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Volatiles and fatty acid analyzes of *Tripleurospermum decipiens* (Fisch & C. A. Mey) Bornm and investigation of the extracts for antimicrobial and enzyme inhibitory activities

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ABSTRACT: In this study, the volatiles were obtained from aerial parts of *Tripleurospermum decipiens* (Fisch & C.A.Mey) Bornm with microsteam distillation - solid phase microextraction (MSD-SMPE) technique. The fatty acids from *T. decipiens* were obtained by using the microextraction method with subsequent transesterification with boron trifluoride reagent. The chemical compositions of the volatiles and fatty acid methyl esters was analyzed by Gas Chromatography (GC) and Gas Chromatography/ Mass Spectrometry (GC / MS) techniques. *n*-Hexane (TDH) and methanol (TDM) extracts of *T. decipiens* were investigated for acetylcholineterase (AChE), α-amylase enzyme inhibitions and antimicrobial activities. *In vitro* inhibition of AChE was monitored by using Ellman's chromogenic agent, and antidiabetic effects of the extracts were spectrophometrically evaluated by inhibition of porcine pancreatic α-amylase enzyme. Antimicrobial activities of the extracts were evaluated against *Salmonella enterica* ATCC 14028, *Bacillus subtilis* subsp *spizizeni* ATCC 6633, *B. subtilis* ATCC 19654, *Staphylococcus aureus* ATCC 6538, *Klebsiella aerogenes* ATCC 13048, *Candida parapsilosis* ATCC 22019 by microdilution method. *In vitro* α-amylase enzyme inhibitory activity was observed for methanol extract (55.6%) at 10 mg/mL. The highest AChE inhibition was recorded for *n*-hexane extract (58.8%) at concentration (10 mg/mL). The extracts were found to be effective against *B. subtilis* ATCC 19654, *B. subtilis* subsp *spizizeni* ATCC 6633, *S. aureus* ATCC 6538 and *C. parapsilosis* ATCC 22019.

KEYWORDS: *Tripleurospermum decipiens*; essential oil; extract; acetylcholineterase; α-amylase; antimicrobial.

1. INTRODUCTION

The use of plants as a herbal drugs for the treatment of various diseases has a long history. In recent years, a large number of plant species have been investigated for medicinal applications. Herbal remedies have been considered as a dietary supplement for disease prevention and used as complementary medicine [1]. In this scope, increasing evidence and knowledge on their potential benefits have highlighted the demand for herbal products [2]. These products were reported for potential against bacterial infections, cancer [3-5], neurodegenerative disorders, as well as prevention of diabetes [6], and cardiovascular diseases [7].

Alzheimer's disease (AD) is an age-linked chronic neurodegenerative disease defined by memory loss, cognitive dysfunction and restriction in daily activities, and acetylcholinesterase and butyrylcholinesterase enzyme inhibitions have been accepted as a significant strategy for the treatment of AD. [11].

In recent years, it has been observed a high percentage rate in Diabetes mellitus (DM) distribution due to people's lifestyle change, and it affects a large number of people around the world. *a*-Glucosidase and α-amylase are key enzymes that catalyze hydrolysis of carbohydrates to glucose monomers. In this case, α-glucosidase and α-amylase inhibitor drugs reduce blood glucose levels and use in the treatment of DM [12-13]. Natural or synthetic drugs have been used to treat high blood glucose levels. Although the mechanism

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of the relationship between Alzheimer's and diabetes has not been fully revealed, a lot of factors play a role which includes genetic factors, lifestyle, socioeconomic causes, cardiovascular diseases, inflammation, diabetes, insulin resistance, and it is thought to affect different pathological processes [8-10].

The Asteraceae family comprises approximately 23.000 species in the world. The genus of *Tripleurospermum* Sch. Bip. is the member of tribe Anthemideae and represented by 31 taxa in the flora of Turkey. Most of the species are distributed worldwide in Europe, Asia, North America, and North Africa [14]. *Tripleurospermum* species have been traditionally used to cure or lower the pain of several diseases as well as for food purposes [15-17]. Uses of *T. parviflorum* (Wiild.) Pobed. as antipyretic [18], and *T. conoclinium* (Boiss.& Bal.) Hayek for prevention of cough are among the well-known traditional uses of *Tripleurospermum* species [19]. Ethnobotanical uses of *Tripleurospermum* species are summarized in Table 1.

Table 1. Etnobotanical uses of *Tripleurospermum* in Turkey.

