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Türkiye kaynaklı *Pulicaria dysenterica* bitkisinin fitokimyasal analizi ve antioksidan ve antikolinesteraz aktiviteleri

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Özet

Bu çalışma *Pulicaria dysenterica* bitkisi ile ilgili ilk antioksidan ve antikolnesteraz aktivite çalışmadır. Sunulan çalışma *Pulicaria dysenterica* bitkisinin fitokimyasal analiz ve biyolojik aktivite çalışması ile ilgilidir. Bitkinin uçucu yağları ve petrol eteri ekstresinin yağ asitleri GC-MS analizi ile belirlendi. Uçucu yağın % 99.7 sini oluşturan 18 uçucu yağ saptandı. Uçucu yağın ana bileşeni terpinolen (%32.6) olarak belirlendi. Petrol eteri ekstresi yağ asidinin %99 unu oluşturan 13 yağ asidi saptandı. Yağ asidinin ana bileşenleri palmitik asit (%34.2), linoleic asit (%16.4) ve stearik asit (%15.7) olarak belirlendi. *P. dysenterica* bitksinin petrol eteri, aseton, metanol ve su ekstrelerinin antioksidan aktivitelerini belirlemek için β-karoten-linoleik asit test sistemi, DPPH serbest radikali giderimi, ABTS katyon radikal giderim ve bakır indirgeme antioksidan kapasitesi yöntemleri kullanıldı. ABTS katyon radikali giderimi aktivitesi yönteminde 100 µg/mL konsantrasyonda su ekstresi güçlü antioksidan aktivite gösterdi. Ekstrelerin asetil- ve bütiril-kolinesteraz inhibisyon aktiviteleri için Ellman metodu kullanıldı. Petrol eteri ekstresi 200 µg/mL konsantrasyonda asetilkolinesteraz enzimine karşı % 65.33 oranında aktivite gösterdi. Sonuçlar *P. dysenterica* bitkisinin ABTS katyon radikali giderici kaynağı olarak kullanılabileceğini gösterdi.

Phytochemical analysis and antioxidant and anticholinesterase activities of *Pulicaria dysenterica* from Turkey

Abstract

This study is the first antioxidant and anticholinesterase activity report on phytochemical analysis and biological activity *Pulicaria dysenterica*. The essential oil analysis and fatty acids of the petroleum ether extract of *P. dysenterica* was determined by GC/MS analysis. The major essential oil was identified as terpinolen (32.6%). Also thirteen fatty acids were identified, constituting 99.0% of the petroleum ether extract. Main components were identified as palmitic acid (34.2%), linoleic (16.4%) and stearic acid (15.7%). β-carotene-linoleic acid test system, DPPH free radical scavenging assay, ABTS cation radical scavenging assay and cupric reducing antioxidant capacity assays were used for studying the antioxidant activity of the petroleum ether, acetone, methanol and water extracts of this plant. The water extract exhibited strong ABTS cation radical scavenging activity at 100 µg/mL. Ellman method was used to indicate the acetyl- and butyryl-cholinesterase inhibitory activities of the crude extracts. This is where, the petroleum ether extract exhibited 65.33% inhibitory activity against acetylcholinesterase at 200 µg/mL. The results showed that *P. dysenterica* is a potential source of ABTS cation radical scavenging products.
Key Words: Asteraceae, *Pulicaria dysenterica*, essential oil, fatty acid, antioxidant, anticholinesterase

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1. Introduction

The genus *Pulicaria* Gaertner belongs to the family Asteraceae and represented by 80 species worldwide and 6 species in Turkey, distributed from Europe into North Africa and Asia. *Pulicaria dysenterica* (L.) Bernh. is widespread in Turkey but rare in South East Anatolia [1, 2]. In Turkish folk medicine the local names of *P. dysenterica* are Dizanteri otu [3] and Yara otu [2, 4] and is use as diuretic and in relieving constipation. Also the species is uses to treat to dysentery [3], promote maturation of abscesses [4].

Literature survey showed that the chemical composition of *Pulicara* species is consisted of flavonoids [5, 6], sesquiterpenoids [7], diterpenes [8], phenolics [9] and caryophyllenes and caryophyllen derivatives [10, 11]. Different biological activities have been reported of some *Pulicaria* species such as cytotoxic [12, 13], antibacterial [14, 15], antispasmodic [16], and antihistaminic [17] activities. There is no antioxidant and anticholinesterase activity reported for *Pulicaria* species. According to report of Triana et al several species of *Pulicaria* genus are used as insect repellents and treatment of dysentery. *P. odora* is used to treat back-pain, intestinal disorders and menstural cramps in Moroccon traditional medicine. *P. dysenterica* has been used against diarrhea, dysentery and insecticide in Europe, also decoction of the plant is used as an antidiarrhoel agent in Iranian's folk medicine.

