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Antimicrobial and lipase inhibition of essential oil and solvent extracts of *Cota tinctoria* var. *tinctoria* and characterization of the essential oil

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Abstract: The essential oil (EO) of *Cota tinctoria* var. *tinctoria* was analyzed using GC-FID / MS. A total of 51 compounds were determined from this taxon, accounting for 99.79% in hydrodistillation. Monoterpenes were the primary chemical class for the volatile organic compounds in the EO (36.1%, 13 compounds). Borneol (18.1%), camphor (14.9%), and ***β***-pinene (11.3%) were the major components in the EO of *C. tinctoria* var. *tinctoria.* The antimicrobial activities of EO and *n*-hexane, acetonitrile, methanol, and water solvent extracts of the taxon were screened in vitro against ten microorganisms. The EO yielded the best activity (15 mm, 372.5 MIC, 59600 µg/µL) against *Mycobacterium smegmatis*. The acetonitrile extract was the most active against the *Staphylococcus aureus* and *Bacillus cereus* with 274 µg/mL MIC value. IC_{50} values for the lipase enzyme inhibitory activity of EO and solvent extracts (*n*-hexane, acetonitrile, methanol, and water) were found to be 59.80 ± 4.3285 µg/mL 68.28 ± 3.1215 µg/mL, 52.60 ± 3.7526 µg/mL, 48.73 ± 2.8265 µg/mL, and 99.50 ± 5.5678 µg/mL, respectively.

Key words: Antimicrobial, *Cota tinctoria* var. *tinctoria*, GC-FID/MS, lipase inhibition, volatile compounds.

1. Introduction

In the Flora of Turkey, *Cota* J. Gay was classified as a section in the genus *Anthemis* L. together with the sect. *Anthemis* and sect. *Maruta* (Cass.) Griseb. [1]. The sect. *Cota* was accepted as a genus after changing generic and infrageneric concepts [2-4]. Thus, the studied taxon is formerly known as *Anthemis tinctoria* var. *tinctoria* which has already been reported as synonym of *Cota tinctoria* var. *tinctoria* [5]. All *Cota* taxa are mainly distributed in North Africa, Caucasia, Europe (except north of Europe), and Central Asia [6,7]. The genus *Cota* includes up to 17 species and 20 taxa, and 9 of them are endemics [8].

Several *Anthemis* species have been used as a traditional medicine to treat psoriasis (*A. cotula*) [9], diaphoretic, carminative (*A. nobilis*) [10], abdominal pain, kidney disease (*A. cretica*) [11]. Infusions and decoctions of *A. creatica* ssp. *tenuiloba* and *A. austriaca* have been reported to be used against abdominal pain, hemorrhoids, and colds [12]. It has been reported that *A. wiedemanniana* plant has sedative, antispasmodic effects and is used in urinary problems [13]. It is known that the decoction prepared from the flowers of *C. tinctoria* var. *tinctoria* is used for antidiabetic and antispasmodic purposes [13]. Some *Anthemis* species showed various biological activities such as antioxidant [14], antiproliferative [15], antidiabetic [16], antiprotozoal [17], antispasmodic [18].

According to the literature, even though Anthemideae is one of the most phytochemically investigated tribes of the Compositae, only essential oils of *Anthemis nobilis* L. [19], *A. aciphylla* Boiss. var. *discoidea* Boiss. [20], *A. altissima* L., *A. chia* L., *A. tomentosa* L., *A. weneri* L. ssp. *weneri* Stoj. & Acht., *A. auriculata* Boiss., *A. melanolepis* Boiss., *A. cotula* L. and *A. tinctoria* L. var. *parnassica* [21], *A. hyalina* DC. [22], *A. tinctoria* L. [23], *A. cretica* L. ssp. *leucanthemoides* (Boiss.) Grierson [24], *A. ruthenica* M.B. and *A. arvensis* L. [25], *A. wiedemaniana* Fish. Et. Mey. [26], *A. melampodina* auct. Non Delili [27], *A. carpatica* Willd. [28], *A. xylopoda* O. Schwarz [29], *A. montana* L. ssp. *carpatica* [30], *A. altissima* L. [31], *A. triumfetti* (L.) DC. [32], *A. altissima* L. var. *altissima* [33], *A. cretica* subsp. *messanensis* (Brullo) Giardina & Raimondo, *Anthemis arvensis* L. subsp. *arvensis*, and *A. cretica* subsp. *columnae* (Ten.) Freze´n [34], *A. dipsacea*, *A. pectinata* var. *pectinata* and *A. pseudocotula* [19] have been investigated so far.