Latin name	Local name	Part used	Etnobotanical use	Utilization method	References
T. parviflorum (Willd.) Pobed.	Beybunik, Kır papatyası, Papatya	Cap.	Food	Eaten fresh (with salt)	[15]
T. parviflorum	Papatya	Flower	Vaginitis	Dec.	[46]
T. conoclinium (Boiss.& Bal.) Hayek	Papatya	Aerial part	Cough	Dec.	[19]
T. parviflorum	Papatya	Flower	Throat diseases	Dec.	[16]
T. parviflorum	Papatya	Cap.	Hair care /Colds, cough, antipyretic, stomach ache	Inf /Int. Dec./Ext.	[18]
T. monticolum (Boiss. & Huet) Bornm.	Kır papatyası	Cap.	Hair care, Colds, cough, antipyretic, stomach ache	Dec. Inf.	[18]
T. sevanense (Manden.) Pobed.	Papatya	Cap.	Hair care	Dec./Ext.	[18]
T. oreadas (Boiss.) Rech. f. var. *oreades*	Papatya	Flower	To ease respiration and gastric pain	Inf.	[47]
T. oreades (Boiss.) Rech var. *oreades*	Papatya, Oşoş	Flower	Dyspnea, abdominal pain, gynecologicaldiseases, arrhythmia, urinary system infections	Dec	[48]
T. parviflorum	Koyun gözü, papatya	Aerial part	Diabetes	Dec.	[49]
T. callosum (Boiss. & Heldr.) E. Hossain	Papatya	Flower	Urinary tract disorders, kidney stones	Inf	[50]
T. disciforme (C.A.Mey.) Sch. Bip.	Papatya, Beybun	Cap. Leaves	Wound healing	Inf	[17]
T. caucasicum (Willd.) Hayek	Beybun	Cap.	Diabetes disease, headache, Toothache	Inf	[17]
T. transcaucasicum (Manden.) Pobed	Beybun	Cap, Leaves	Acne, diabetes, headache	Inf	[17]

Cap.: Capitulum, Inf: Infusion, Dec: Decoction, Int: Internal, Ext: External

Phytochemical investigations on *Tripleurospermum* species have generally been focused on essential oil content [20-24]. A summary of previous studies on essential oil chemical composition of *Tripleurospermum* species is reported in Table 2. In general, *β*-farnesene (22.5%), *β*-sesquiphellandrene (17.9%) [21], neryl acetate (12.8%), *(E)-β*-farnesene (12.5%), phytol (12.1%) [24], globulol (13.5%) and sesquiphellandrene (9.3%) [25]; *(Z)-β*-farnesene (18.2%), 1-epi-cubenol (16.1%) [22] were reported as common volatiles compounds while dioxoipran derivative [26] and flavonoids were detected as non-volatile constituents in the extracts [27]. Fatty acid compounds of *Tripleurospermum* species are summarized in Table 3.

Table 2. Literature overview on essential oil compounds of *Tripleurospermum* species.

Species	Major Compound	References
T. decipiens (Fisch. & Mey.) Bornm.	*(2Z, 8Z)*-Matricaria ester (57.9- 70.0%) *(2E,8Z)*-Matricaria ester (1.7-8.1%) *(Z)-β*-Farnesene (0.3-7.5%) *(2E,8E)*-Matricaria ester (0.4- 2.3%) *(2Z,8E)*-Matricaria ester (0.5-1.4%) *β*-Sesquiphellandrene (2.7- 10.4%) *p*-Methoxy-*p*-cyclopropylstyrene (18.8%)	[36]
T. disciforme	*(E)-β*-farnesene (15.6%); sesquiphellandrene (15.4%) viridifloren (13.0-41.2%); *trans*-matricaria ester (31.9-51.0%); *trans*-matrix ester (39.9%); *cis*-calamene (22.9%)	[20]
T. disciforme	*β*-Farnesene (22.5%) ; *β*-sesquiphellandrene (17.9%) *p*-methoxy *β*-cyclopropylstyrene (16.6 %)	[21]
T. corymbosum E. Hossain	*(Z)-β*-Farnesene (18.2%); 1-epi-cubenol (16.1%)	[22]
T. insularum Inceer & Hay.-Ayaz	Globulol (13.5%); *β*- sesquiphellandrene (9.3%)	[25]
T. parviflorum	*β*-Farnesene (18.4%); *β*- sesquiphellandrene (10.1%)	[23]
T. inodorum (L.) Sch. Bip	The flower oil; artemisia ketone (14.4%), terpinen-4-ol (5.5%); The leaf oil; caryophyllene oxide (16.0 %) and phytol (12.1 %); The stem oil; neryl acetate (12.8 %), *(E)-β*-farnesene (12.5 %), phytol (12.1 %)	[24]

Table 3. Literature overview on fatty acid compounds of *Tripleurospermum* species.