Depending on the life expectancy and the aging of the world's population various health problems gains importance both socially and economically. Nowadays, best results in the treatment of Alzheimer's disease, has been obtained from a single drug group, acetylcholinesterase (AChE) inhibitors together with prolonging the human life, the occurrence of chronic diseases (cancer, cardiovascular disease, Alzheimer's, and so on.) have increased and consequently the antioxidants have gained more attentions nowadays [18]. On the other hand synthetic antioxidants are used to extend the shelf life of foods and prevent degradation. BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate) and TBHQ (t-butylhydroquinone) are commonly used synthetic antioxidants. However, there are some studies which demonstrate that synthetic antioxidants and by-products formed of them can lead to various diseases [19]. For this reason, reporting new antioxidant substances as an alternative for the synthetic ones have gained importance in this field. The antioxidants may also releaf the progression of Alzheimer's disease among elderly people all around the world [20, 21].

A literature survey showed that there is no report on antioxidant and anticholineterase activity on *P. dysenterica* (L.). The aim of this study was to evaluate the antioxidant and anticholinesterase activities of the petroleum ether (PDP), acetone (PDA), methanol (PDM) and water (PDW) extracts of *P. dysenterica.* The essential oil of the plant and fatty acid composition of the petroleum ether extract were determined by GC/MS analysis. Total phenolic and flavonoid contents of these crude extracts were determined as pyrocatechol and quercetin equivalents, respectively. β-Carotene-linoleic acid test system, DPPH free radical scavenging, ABTS cation radical decolorisation and cupric reducing antioxidant capacity assays were used to indicate the antioxidant activity. The anticholinesterase potential of the extracts were determined by Ellman method.

2. Experimental

2.1. Plant material

The aerial parts of *P. dysenterica* (L.) were collected from Şile-Istanbul in August 2012 and identified by Dr. Yeter Yeşil (Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University). A voucher specimen was deposited in the Herbarium of Istanbul University (ISTE 98052).

2.2. Isolation of essential oils

The dried aerial parts of *P. dysenterica* were cut into small pieces and subjected to hydro-distillation with water for 4 h, using a Clevenger-type

apparatus to produce essential oils which were dried over anhydrous sodium sulphate and stored at +4°C until required. The essential oils were diluted by dichloromethane (1:3, v/v) before the GC run.

2.3. GC/MS conditions for essential oil analysis

GC/MS analyses were performed on Thermo Electron Trace 2000 GC model gas chromatography and Thermo Electron DSQ quadrupole mass spectrometry. A nonpolar Phenomenex DB5 fused silica column (30 m x 0.32 mm, 0.25 µm film thickness) was used with helium at 1mL/min (20 psi) as a carrier gas. The GC oven temperature was kept at 60°C for 10 min and programmed to 280°C at a rate of 4°C/min and then kept constant at 280°C for 10 min. The split ratio was adjusted to 1:50, the injection volume was 0.1 µL and EI/MS was recorded at 70 eV ionization energy. Mass range was *m/z* 35-500 amu. Identification of the compounds was based on the comparison of their retention times and mass spectra with those obtained from authentic samples and/or the NIST and Wiley spectra as well as the literature data.

2.4. Esterification of total fatty acids

A hundred milligram of the sample was refluxed in 0.1M KOH solution in ethanol (2 mL) for 1h. The solution was cooled and 5mL water was added. The aqueous mixture was neutralized with 0.5 mL HCl solution and extracted with hexane:diethyl ether (1:1; 3.5 mL). The organic layer was separated and washed with water (10 mL), and dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure on a rotary evaporator to give fatty acid methyl esters [22].

2.5. GC/MS conditions for fatty acid analysis

Helium was used as carrier gas at a constant flow rate of 1 mL/min. 1 µL of sample was injected. The GC temperature program was set as follows; 150 °C hold for 5 min, ramp to 250 °C at 3 °C/min and hold for 10 min. The temperature of the MS transfer line was set at 230 °C. Using scan mode a mass range from 50 to 650 *m/z*. Used column, Rtx-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm. Thermo Scientific Polaris Q GC-MS/MS was used in this study.

