C/min. The carrier gas was helium, diluted samples (1/100 in acetone, v/v) of 1.0 μL were injected and in the splitless mode.

The GC-MS analysis of the essential oil was equipped using a Hewlett-Packard 5890 II GC, performed with a HP-5 MS capillary column (30 m x 0.25 mm i.d., 0.25 µm) and a HP 5972 mass selective detector. Helium was the carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. The oven temperature was programmed as in the GC-FID analysis and then diluted samples (1/100, v/v, in acetone) of 1.0 µL were injected, in the splitless mode. The components were defined based on the comparison of their relative retention time and mass spectra with those of commercial standards (for the main components), NBS75K library data of the GC-MS system, and literature data (Adams, 2001). The results were also verified by the comparison

of the compounds elution order with their relative retention indices on nonpolar phases checked in the literature.

2.6. Total phenolic content

Each extract was dissolved in methanol (5 mg/ mL). 50 µL sample was placed into a test tube and added Folin–Ciocalteu's reagent/water (750 µL, 1:14) mixture. The reaction was finished completely 3 min after by supplementing 200 µL of 20% Na_2CO_3. After the solution was left in the dark for 30 minutes, absorbance of the reaction mixture was measured at 760 nm (each measure in triplicate). Methanolic solution of gallic acid was used as standard for preparation of calibration graph and total phenol amounts in extracts were expressed in mg gallic acid/100 g dry matter (Singleton and Rossi, 1965).

2.7. Proanthocyanidin content

Stock solutions of each extract were prepared in methanol (4 mg/mL). 0.5 mL sample was added to a mixture of 0.5 mL MeOH, 6 mL n-BuOH/concentrated HCl (95:5 v/v) and 0,2 mL of a 2% $NH_4Fe(SO_4)_2.12H_2O$ solution in 2 M HCl. Absorbance value of the solution was measured 550 nm using a blank (n-BuOH/HCl mixture) before and after heating for 40 min at 95.0 ± 0.2 ^oC (each measure in triplicate). A series of dilutions of cyanidin chloride in n-BuOH/HCl was assayed; proanthocyanidin amounts in extracts were calculated from the following equation (Porter et. al., 1986).

$$\text{Proanthocyanidine} = \frac{(A_{550nm})_{sample} - (A_{550nm})_{control}}{\varepsilon . L}.(MW).(DF).1000$$

where;

$(A_{550})_{sample}$ = Absorbance of the sample at 550 nm

$(A_{550})_{control}$ = Absorbance of the control at 550 nm

ε = Molar absorptivity coeficiency of cyanidin (17,360/ M. cm)

L = Cuvette length (1 cm)

MW = Molecular weight of cyanidine (287 g/ mol)

DF = Dilution factor (g/L)

1000 = Factor for conversion from gram to milligram.

2.8. Flavonoid content

A 1.5 ml of a 2% methanolic solution of $AlCl_3.6H_2O$ were added to 0.5 mL sample (4 mg/mL), then sealed tubes were kept in the dark for 10 min. Absorbance value of the solution was read at 430 nm using a blank (methanolic $AlCl_3$, each measure in triplicate). A series of methanolic dilutions of rutin was prepared and assayed; flavonoid amounts in extract were expressed in mg rutin/100 g dry matter (Lamaison and Carnat,1991).

2.9. Anthocyanin content

Two 40 µL portions of methanolic stock solution (6 mg/mL) were put into the sealed tubes and nine hundred and sixty microlitres of pH 1.0 (25 mL of 1.49% KCl + 67 mL of 1.7% HCl, pH corrected with HCl) or pH 4.5 (1.64% AcONa, pH corrected with AcOH) buffer solutions were each added. The absorbance values of these solutions were recorded at 517 nm for both pH values. Each measure was made in triplicate and total antocyanin content was calculated from following equation (Giusti and Wrolstad, 2001).

$\Delta A = [(A_{510}nm - A_{700}nm)]_{pH} = 1.0 - [(A_{510}nm - A_{700}nm)]_{pH} = 4.5$

$TACY = (\Delta A \times MW \times DF \times 1000)/\varepsilon) \times 0.1$

TACY = total anthocyanins expressed as mg cyanidin 3-glucoside/100g sample

MW = molecular weight of cyanidin 3-glucoside (449.2 g/L).

DF = dilution factor to express the samples on a per gram of plant

1000 is the conversion factor for grams to mg.

ε = molar absorbance coefficient of cyanidin 3-glucoside (26,900 / M.cm).

0.1 is the conversion factor for per 1000 grams to 100 grams basis.