Species	Fatty acids	References
T. callosum	Linoleic acid (16.2%), Hexadecanoic acid (17.9%)	[51]
T. callosum	Hexadecanoic acid (6.2%)	[51]
T. parviflorum	Palmitic acid (38.6%) Linoleic acid (20.6%) Oleic acid (9.6 %)	[39]
T. tenuifolium	Palmitic acid (47.6%) Linoleic acid (18.4%) Oleic acid (8.5 %)	[39]
T. decipiens	Pentadecanoic acid (55.2%) Tetracosanoic acid (64.3%)	[37]

T. decipiens is known as "Sarı papatya" and it grows in Northwest, West, Central and South Anatolia in Turkey. In this study, we aimed to reveal the chemical composition of the essential oil and fatty acid content and biological properties of the extracts of *T. decipiens.* The chemical composition of the essential oil and fatty acid was determined by Gas Chromatography (GC) and Gas Chromatography / Mass spectrometry (GC / MS) methods. The extraction of fatty acids from *T. decipiens* has been carried out using the "Fatty Acid Extraction" Kit. Methyl esters of the fatty acids were obtained with Boron trifluoride-methanol solution and analyzed by GC-FID/MS (28). *In vitro* AChE and α-amylase enzyme inhibitions of the extracts were determined by spectrophotometric method with Ellman's and KI/I_2 reagents, respectively. Antimicrobial activities of the extracts were evaluated against *Salmonella enterica* ATCC 14028, *Bacillus subtilis* subsp. *spizizeni* ATCC 6633, *Bacillus subtilis* ATCC 19654, *Staphylococcus aureus* ATCC 6538, *Klebsiella aerogenes* ATCC 13048, *Candida parapsilosis* ATCC 22019 by microdilution method.

The aim of this paper was to give characterization of the volatile oil and fatty acid compositions and biological properties of the apolar and polar extracts of *T. decipiens*. To the best of our knowledge, there is no previous report on anti-microbial, anti-acetylcholinesterase, anti-amylase activities for *T. decipiens.*

2. RESULTS AND DISCUSSION

2.1. Chemical composition of the volatiles of *Tripleurospermum decipiens*

In the present study, we investigated the volatiles composition from aerial parts of *T. decipiens* with MSD-SPME technique in tandem with GC-MS/FID techniques. The list of the volatile compounds identified in *T. decipiens* with their relative retention indices and relative percentages is reported in Table 4.

Forty-eight volatile compounds have been identified in aerial parts of *T. decipiens*, representing for 94.3% of the total volatiles. The main volatile compounds were presented by isomeric polyacetylenic constituents representing 61.7% of total volatiles. The main constituents were found to be as *(2Z,8Z)*-matricaria ester (31.4%), *(2E,8Z)*-matricaria ester (12.3%), *(2E,8E)*-matricaria ester (10.9%) and *(2Z,8E)*-matricaria ester (6.7%). The main compound groups detected in the volatiles of *T. decipiens* are presented in Table 5. The oxygenated monoterpenes comprised the second major compound group (9.2%). The main representatives of this group were found to be as linalool (2.4%) and linalyl acetate (1.5%). Modhephene (2.9%), *(Z)-β*-farnesene (0.5%) and *a*-isocomene (0.3%) were determined in the group of the sesquiterpene hydrocarbons (3.7 %). The oxygenated sesquiterpenes (1.7%) were presented by caryophyllene oxide (1.0%), spathulenol (0.4%) and hexahydro-farnesylacetone (0.3%). Aliphatic ketones constituted 5.9% of the total volatiles with 6-methyl-5-hepten-2-one (4.1 %) as the main representative.

Literature overview on essential oil compounds of *Tripleurospermum* species was given in Table 2. The dried flowers of *T. decipiens* from two different populations were investigated for essential oil compositions by Kürkçüoğlu et al. [36]. According to this study, in the flower essential oil *(2Z,8Z)*-matricaria ester (57.9-70.0%), *β*-sesquiphellandrene (2.7-10.4%), *(2E,8Z)*-matricaria ester (1.7-8.1%), *(Z)-β*-farnesene (0.3-7.5%), *(2E,8E)*-matricaria ester (0.4-2.3 %), and *(2Z,8E)*-matricaria ester (0.5-1.4 %) were found as the main components. The differences in essential oil contents of previous publications may be due to edafic factors as well as to different techniques applied for obtaining of the volatiles from the plant materials.