2.6. Preparation of plant extracts

The dried and powdered aerial parts of *P. dysenterica* (100 g) were sequentially macerated with petroleum ether (3 x 250 ml), acetone (3 x 250 ml), methanol (3 x 250 ml) and water (3 x 250 ml) for 24 h at room temperature. After filtration, the solvents were evaporated to dryness under vacuum. The petroleum ether extract was analyzed to determine its fatty acid composition by GC/MS (GC/MS conditions were described in the Section 2.3).

2.7. Determination of total phenolic and flavonoid content

The amounts of phenolic [23] and flavonoid [24] contents in the crude extracts were expressed as pyrocatechol and quercetin equivalents, and they were calculated according to the following equations;

Absorbance = 0.0223 pyrocatechol (µg)+ 0.0542 ($R^2 = 0.9989$)

Absorbance = 0.1701 quercetin (µg) - 0.778 ($R^2 = 0.9939$)

2.8. Antioxidant activity

β-Carotene-linoleic acid test system [25], DPPH free radical scavenging activity [26], ABTS cation radical decolorisation [27] and cupric reducing antioxidant capacity (CUPRAC) [28] methods were carried out to determine the antioxidant activity.

2.19. Anticholinesterase activity

A spectrophotometric method developed by Ellman, Courtney, Andres, & Featherstone was established to indicate the acetyl- and butyrylcholinesterase inhibitory activities in vitro [29].

2.10. Statistical analysis

The results of the antioxidant, antimicrobial and anticholinesterase activity assays were mean ± SD of three parallel measurements. The statistical significance was estimated using a Student's *t*-test, p values < 0.05 were regarded as significant.

3. Results and Discussion

This paper is the first report on the biological activity studies of *P. dysenterica*. The essential oil composition of the plant was determined by GC/MS analysis. Eighteen essential oils were identified, constituting 99.7% of whole essential oil ingridients of the plant (Table 1). The major essential oil was identified as terpinolen (32.6%). According to Basta et al. report, main essential oils of *P. dysenterica* from Greece were determined as (Z)-nerolidol, caryophyllen oxide and (E)-nerolidol [30]. Essential oil composition of *P. gnahalodes* were calamenene-10-one, longifolol, curcumen-15-al-ar [31]. The fatty acid content of petroleum ether extract was determined by GC/MS analysis. As seen in Table 2, 13 components were identified, constituting 99.0% of the petroleum ether extract. According to the results, a large rate in the amounts of palmitic acid (34.2%), linoleic acid (16.4%) and stearic acid (15.7%) were identified in the extract. This study is also the first report on *P. dysenterica* fatty acid composition. There are no study on fatty acid composition of *Pulicaria* species in the literature as well.

Table 1. *Chemical composition of the essential oil from Pulicaria dysenterica*

Rt (min)[a]	Constituents[b]	Composition%	RI[c]
18.98	Terpinolen	32.6	1086
24.05	1,3-di-tertbutyl benzene	1.6	1249
30.48	Valencene	2.4	1484
30.67	β-selinene	2.7	1490
30.87	α-selinene	9.0	1498
33.13	Caryophyllene oxide	3.2	1583
35.52	2-methyl heptadecane	1.9	1746
36.45	Octadecane	1.9	1800
36.87	1-nonadecanol	1.7	2156
38.22	Z-14-octadecen-1-ol acetate	8.9	2185
38.34	Z-8-octadecen-1-ol acetate	4.0	2185
39.96	Heneicosane	2.5	2109
40.13	2,5-di-tert octyl-p-benzoquinone	4.4	2259
40.61	Arachidic acid	9.9	2366
40.66	Hexadecanoic acid	4.3	1986
43.63	1-hexacosanol	4.2	2852
44.10	Nonacosane	2.9	2900
44.41	Ethyl iso-allocholate	1.6	3094
	TOTAL	**99.7**	

[a] Retention time (as minute).
[b] Compounds listed in order of elution from a HP-5 MS column. A nonpolar Phenomenex DB-5 fused silica column
[c] RI Retention indice (DB-5 column)

Table 2. *Fatty acid analysis of the petroleum ether extract of Pulicaria dysenterica*