2.10. HPLC analysis of phenolic compounds

Methanolic extract of *Achillea biserrata* was prepared to identify its phenolic composition. A 0.5 g portion of dried plant material was extracted with methanol for 3 hours in a ultrasonic bath and supernatant was evaporated to dryness at 40°C in a evaporator. The residue was re-dissolved and partitioned with ethyl acetate and diethyleter. Organic phases were unified and exactly evaporated then extraction. Before a 20 mg of the extract was exerted HPLC analysis, the extract was dissolved in methanol. HPLC analyses were performed with a Shimadzu LC-UV C_{18} column (150 mm × 4.6 mm id, 5 µm particle size, Agilent USA) at 280 and 315nm. Solvents A (2% AcOH in water) and B (80:20 acetonitrile/water) were run and the flow rate was kept constant at 1 ml/min using gradient conditions. Concentrations of phenolic compounds were quantified by their peak areas against those of standards.

3. Results and discussion

3.1. Extract yields

The extraction yields of *A. biserrata* are shown in Table 2. As seen from Table 2, sequential extraction seems to produce more extract yield on the basis of total amount of extract (hexane, chloroform and water).

Table 2. Extract yields of *A.biserrata*[*]

HE	Methanol		MWE	EO
	CE	*WE*		
3.01	2.90	12.16	4.55	0.40

[*] given as % (w/w), for the essential oil % (v/w).

3.2. Chemical composition of the essential oil

The essential oil yielded (0.4%, v/w) was much higher than the value given in a previous report (0.07%, v/w) (Azaz et. al., 2009).

Chemical composition of the essential oil two complimentary chromatographic analyses were carried out for the identification of essential oil components. GC-FID analysis provides Kovat's Indices which gives more accurate information about the chemical nature of the each component. GC-MS analysis also gives vast information about chemical structure and can be compored with library database for identification of the compounds. GC-FID and GC-MS analyses of *A. biserrata* resulted in the identification of 45 components representing 92.9% of the oil, camphor (19.4%), borneol (17.1%) and eucalyptol (12.8%) are being the basic constituents (Table 2). Results presented here are in accordance with the former reports published elsewhere (Azaz et. al., 2009; Maffei et. al., 1994).

Table 3. Chemical Composition of the Essential Oil of *A.biserrata*

Compound No	K.I.*	Component	% Percentage
1	924	*α*-Thujene	0.2
2	932	*α*-Pinene	1.3
3	946	Camphene	4.8
4	969	Sabinene	0.5
5	974	*β*-Pinene	1.2
6	1014	*α*-Terpinene	0.3
7	1020	*p*-Cymene	0.7
8	1024	Limonene	0.3
9	1026	Eucalyptol	12.8
10	1054	*γ*-Terpinene	1.1
11	1065	*cis*-Sabinene hydrate	0.3
12	1086	Terpinolene	0.2
13	1095	Linalool	0.4
14	1118	*cis*-Menth-2-en-1-ol	0.1
15	1124	Chrysanthenone	0.1
16	1136	*trans*-Menth-2-en-1-ol	0.3
17	1141	Camphor	19.4
18	1165	Borneol	17.1
19	1174	Terpinen-4-ol	3.7
20	1186	*α*-Terpineol	1.8
21	1194	Myrtenol	0.2
22	1207	*trans*-Piperitol	Trace[&]
23	1215	*trans*-Carveol	Trace
24	1226	*cis*-Carveol	0.3
25	1241	Carvacrol methyl ether	0.2
26	1287	Bornyl acetate	7.9
27	1298	Carvacrol	0.1
28	1356	Eugenol	0.3
29	1359	Neryl acetate	0.3
30	1374	*α*-Copaene	0.3
31	1417	*β*-Caryophyllene	0.2
32	1454	*(E)-β*-Farnesene	7.6
33	1462	Cabreuva oxide B,	0.3
	1464	*α*-Acoradiene (overlapped)	
34	1484	D-Germacrene	1.9
35	1500	Bicyclogermacrene	0.1
36	1505	*β*-Bisabolene	0.3
37	1521	*β*-Sesquiphellandrene	3.0
38	1544	*α*-Calacorene	0.3
39	1561	(E)-Nerolidol	0.7
40	1570	Dendrolasin	0.5
41	1577	Spathulenol	0.6
42	1582	Caryophyllene oxide	0.2
43	1594	Salvial-4(14)-en-1-one	0.2
44	1685	α-Bisabolol	0.7
45	1712	Curcunem-15-al	0.1
		Total	92.9

* K.I.: Literature [1] Kovats Index for DB-5 non-polar column. [&] Trace: for percentages ≤ 0.07%

3.3. Antioxidant activity of extracts:

3.3.1. DPPH free radical scavenging activity

In this test antioxidative capacities of *A. biserrata* extracts were by their free radical scavenging potentials and IC_{50} values of all were given in Figure 1.