2.2. Fatty acids compositions

Fatty acid composition of *T. decipiens* was given in Table 6. Gas-chromatographic analysis resulted with eight compounds, representing 100.0 % of fatty acids. Palmitic (42.1%), nonadecanoic (19.7 %) and linoleic (19.0%) acids were found as main ones in *T. decipiens*.

In literature there are several reports about fatty acid composition of *Tripleurospermum* species. Palmitic and linoleic acids generally found as main compounds of different *Tripleurospermum* species (Table 3). Ayaz et al. reported about fatty acid composition of the plant. Pentadecanoic acid (55.2%) and tetracosanoic acid (64.3 %) were reported for *T. decipiens* [37].

2.3. Antimicrobial activity (MIC, µg/mL)

The effects of *T. decipiens* extracts and standards are given in Table 7 against *S. enterica* ATCC 14028, *K. aerogenes* ATCC 13048, *B. subtilis subsp. spizizeni* ATCC 6633, *B. subtilis* ATCC 19654, *S. aureus* ATCC 6538, and *C. parapsilosis* ATCC® 22019. As can be seen from Table 7, *n*-hexane extract more active with MIC =156.25 µg/mL against *B. subtilis* ATCC 19654 and *C. parapsilosis* ATCC® 22019 than the other pathogens. The methanol extract was observed active against *S. enterica* ATCC 14028, *K. aerogenes* ATCC 13048 and *C. parapsilosis* ATCC 22019 with MIC= 312.5 value µg/mL.

Tripleurospermum species have reported on anti-microbial, anti-acetylcholinesterase, analgesic, antioxidant, anti-inflammatory, antiproliferative, anti-ulcer, and cytotoxic activities [25; 27; 38-43]. Generally, there are some reports about antimicrobial activity genus on *Tripleurospermum*. The extract of *T. disciforme* aerial parts was examined against *S. epidermidis, S. aureus, B. cereus and Pseudomonas aeruginosa*. The extract (64 mg/mL) exhibited antimicrobial effects only against *S. aureus* (14 mm) and *S. epidermidis* (12mm) [27]. In another study, antimicrobial activity of *T. disciforme* essential oil was investigated against *Serratia marcescens* PTCC 1330, *Enterobacter aerogenes* PTCC 10009, *Proteus vulgaris* (Lio), *Citrobacter amalonaticus* (Lio), *B. cereus* ATCC 7064, *B. megaterium* PTCC 1672, *S. subrogation* (Lio), and *S. aureus* ATCC 6633. The lowest MIC value (4 µL/mL) of *T. disciforme* oil was observed against *S. aureus* ATCC 6633 and *B. cereus* ATCC 7064 [21]. The ethanol extract of *T. conoclinium* Boiss. Ball. (aerial parts) was tested against *Mycobacterium tuberculosis* H37Rv with MIC ≤100 µg/mL [44]. The antimicrobial activities of *n*-hexane, methanol, ethanol, ethyl acetate

and water extracts of *T. parviflorum* (Willd.) Pobed. were reported against *P. aeroginosa* ATCC 27853, *E. cloacae* ATCC 13047, *E. faecalis* ATCC 29212, *S. aureus* ATCC 6538P, *S. aureus* ATCC 29213, *E. coli* ATCC 29998, *E. coli* ATCC 25922, *E. coli* ATCC 11230, and *C. albicans* ATCC 10239 [45].

Table 4. Chemical composition of the volatiles of *Tripleurospermum decipiens*.