Rt (min)[a]	Constituents[b]	Composition%
9.69	Octanedioic acid, dimethyl ester	2.7
14.39	10-undecenoic acid, methyl ester	1.1
18.60	Myristic acid, methyl ester	2
21.97	Pentadecanoic acid, methyl ester	1.9
25.27	Palmitic acid, methyl ester	34.2
28.86	11,13-dimethyl-12-tetradecen-1-ol acetate	1.3
29.75	Phytol	3.3
30.64	Linoleic acid, methyl ester	16.4
30.77	Oleic acid, methyl ester	5.7
30.86	Linolenic acid, methyl ester	8.5
31.54	Stearic acid, methyl ester	15.7
37.38	Arachidic acid, methyl ester	3.6
39.36	Docosane	3.6
	TOTAL	**99.0**

[a] Retention time (as minute)
[b] Compounds listed in order of elution from a HP-5 MS column. A nonpolar Phenomenex DB-5 fused silica column

The petroleum ether, acetone, methanol and water extracts prepared from the aerial parts of *P. dysenterica* were screened for their possible antioxidant activity by using four complementary methods, namely β-carotene bleaching, DPPH free radical scavenging assay, ABTS cation radical decolorisation assay and cupric reducing antioxidant capacity. Total phenolic and flavonoid contents of the extracts were determined as pyrocatechol (PEs) and quercetin (QEs) equivalents, respectively. As shown in Table 3, the phenolic contents of the methanol extract are higher than those of the petroleum ether, acetone and water extracts. The flavonoid contents of acetone extract are higher than the other extracts.

Table 3. *Total phenolic and flavonoid contents of Pulicaria dysenterica extracts[a]*

Extracts	Phenolic content (μg PEs/mg extract)[b]	Flavonoid content (μg QEs/mg extract)[c]
Petroleum ether extract (PDP)	48.41 ± 0.28	29.82 ± 0.59
Acetone extract (PDA)	118.29 ± 0.07	55.76 ± 0.83
Methanol extract (PDM)	374.39 ± 1.73	52.00 ± 0.15
Water extract (PDW)	114.23 ± 0.62	49.14 ± 0.73

[a] Values expressed are means ± S.D. of three parallel measurements ($p < 0.05$)
[b] PEs, pyrocatechol equivalents ($y=0.0223x + 0.0542$ $R^2=0,9989$)
[c] QEs, quercetin equivalents ($y=0.1701x – 0.0778$ $R^2=0,9939$)

As shown in ***Figure 1***, the acetone and water extracts exhibited moderate lipid peroxidation activity in β-carotene bleaching method at 100 μg/mL. DPPH free radical scavenging activity of the extracts were very weak (***Figure 2***). The acetone and methanol extracts of P. dysenterica exhibited moderate inhibition while the water extract exhibited over 80% inhibition in ABTS cation radical scavenging assay at 100 μg/mL (Figure 3). None of the extracts was found to be active in CUPRAC. (***Figure 4***)