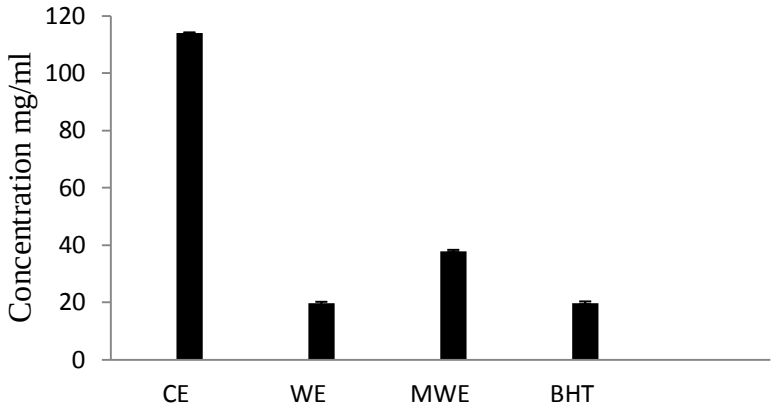


Figure 1. IC_{50} values of the extracts in DPPH assay.

There is an opposite relationship between the extract concentration and inhibition percentage. The lowest IC_{50} value showing the highest antioxidant activity was observed in WE providing 50% inhibition of the free radicals at 19.6 µg/mL concentration. Scavenging of CE was very low, whereas both the essential oil and hexane extract showed no scavenging at all and MWE extract seems to be quite effective.

The antioxidant activities of some other *Achillea* species have been already investigated and published elsewhere (Giorgi et al. 2009; Bozin et al. 2008; Alexandru et al. 2007; Nickavar et al. 2006; Conforti et al. 2005; Candan et al. 2003). However, extensive literature search showed that no study is presented so far present related to antioxidant activities of *A.biserrata* and thus the study will be a first report.

3.3.2. Inhibition of linoleic acid oxidation

Extracts were tested for their ability to inhibition of the oxidation of linoleic acid ın β-carotene-linoleic acid system and their inhibition values are shown in Figure 2.

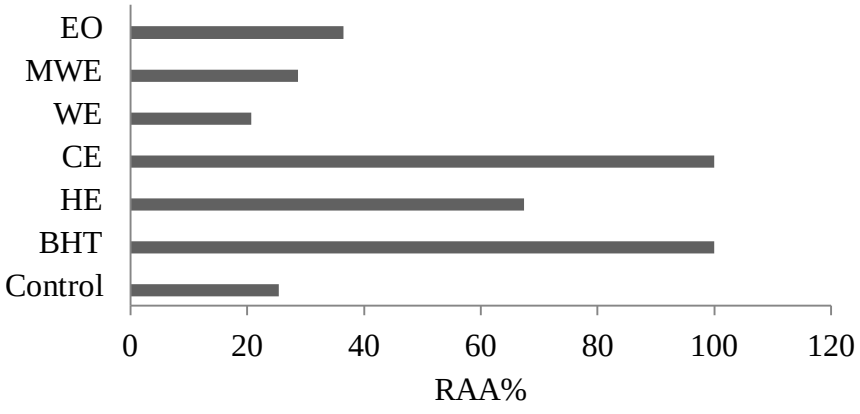


Figure 2. Antioxidant activity of *A.biserrata* extracts and the essential oil, given as inhibition percentage in β-carotene-linoleic acid assay.

Inhibition values of polar extracts (WE and MWE) and the essential oil were 20.63%, 28.65% and 36.49 % inhibitions at 2 mg/mL concentrations, respectively (Figure 2). On the other hand nonpolar extracts (CE and HE) seems to be effective at this system. Our results in accordance with prior observation, e.g. using different solvents produced better classification of the compounds.

3.4. Phenolic contents of the extracts

Antioxidant activity is directly related to phenolic contents of the extracts. Measured total phenolic constituents of the extracts together with their total flavonoid, proanthocyanidins and anthocyanins are given in Table 4.

Table 4. Total phenolics, proanthocyanidins, flavonoid, and anthocyanins content of *A.biserrata* extracts.