No	RRI	Compound	%
1	1335	*(E)*-2-Heptenal	0.7
2	1348	6-Methyl-5-hepten-2-one	4.1
3	1400	Nonanal	1.7
4	1400	Tetradecane	0.6
5	1415	4,8-Dimethyl-1,3,7-nonatriene	0.1
6	1416	3-Octen-2-one	0.5
7	1441	*(E)*-2-Octenal	0.8
8	1452	1-Octen-3-ol	0.8
9	1474	Dihydromyrcenol, 6,10-	0.3
10	1479	*(E,Z)*-2,4-Heptadienal	0.6
11	1496	2-Ethyl hexanol	0.8
12	1506	Decanal	1.2
13	1507	*(E,E)*-2,4-Heptadienal	0.6
14	1532	Camphor	0.3
15	1532	*a*-Isocomene	0.3
16	1553	Linalool	2.4
17	1565	Linalyl acetate	1.5
18	1525	Modhephene	2.9
19	1573	*(E,E)*-3,5-Octadien-2-one	0.4
20	1602	6-Methyl-3,5-heptadien-2-one	0.9
21	1611	Terpinen-4-ol	0.3
22	1655	*(E)*-2-Decenal	0.9
23	1664	Nonanol	0.3
24	1668	*(Z)*-β-Farnesene	0.5
25	1694	Neral	0.3
26	1709	*a*-Terpinyl acetate	0.4
27	1715	*(E,E)*-2,4-Nonadienal	0.2
28	1719	Borneol	0.2
29	1740	Geranial	0.6
30	1764	*(E)*-2-Undecenal	0.5
31	1808	Nerol	0.3
32	1827	*(E,E)*-2,4-Decadienal	0.3
33	1857	Geraniol	0.8
34	1868	*(E)*-Geranyl acetone	0.4
35	1871	Neryl isovalerate	0.3
36	1873	1-Isobutyl 4-isopropyl 3-isopropyl-2,2-dimethyl succinate (= 2,2,4-trimethyl-3-carboxyisopropyl-isobutyl pentanoate)	0.6
37	2008	Caryophyllene oxide	1.0
38	2015	*trans-β*-Ionone-5,6-epoxide	0.6
39	2131	Hexahydro-farnesylacetone	0.3
40	2144	Spathulenol	0.4
41	2192	Nonanoic acid	0.8
42	2210	(2E,8Z)- Matricaria ester	12.3
43	2221	Isocarvacrol (=*4-Isopropyl-2-methyl phenol*)	0.5
44	2227	8Z-2,3-Dihydromatricaria ester	0.4
45	2298	Decanoic acid	0.6
46	2291	*(2E,8E)*-Matricaria ester	10.9
47	2336	*(2Z,8Z)*-Matricaria ester	31.4
48	2435	(2Z,8E)-Matricaria ester	6.7
		Total	94.3

RRI, relative retention index experimentally calculated based on retention of *n*-alkanes; %, calculated from flame ionization detector data. The data are presented as relative % by weight for each component detected in *T. decipiens*.

There is no previous biological studies for *T. decipiens* in the literature, although considerable reports conducted due to the widespread traditional use of *Tripleurospermum* species. To the best of our knowledge, the present work is the first report on antimicrobial activity of *T. decipiens* extracts.

Table 5. Distribution of the main compound groups detected in the volatiles of *Tripleurospermum decipiens*.

Compound group	%
Aliphatic aldehydes	7.5
Aliphatic ketones	5.9
Oxygenated monoterpenes	9.2
Sesquiterpene hydrocarbons	3.7
Oxygenated sesquiterpenes	1.7
Isomeric polyacetylenic constituents	61.7
Fatty acids	1.4

Table 6. Fatty acid composition of *Tripleurospermum decipiens.*

No	RRI[a]	RRI[b]	Compound	%[c]
1	1600	1604[d]	10:0 (Methyl caprate)	2.1
2	1810	1807[e]	12:0 (Methyl laurate)	1.1
3	2018	2014[f]	14:0 (Methyl myristate)	3.5
4	2223	2223[13]	16:0 (Methyl palmitate)	**42.1**
5	2436	2445[14]	18:0 (Methyl stearate)	6.7
6	2468	2472[14]	18:1ω9 (Methyl oleate)	5.8
7	2509	2502[15]	18:2ω6 (Methyl linoleate)	**19.0**
8	2512	2513[14]	19:0 (Methyl nonadecanoate)	19.7
			Total:	100.0

RRIa: relative retention index experimentally calculated based on retention of n-alkanes, **RRIb**: relative retention index reported in literature for constituents analyzed on polar column; c The data are presented as relative % by weight for each component detected in Tripleurospermum decipiens; %, calculated from flame ionization detector data; d [52], e [53]- f [54]

2.4. α-Amylase inhibitory activity

In the present work we evaluated the inhibitory potential of *T. decipiens* extracts towards α-amylase, key enzyme in carbonhydrate methabolism. The evaluation was carried out *in vitro* using microplate titer assay.