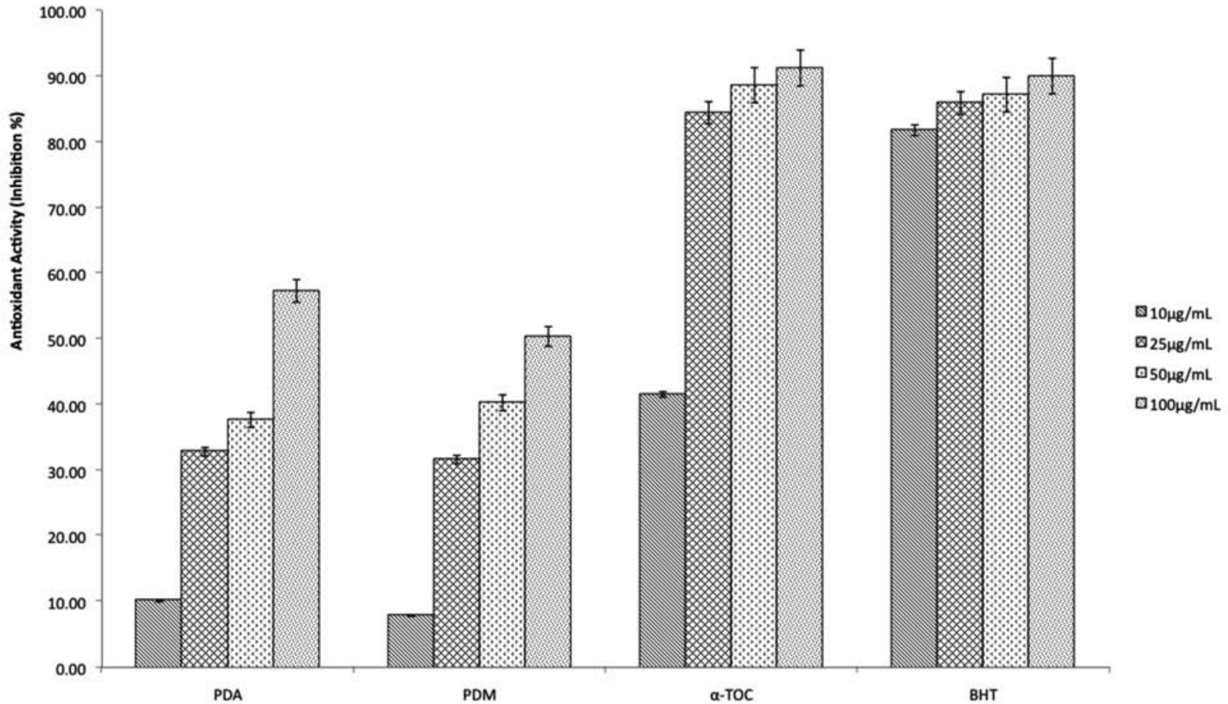

Figure 1. *Inhibition (%) of lipid peroxidation of the Pulicaria dysenterica extracts, α-tocopherol and BHT by β-carotene bleaching method. Values are means ± S.D., n=3, p<0.05, significantly different with Student s t-test*

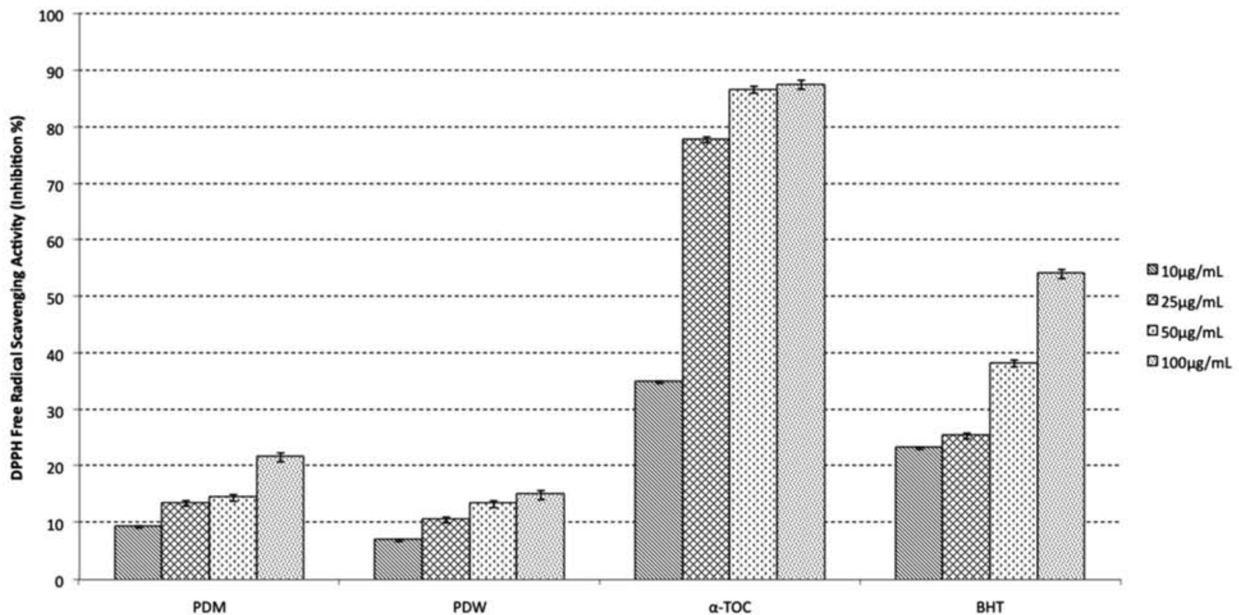

Figure 2. *Inhibition (%) of DPPH free radical scavenging of the Pulicaria dysenterica extracts, α-tocopherol and BHT Values are means ± S.D., n=3, p<0.05, significantly different with Student s t-test*

