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Cytotoxic, antimicrobial activities, AChE and BChE inhibitory effects of compounds from *Tanacetum chiliophyllum* (Fisch. & Mey.) Schultz Bip. var. *oligocephalum* (D.C.) Sosn. and *T. chiliophyllum* (Fisch. & Mey.) Schultz Bip. var. *monocephalum* Grierson

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ABSTRACT

Tanacetum L. species traditionally used for insecticidal purposes as well as in folk medicine for their antitumor, antimicrobial, antifungal activities. In our previous study a novel sesquiterpene lactone and triterpene lactone together with 12 known flavonoids, coumarin and a triterpene were isolated from *T. chiliophyllum* var. *oligocephalum* and *T. chiliophyllum* var. *monocephalum* extracts which have insecticidal and antimicrobial activity. In this study, cytotoxic, antimicrobial activities and acetylcholinesterase (AChE), butyrylcholinesterase (BChE) inhibitory effects of pure compounds isolated from these plants were investigated. The tested compounds showed AChE and BChE inhibition which ranged between 7.20–80.37% and 9.19%–76.99% respectively. The highest AChE and BChE inhibition was observed for ulubelenolide which afforded 80.37% and 76.99% inhibition respectively. The cytotoxic effect of the compounds ranged between 22.34–49.77 μg/mL IC_{50} values. Highest cytotoxic activity was observed against MCF-7 and HEK 293 cell line by 5–hydroxy-3′,4′,7-trimethoxy flavone and 5-hydroxy-3′,4′,6,7-tetramethoxyflavone that produced 25.80 ± 0.17 and 22.34 ± 0.70 IC_{50} values respectively. Compounds eupatilin, cirsilineol, 5–hydroxy-3′,4′,7-trimethoxy flavone and ulubelenolide showed significant antimicrobial effect on *C. albicans* with 7.8 μg/mL MIC. The new compound ulubelenolide afforded high AChE and BChE inhibition as well as high antifungal activity. In our opinion activity of this substance should be evaluated further against other fungal species.

1. Introduction

Tanacetum L. species of Asteraceae finds uses in the treatment of various illnesses in folk medicine (D'Amelio and Roton, 1999). Previous research on this genus presents that essential oils, extracts and pure compounds from the species of this genus have antitumor, cytotoxic, antimicrobial, phytotoxic, migraine prophylactic, and insecticidal activities, as reviewed in a previous comprehensive chapter (Gören et al., 2002). Therefore, this genus is extensively investigated by many researchers. The genus *Tanacetum* finds habitat mostly in the moderate climatic zones in Northern hemisphere and in some parts of Southern hemisphere. In Turkey genus *Tanacetum* is represented by 45 species and altogether by 60 taxa (Davis, 1975). *T. chiliophyllum* (Fisch. & Mey.) Schultz Bip. species naturally grows in Iran, Azerbaijan, Armenia and East Turkey (Davis, 1975). In Turkey, it is represented with four varieties including var. *monocephalum* Grierson, var. *oligocephalum* (DC.) Sosn., var. *chiliophyllum* and var. *heimerlei* (Náb.). The varieties of *T. chilophyllum* find a natural habitat on volcanic, limestone slopes between 1200 and 3200 m altitude in Eastern and Southeastern Turkey (Davis, 1975).

Previously from the *T. chiliophyllum* varieties following new compounds were isolated ulubelenolide (Polatoglu and Gören, 2015), 1-*epi*-chiliophyllin, (Polatoğlu et al., 2011), chiliophyllin and heimerlein (Gören and Tahtasakal, 1993). Furthermore, known sesquiterpene