α−Amylase enzyme inhibition results for *T. decipiens n*-hexane and methanol extracts are given in Table 8. The methanol extract inhibited α-amylase (55.6%, IC_{50} 7.8 mg/mL) better than *n*-hexane extract (32.9%). Comparison of the obtained IC_{50} values demonstrated that the crude methanol extract of *T. decipiens* had weaker activity than pure standard inhibitor (acarbose), these results have been evaluated as promising items for continuation of investigations. There is no previous report about α-amylase inhibitory activity of *T. decipiens.*

2.5. Acetylcholinesterase inhibitory activity

In the present work we evaluated the inhibitory potential of *T. decipiens* extracts towards acetylcholinesterase, key enzyme in pathogenesis of Alzheimer's disease. The evaluation was carried out *in vitro* using microplate titer assay. It has been shown that *n*-hexane extract of *T. decipiens* demonstrated inhibitory effect (IC_{50} 6.8 mg/mL) towards AChE enzyme while no AChE inhibition was observed for the methanol extract (Table 8). Although, comparison of the obtained IC_{50} values demonstrated that the crude hexane extract had weaker activity than pure standard inhibitor (galanthamin), these results have been evaluated as promising items for continuation of investigations. In the literature, it was observed that AChE

activity studies mostly focused on *T. disciforme*. Methanol extract of *T. disciforme* at 5 µg/mL was reported to inhibit AChE enzyme (71.18±4.9%) [40]. Servi et al. reported about anti-AChE activity of the flower essential oil of *T. inodorum* (53.35 % at 20 mg/mL) [24]. To the best of knowledge, there is no previous report on AChE inhibitory activity of *T. decipiens*.

Table 7. Antimicrobial activity of *Tripleurospermum decipiens* extracts (minimum inhibitory concentration, µg/mL).

	Microorganism					
Samples	***S. enterica* ATCC 14028**	***B. subtilis subsp. spizizeni* ATCC 6633**	***B. subtilis* ATCC 19654**	***S. aureus* ATCC 6538**	***K. aerogenes* ATCC 13048**	***C. parapsilosis* ATCC 22019**
TDH	312.5	312.5	156.25	312.5	312.5	156.25
TDM	312.5	625	625	625	312.5	312.5
Cefuroxime	16	2	2	2	64	-
Moxifloxacin	0.125 >	0.125 >	0.125 >	0.125 >	0.125 >	-
Ampicilline	0.25	0.5	0.125 >	2	16	-
Fluconazole	-	-	-	-	-	> 64

TDH: *T. decipiens n*-hexane extract, TDM: *T. decipiens* methanol extract, (-): Not tested

Table 8. Enzyme inhibitory activities of *Tripleurospermum decipiens* extracts.

Assays	**TDH** IC_{50}, mg/mL	**TDM** IC_{50}, mg/mL	**Acarbose** IC_{50}, mg/mL	**Galantamine** IC_{50}, mg/mL
α-Amylase inhibitory activity	>10	7.8	0.08	-
Acetylcholinesterase inhibitory activity	6.8	N/A	-	0.01

TDH: *T. decipiens* n-hexane extract, TDM: *T. decipiens* methanol extract; [a] the extracts were tested in concentration 10 mg/mL; N/A: Not active.

3. CONCLUSION

The present study is the first investigation of *Tripleurospermum decipiens* extracts for anti-microbial, anti-acetylcholinesterase, and anti-α-amylase activities. The *n*-hexane extract of *T. decipiens* has a reasonable potential activity against *B. subtilis* ATCC 19654 and *C. parapsilosis* ATCC 22019 eventhough its antimicrobial activity is not very strong. The extract also shows acetylcholinesterase inhibitory activity.

4. MATERIALS AND METHODS

4.1. Plant material

Aerial parts of *Tripleurospermum decipiens* were collected in Eskişehir province of Turkey (Şelale-Kirazlı road 2nd km, roadside, calcareous soil) on July 02, 2019. The plant material was dried under the shade and identification was performed by Prof. Dr. Y. B. Köse (Anadolu University). The voucher specimen was kept in Anadolu University Herbarium (ESSE 15609).

4.2. Chemicals

5,5-Dithio-bis-(2-nitrobenzoic acid (DTNB), acetylcholinesterase (AChE) from *Electrophorus electricus* (electric eel, Type VI-S, 200–1000 units/mg protein), bovine serum albumin (BSA), acetylthiocholine iodide (ATCI), galanthamine hydrobromide from *Lycoris* sp., α-amylase from porcine pancreas (Type VI-B, ≥10 units/mg solid) and acarbose were purchased from Sigma-Aldrich (St. Louis, MO, USA). Starch-soluble extra pure iodine and potassium iodide were purchased from Merck (Darmstadt, Germany). Sodium phosphate, disodium phosphate, aluminum chloride, ultrapure water, methanol (MeOH), dimethyl sulfoxide (DMSO) were extra pure analytical grade. A C9–C40 n-alkane standard solution was purchased from Fluka (Buchs, Switzerland).