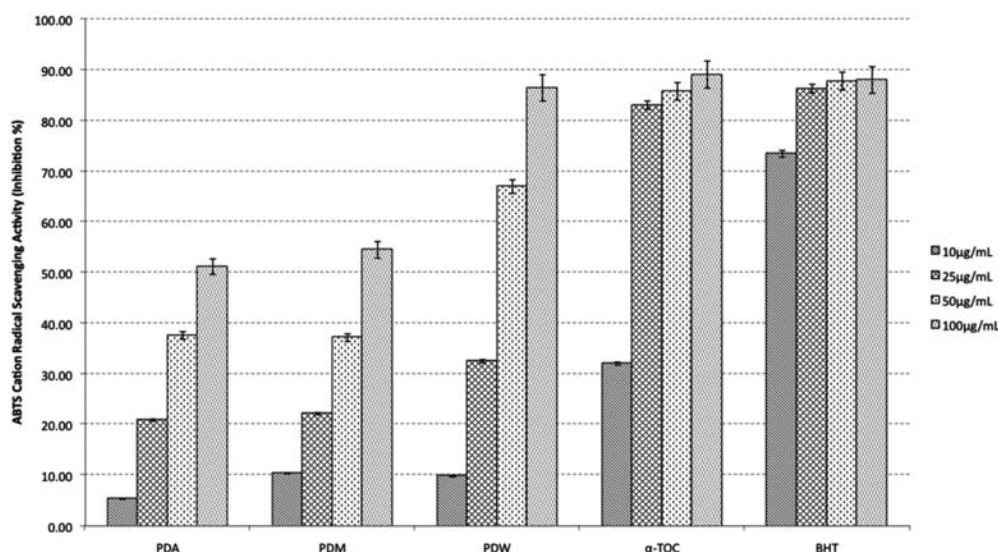

Figure 3. *Inhibition (%) of ABTS cation radical scavenging of the Pulicaria dysenterica extracts, α-tocopherol and BHT. Values are means ± S.D., n=3, p<0.05, significantly different with Student s t-test*

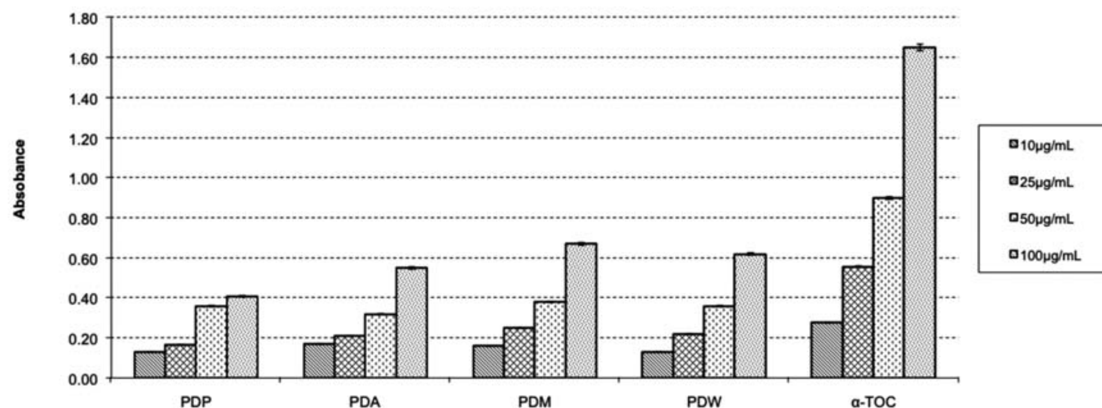

Figure 4. *Cupric reducing antioxidant capacity of the Pulicaria dysenterica extracts and α-tocopherol. Values are means ± S.D., n=3, p<0.05, significantly different with Student's t-test*

As shown in ***Table 4***, the petroleum ether extract exhibited as high inhibition (65.33%) while the acetone, metanol and water extracts posses moderate activity against acetylcholinesterase enzyme at 200 µg/mL. None of the extracts was found to be active against butyrylcholinesterase.

4. Conclusion

This study is the first antioxidant and anticholinesterase activity report on *P. dysenterica*. The results showed that *P. dysenterica* can be used as ABTS cation radical scavengers and acetylcholinesterase inhibitory source. Therefore, phytochemical and biological studies of this genus should be intensified.

Table 4. *Anticholinesterase activity of Pulicaria dysenterica extracts at 200 µg/mL*[a]

Extracts	Inhibition % against AChE	Inhibition % against BChE
Petroleum ether extract	65.33 ± 2.10	7.98 ± 0.20
Acetone extract	56.10 ± 0.76	7.78 ± 0.12
Methanol extract	54.14 ± 1.76	9.74 ± 0.45
Water extract	40.91 ± 0.35	NA
Galanthamine[b]	85.55 ± 0.55	79.47 ± 0.80

"[a] Values expressed are means ± S.D. of three parallel measurements (p<0.05) [b] Standard drug NA: Not active

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