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lactones (spiciformin, deacetyllauerenbiolide, 1α-hydroperoxy-1-desoxo chrysanolide, tabulin, tanachin, tamirin, and dentatin), flavones (5-hydroxy-3′,4′,6,7-tetramethoxyflavone, eupatilin (6-hydroxyluteolin-6,3′,4′-trimethylether), cirsimaritin (scuttellarin-6,7-dimethylether), cirsilineol, 5–hydroxy-3′,4′,7-trimethoxy flavone, desmethoxy-centaureidin, jaceosidin, scutellarein-6,7,4′-trimethyl ether, 6-hydroxyluteolin-6,3′4′-trimethyl ether, 6-hydroxyluteolin-6,3′-dimethyl ether, scutellarein-6,7-dimethyl ether, 4′,5,7-trihydroxy-3′,8-dimethoxyflavone, 4′,5,7-trihydroxy-8-methoxyflavone, quercetagetin-3,6,4′-trimethyl ether, quercetagetin-3,6,7-trimethyl ether) and triterpenes (neolupenyl acetate, taraxasterol acetate) were identified from the *T. chiliophyllum* varieties (Gören and Tahtasakal, 1993, 1994; Matsakanyan and Revazova, 1974; Polatoğlu et al., 2011; Polatoglu et al., 2013; Polatoglu and Gören, 2015). In our previous investigation on the ethyl acetate and methanol extracts of *T. chiliophyllum* var. *oligocephalum* the ethyl acetate extract afforded high antimicrobial activity against *Bacillus cereus* (MIC: extract: 125 μg/mL; Chloramphenicol: 125 μg/mL) and considerable inhibition on *S. aureus*, meticillin-resistant *S. aureus*, *S. epidermis*, *S. typhimurium* (extract produced two-fold MIC than the positive control chloramphenicol) (Polatoglu et al., 2013). Additionally, ethyl acetate extracts of *T. chiliophyllum* var. *oligocephalum* and *T. chiliophyllum* var. *monocephalum* produced considerable contact toxicity against the granary pest *Sitophilus granarius* (81.73 ± 0.14%, 65.05 ± 0.28% mortality after 24 h of application respectively.) (Polatoğlu et al., 2011; Polatoglu et al., 2013). These extracts afforded one new sesquiterpene lactone and one new triterpene lactone together with 12 other known flavonoids, coumarin and triterpene from *T. chiliophyllum* var. *oligocephalum* and *T. chiliophyllum* var. *monocephalum* ethyl extracts. Previous reports also indicate cytotoxicity of some of these compounds namely 5-hydroxy-3',4',6,7-tetramethoxyflavone (9KB, P-388) (Dobberstein et al., 1977); eupatilin (AGS, HeLa, HL-60, MK-1, MKN-1, B16F10) (Choi et al., 2009; Nagao et al., 2002; Park et al., 2013; Seo and Surh, 2001); cirsimaritin (GBC-SD, GBC-SD18H, BGC-823, SMMC-7721, PBMC, T-lymphocytes) (Quan et al., 2010); cirsilineol (Caov-3, Colon 26, HeLa, HCT-116, K-562, MCF-7, A431, A2780, PC3, Raji, Skov-3, SW1116, THP-1) (Hajdú et al., 2014; Lin et al., 2015; Sheng et al., 2008; Tsasi et al., 2016); 5-Hydroxy-3′,4′,7-trimethoxyflavone (Hep 62, MCF-7, Vero, WI-38) (Alarif et al., 2013); isofraxidin (P-388) (Borris et al., 1980), jaceosidin (Caov-3,HeLa, PC3, T24, Skov-3, MCF10A-*ras,* HPV-16) (Kim et al., 2007; Lee et al., 2005; Li and Tan, 2013; Lv et al., 2008); 4′-hydroxy wogonin (HL-60, K562, MGC-803, SH-SY5Y, SW1116, SMMC-7221, SW480, Hep62, KB) (Sonoda et al., 2004; Yao et al., 2011). Considerable biological activity results which we have obtained in our previous studies prompted us to investigate AChE and BChE inhibition effects that could be related to observed insecticidal activities and further cytotoxicity of the active principles against PC3, U87MG, HEK293 and MCF-7 cell lines. Additionally, due to antimicrobial activity results and the type of compounds isolated from these species, we have suspected that these compounds could produce considerable antimicrobial activities. Therefore, here we present cytotoxic, antimicrobial activities, AChE and BChE inhibitory effects of pure substances obtained from *T. chiliophyllum* varieties.