4.3. Preparation of extracts

The dried aerial parts of *T. decipiens* (20 g) were subjected to maceration with *n*-hexane (200 mL× 3) and methanol (200 mL×3) respectively at room temperature for 24 h. The solvents were evaporated *in vacuo*. The dried extracts were kept at 4°C before biological activities tests.

4.4. Extraction of volatiles with microsteam distillation-solid phase microextraction (MSD-SPME)

The extraction of the volatiles was carried out from *T. decipiens* aerial parts using MSD-SMPE technique [29]. The ground aerial parts of the plant material (0.5 g) were put in 25 mL round bottom flask, and 3 mL of water was added. The flask was fitted with a Claisen distillation head with plug and a condenser set up for refluxing rather than distillation. The threaded plug was used for SPME fiber assembly. A manual SPME holder and Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) fiber "blue type" (65 µm) were used for the extraction of volatiles. Previously, the fiber was conditioned at 250 °C for 10 min before the experiment. Different extraction periods were applied to optimize experiment conditions. The extraction time for the volatiles was 3 min. After trapping the volatiles were subsequently subjected to GC-MS/FID analyses. Thermal desorption of analytes from the fiber coating was performed by injection of the fiber in the injection port (at 250°C) for 5 min.

4.5. Lipid extraction and fatty acid derivatization

The Lipid Extraction Kit used for the extraction of the total lipids from *T. decipiens.* According to protocol, 0.15 g mill-ground plant material was treated with a 3 mL solvent containing chloroform: methanol (2:1). After homogenizing and vortexing of mixture, 0.5 mL of an aqueous buffer of the kit was added, and the sample was mixed by a vortex again. Subsequently, the extraction solution was poured into a syringe system containing a filter. The eluted solvent contained the chloroform phase with total lipids. Then, aliquot 200 µL of the total lipids were dried under a stream of nitrogen for subsequent transesterification. After drying, 1 mL of BF_3-MeOH solution and 0.3 mL of *n*-hexane were added. The mixture was heated at 95°C for one hour under reflux. Then, 1 mL of *n*-hexane and 1 mL of distilled water were added. The mixture was vortexed and centrifuged at 500 × g for 5 min. The top hexane layer was transferred into a vial and then injected into the GC-MS/FID system.

4.6. Gas chromatography analysis

GC-MS analysis was examined by an Agilent 6890N GC and Agilent 5975 GC/MSD systems (Agilent Technologies, USA). HP-Innowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness, Agilent, USA) was used with a helium carrier gas at 0.8 mL/min as reported previously [29].

4.6.1. Identification of the volatile constituents

The volatile constituents and fatty acid methyl esters were determined by co-injection with standards. In addition, compound identities were confirmed by comparison of their mass spectra with those in the Wiley GC/MS Library (Wiley, NY, USA), MassFinder software 4.0 (Dr. Hochmuth Scientific Consulting, Hamburg, Germany), Adams Library, and NIST Library. Confirmation was also achieved using the in-house "Başer Library of Essential Oil Constituents" database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions. A C_8–C_{40} n-alkane standard solution was used to spike the samples for the determination of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

4.7. Antimicrobial activity (MIC, µg/mL)

The extracts of *T. decipiens* were tested for an antimicrobial activity against following microbial strains: *S. enterica* ATCC 14028, *K. aerogenes* ATCC 13048, *B. subtilis* subsp. *spizizeni* ATCC 6633, *B. subtilis* ATCC 19654, *S. aureus* ATCC 6538, and *C. parapsilosis* ATCC 22019 according to previously reported microdilution method [30-31]. The extracts were dissolved in DMSO. The antimicrobial activity of the extracts was evaluated in comparison to moxifloxacin, cefuroxime, ampicillin, and fluconazole (obtained from Sanovel Pharm. Ind.). The Minimum Inhibitory Concentration (MIC) values of the extracts was evaluated with a slight modification of broth microdilution method [32-33]. The extracts were diluted two-fold initially with a final concentration range of 2500 to 19.53 µg/mL. Standard antimicrobial drugs ampicillin, cefuroxime, and fluconazole (64-0.125 µg/mL) were prepared within DMSO and water. The positive growth controls (to assess the presence of turbidity) were performed in wells not containing antimicrobial drugs. The microbial

growth was observed by adding 20 µL of resazurin (0.01%) with minor modifications of CLSI standards. A change from blue to pink indicated a reduction of resazurin, so it showed microbial growth. MIC determined as the lowest drug concentration that prevented this color change. The tests were carried out in triplicate for calculation of the mean of MIC.