Extract	Total phenols[a] (% w/w)	Proantocyanidins[b]	Flavonoids[c]	Anthocyanins[d]
CE	2.78±0.03	7125.00±0.10	0.93±0.007	879.99±89.71
WE	3.39±0.05	5808.33±591.78	1.12±0.002	n.d
MWE	3.10±0.10	2733.33±591.78	1.10±0.002	310.00±12.40

[a] Total phenol: Folin–Ciocalteu (%)
[b] Proanthocyanidins: butanol–HCl methods (mg l^{-1})
[c] Flavonoids: $AlCl_3$ method (%)
[d] Anthocyanins: direct colorimetry (mg l^{-1})

Total phenolics of the CE and WE and MW extracts were 2.78%, 3.39%, 3.10% respectively and the radical scavenging activity of water extract was higher than CE and MWE. Total phenolic constituent of the methanol, ethanol and water extracts of other *Achillea* species had been reported previously and found to be even higher amounts of total phenol (Giorgi et al. 2009; Alexandru et al. 2007; Conforti et al. 2005). Our findings are in good agreement with the literature. In this experiment with an automated Soxtherm apparatus gave higher extract yield with higher total phenolics and as a result better activity was observed comparable to those available in the current literature.

Proanhtocyanidin contents of the extracts varied depending on polarity of the solvent. It should be noted that WE and MWE extracts showed strong free radical scavanging activity and their proanthocyanidine contents were also high. The highest content was found in the MWE extract (7125 mg/ L). Although the WE showed the highest scavenging action the proanthocyanidine content of WE was lower than MWE. Thus, it can be concluded that no direct correlation is available between radical scavenging activity and proanthocyanidine content.

With regard to data given in Table 3, the highest flavonoid content was found for WE and total flavonoid contents of CE, WE and MWE were similar; ranging from 0.93% to1.12%. In a previous study, the free radical activity had been of ethanol extracts of six *Achillea* species compared with the total flavonoid contents but no significant relationship was found (Nickavar et al. 2006).

Total anthocyanin of the CE was quite high (879.33 mg/ L). Together with aforesaid proanthocyanidine content of the extract in question may account for the reason why this extract exerted superior activity in the inhibition of linoleic acid assay.

3.5. HPLC analysis of methanolic extract

HPLC analysis was exerted and HPLC chromatograms of phenolics standards and the ME extract were given in Figure 3.