2. Results and discussion

The highest AChE inhibition was observed for ulubelenolide **9**, (80.37 ± 6.02%) which also afforded the highest BChE inhibition (76.99 ± 2.76%). The concentration of ulubelenolide (Mw: 438.68 g/mol) in the assay solution was 114.5 μM which was 16.5-fold higher concentration than the positive control galantamine that has the same AChE activity at 6.94 μM concentration. The concentration of the **9** in BChE inhibition assay was 6-fold higher than the galantamine (19.11 μM) that produced the same inhibition. The tested compounds (120 μL from 1 mg/mL solution) **1**, **3**, **4**, **5**, **6**, **7**, **10**, **11** and **12** showed low AChE inhibition ranging between 7.20–23.64%. The compounds **2** and **8** did not produce any AChE inhibition. Compounds **10** and **12** produced low BChE inhibition (9.23 and 9.19% respectively); however, 4′,5,7-trihydroxy-3′8-dimethoxyflavone produced considerable BChE inhibition that was twice higher than its AChE inhibition at the same concentrations (30.25 ± 9.65 and 14.98 ± 3.98% respectively). Compounds **1–8** did not produce any BChE inhibition at the studied concentrations (120 μL from 1 mg/mL solution) in the assay. The AChE and BChE results obtained from the compounds **1–12** were given in Table 1. Previously the extracts of *T. chiliophyllum* var. *monocephalum* (**9–12**) and var. *oligocephalum* (**1–8**) were tested for their insecticidal activity against *Sitophilus granarius* and latter afforded the highest activity. The insecticidal compounds mostly act on the nervous system of the insects and are suspected to inhibit enzymatic systems such as AChE, BChE (Lopez and Pascual-Villalobos, 2010), microsomal P-450 monooxygenase and glutathione *S*-transferase (Lopez et al., 2010).

Table 1
AChE, BChE inhibitory activities of the compounds 1–12.

Compounds	AChE Inhibition%	BChE Inhibiton%
Galanthamine	100 < [a]	86.95[b]
1	23.12 ± 0.56[d]	–[c]
2	–	–
3	23.64 ± 2 .57	–
4	22.08 ± 3.62	–
5	17.84 ± 1.05	–
6	7.20 ± 0.79	–
7	11.20 ± 2.28	–
8	–	–
9	80.37 ± 6.02	76.99 ± 2.76
10	14.13 ± 2.52	9.23 ± 3.23
11	14.98 ± 3.98	30.25 ± 9.65
12	11.55 ± 1.93	9.19[e]

[a] AChE inhibition% is calculated for 1 mg/mL galanthamine concentration (application volume 20 μL) from the standard curve obtained from standard dilutions of galanthamine ($n = 3$; $y = 24.968\ln(x) + 32.003$; $R^2 = 0.9934$). The inhibition percent is higher than 100%.
[b] BChE inhibition% is calculated for 1 mg/mL galanthamine concentration (application volume 20 μL) from the standard curve obtained from standard dilutions of galanthamine ($n = 2$; $y = 18.389\ln(x) + 22.729$; $R^2 = 0.993$).
[c] Not available.
[d] Results are given as mean ± standard deviation of at least three replications.
[e] Result is given according to single measurement.

The cytotoxic effect and IC_{50} value of compounds on a selection of cells were examined by using different concentrations of the compounds by MTT assay. The results of the MTT assay were given in Table 2. The MTT assay results showed that the 5-hydroxy-3′,4′,6,7-tetramethoxyflavone (**1**) showed moderate cytotoxic activity against PC3, U87MG and HEK293 with IC_{50} values of 36.61 ± 0.05, 46.47 ± 0.07 and 22.34 ± 0.70 μg/mL, respectively. This compound was not active against the MCF-7 cells. Highest cytotoxic activity of **1** was observed against HEK293 cell line. Among all the tested compounds **1** produced highest activity against this cell line. Compound 5–hydroxy-3′,4′,7-trimethoxy flavone (**5**) also exhibited cytotoxicity on the tested cell lines with higher IC_{50} values which varied from 25.80 ± 0.17 to 49.77 ± 0.51 μg/mL. Compound **5** afforded its highest activity against MCF-7 cell line with IC_{50} value of 25.80 ± 0.17 μg/mL. Among all the tested compounds **1** produced the highest activity against PC3 cell line and **5** produced the highest activity against U87MG cell line. Compounds **1** and **5** contains structurally similar aspects such as hydroxyl group on C5, and methoxy groups on C7, C3′ and C4′. The only difference between **1** and **5** is the methoxy group on C6 in the first one. The compounds **1** was previously reported to have cytotoxic activity against 9KB, P388 (Dobberstein et al., 1977) and **5** against Hep 67, MCF-7, Vera, WI-38 (Alarif et al., 2013). Previously the tested compounds except for **9** and **10** afforded cytotoxic activity against many other cell lines which was mentioned before. In the present research to our surprise except for the compounds **1** and **5**,