4.8. Determination of α-amylase inhibition

The antidiabetic activity of *T. decipiens* extracts was determined upon inhibition of the α-amylase enzyme that involved in hydrocarbon's metabolism. The iodine/potassium iodide (I_2/KI) method was used [34]. The extracts stock solutions (10 mg/mL) were prepared in methanol (with 10% DMSO). In the experiment, the enzyme (0.8 U/mL) was prepared in sodium phosphate buffer (20 mM, pH 6.9). The substrate solution (0.05%) was prepared by dissolving of soluble potato starch (10 mg) in 20 mL ultrapure water then boiling for 10 min and cooling to room temperature before use. In the experiment, 20 mM sodium phosphate buffer (pH 6.9) was pipetted in the 96-well flat bottom plates with multichannel automatic pipette (Eppendorf Research® plus, Germany), then 25 µL of sample solution and 50 µL of α-amylase (0.8 U/mL in buffer) were added and incubated for 10 min at 37°C. After incubation, 50 µL of substrate solution was added to the mixture. The mixture was subjected to a second incubation for 10 min at 37°C. The reaction was stopped by addition of 25 µL of HCl solution (1 M). Finally, 100 µL of I_2/KI reagent was added to the wells. The sample blanks contained all reaction reagents and 50 µL of buffer instead of enzyme. The control wells contained all reaction reagents and 25 µL of solvent (instead of the sample solution). The absorbance values were recorded at 630 nm. The percentage inhibition of the α-amylase activity (Inh%) was calculated according to Equation 1. Acarbose (inhibitor of α-amylase) prepared in concentration 0.25 mg/mL was used as the positive control. For determination of IC_{50} values the microdilution tests have been carried out. Minimum of twelve different concentrations have been prepared by means of sequential dilution of the stock solution of the extract were used for calculating the IC_{50} value. In total, twelve diluted solutions of the sample stock solution (10.0; 5.0; 2.5; 1.25; 0.625; 0.312; 0.156; 0.078; 0.039; 0.019; 0.009; 0.0048 mg/mL) were subjected to evaluation for enzyme inhibitory effect. IC_{50} calculations were done by using Sigma Plot 12.0 software. The percentage of inhibition was calculated according to Eq. 1:

$$\text{Inhibition } \% = \frac{(Abs_{contr} - Abs_{contr\ blank}) - (Abs_{sample} - Abs_{sample\ blank})}{(Abs_{contr} - Abs_{contr\ blank})} \times 100 \qquad \text{(Eq. 1)}$$

$Abs_{control}$: The absorbance of control; $Abs_{control\ blank}$: The absorbance of blank
Abs_{sample} The absorbance of sample; $Abs_{sample\ blank}$: The absorbance of blank

4.9. Determination of AChE inhibition

Acetylcholinesterase (AChE) inhibition of the extracts was evaluated according to Ellman's method [35] with a slight modification. The extracts stock solutions (10 mg/mL) were prepared in methanol (with 10% DMSO). In experiment, 25 µL of the sample solution, 50 µL of buffer, and 25 µL of AChE (0.22 U/mL) solution were added into the 96-well (flat-bottom) plates, and incubated at 25°C for 15 min. After that, 125 µL of Ellman's reagent, DTNB (3.0 mM) and 25 µL of the substrate ATCI (15 mM) were added. The mixture was allowed to stand at 25°C for 15 min, and the absorbance was recorded at 412 nm by a microplate reader (Biotek Powerwave XS, USA). Galanthamine solution was prepared in concentration 0.02 mg/mL and used as the positive control. Similarly, a blank control was prepared by adding the sample solution to all reaction reagents and added 25 µL of the buffer instead of the enzyme. The control wells contained all the reagents without the sample. For determination of IC_{50} values the microdilution tests have been carried out. The percentage of inhibition was calculated according to Eq. 2. The mean standard error (±SEM) was used for evaluation of data.

$$\text{Inh } \% = \frac{Abs_{contr} - Abs_{sample}}{Abs_{contr}} \times 100 \qquad \text{(Eq. 2)}$$

where, $Abs_{control}$ and Abs_{sample} are the absorbance values of the control and the sample.

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