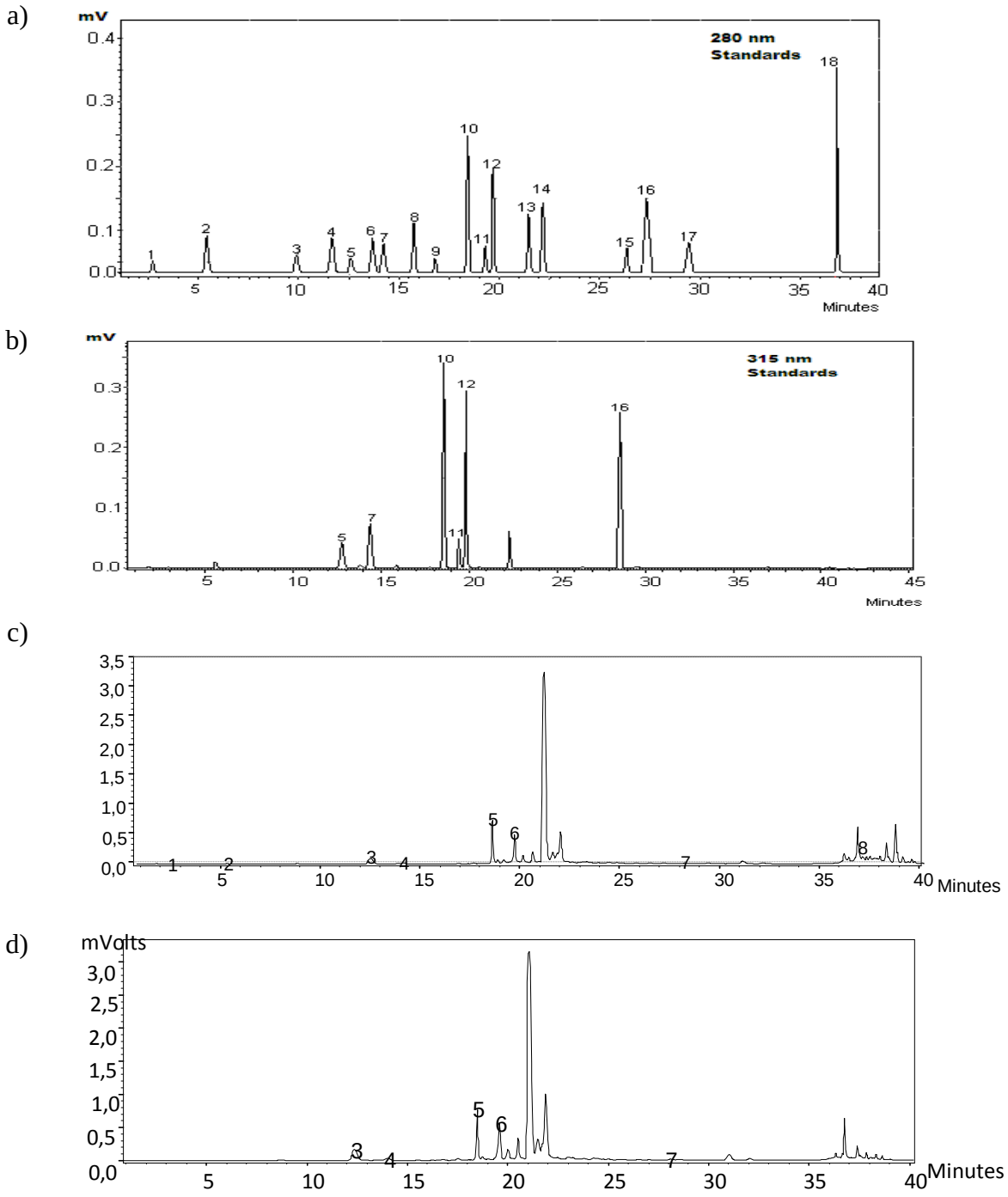


Figure 3. HPLC chromatograms of the phenolic standards at a) 280 nm b) 315 nm detection. Peak identification: (1) Gallic acid, (2) Protocatechuic acid, (3) *p*-OH benzoic acid, (4) Catechin, (5) Chlorogenic acid, (6) Vanillic acid, (7) Caffeic acid, (8) Syringic acid, (9) Epicatechin, (10) *p*-coumaric acid, (11) Rutin, (12) Ferulic acid, (13) Benzoic acid, (14) *o*-coumaric acid, (15) Absisic acid, (16) Quercetin, (17) t-cinnamic acid, (18) Propiyl paraben (IS). HPLC chromatogram of the *A.biserrata* at c) 280 nm and d) 315 nm dedection. Peak identification: (1) Gallic acid, (2) Protocatecuic acid, (3) Chlorogenic acid, (4) Caffeic acid, (5) *p*-Coumaric acid, (6) Ferulic acid, (7) Quercetin, (8) Propyl paraben.

Main phenolic compounds were determined as protocatecuic acid (177.83 µg/g dry material), chlorogenic acid (164.78 µg/g), p-coumaric acid (86.08 µg/g), gallic acid (34.78 µg/g), caffeic acid (12.32 µg/g), quarcetin (1.90 µg/g) (Table 5). Benetis *et.al* (2008)

determined chlorogenic acid, vicenin-2, luteolin-7-O-glucoside, apigenin-7-O- glucoside, luteolin, luteolin-37-di-O-glucoside at high concentrations and the total amount of phenolic compounds yarrow flowers in different communities had been reported to range from 13 to 27.8 mg/g, ın the literature (Benetis et al. 2008). Protocatecuic acid and chlorogenic acid were main two phenolic acids and thought to be responsible for the radical scavenging activity exerted in this study.

Table 5. Phenolic contents of *A. biserrata*

Phenolic Compounds	**Concentration (µg/g dry material)**
Gallic acid	34.78
Protocatecuic acid	177.83
p-OH Benzoic acid	-
Catechin	-
Chlorogenic acid	164.78
Vanillic acid	-
Caffeic acid	12.32
Syringic acid	-
Epicatechin	-
p-Coumaric acid	86.08
Ferulic acid	-
Benzoic acid	-
Rutin	-
o-Coumaric acid	-
Absisic acid	-
t-Cinnamic acid	-
Quercetin	1.90

4. Conclusion

The free radical scavenging action of *A.biserrata* rises with increasing polarity of the solvent system. Water extract was shown perfect activity which is comparable with synthetic antioxidant BHT and phenolic constituent and presence of key phenolic compounds responsible from the activity. Total phenolic constituents of polar extracts are crucially higher than non-polar ones. Furthermore, non-polar solvents seems to be more suitable to attain active principles, which are highly effective for the interception of dien formation.

Acknowledgements

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