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Obesity is a long-term problem which causes a series of psychological and physiological problems. Many kinds of chronic metabolic diseases are caused by obesity. Porcine pancreatic lipase (PPL) inhibitors have been of interest in obesity treatment research in recent years. PPL inhibitors from natural products have a wide range of sources and low toxicity and have therefore attracted interest. Because of these advantages, they could lead to new health products in the pharmaceutical industry [35].

To the best of our knowledge, total phenolic and flavonoid contents, antioxidant activity, butyrylcholinesterase, acetylcholinesterase, and tyrosinase enzyme inhibition of methanol extract of *C. tinctoria* var. *tinctoria* were mentioned [36]. Cytotoxic activity of ethanol extract of *C. tinctoria* flowers was investigated [37]. In another study, phenolic constituents, cytotoxic activity, and dyeing properties for the ethanol and aqueous extracts of stem, flower, and root of the plant were studied [38]. However, antimicrobial activity and lipase enzyme inhibition for the essential oil and solvent extracts (*n*-hexane, acetonitrile, methanol, and water) of *C. tinctoria* var. *tinctoria* were not studied. Hence, we focused our study on the essential oil composition with GC and GC-MS analysis, antimicrobial and lipase enzyme inhibition activities of the EO and the solvent extracts of *C. tinctoria* var. *tinctoria* were screened in vitro against ten microorganisms and porcine pancreatic lipase, respectively.

2. Materials and methods

2.1. Plant materials

Aerial part of *C. tinctoria* var. *tinctoria* (160 g, wet) was harvested from Şiran, Gümüşhane at a height of 1650 m in May 2018. The plant was collected by Prof. Nurettin Yaylı and authenticated by Prof. Salih Terzioğlu. Voucher specimen (KATO: 19258) has been deposited in the Herbarium of the Faculty of Forestry, Karadeniz Technical University, Turkey.

2.2. Chemicals and reagents

All solvents (methanol, acetonitrile, and *n*-hexane) and chemicals (Tris-HCl and *p*-nitrophenyl butyrate) used were purchased from Sigma-Aldrich in analytical grade.

2.3. Hydrodistillation procedure for the obtaining of EO

The aerial part of *C. tinctoria* var. *tinctoria* (106 g, dry) was grounded with plant mill into small pieces and then hydrodistillated (HD) with a modified Clevenger-type apparatus with cooling bath (–15 °C, 3 h), yield (*v/w*): 0.079%. After the HD, EO was extracted with *n*-hexane (0.5 mL) and dried over anhydrous Na_2SO_4 and stored in a dark glass bottle in the refrigerator at 4 °C prior to the GC-MS analysis.

2.4. Solvent extractions (*n*-hexane, acetonitrile, methanol, and water solvents) of *C. tinctoria* var. *tinctoria*

The aerial parts of the plant (40 g, dry) were blended into small pieces. Blended material (5 g, each) was extracted (25 mL × 3; 12 h each) using maceration method at room temperature with analytical grade *n*-hexane, acetonitrile, methanol, and water solvents in flasks (50 mL) separately. After the suction filtration, the same extracts were combined and solvents were evaporated or lyophilized to yield crude *n*-hexane (0.0722 g), acetonitrile (0.0439), methanol (0.5234 g), and water extract (0.0435 g) [39, 40].

2.5. Gas chromatography-mass spectrometry (GC-FID/MS)

GC-MS analysis of the EO was carried out by a Shimadzu QP2010 ultra, having Shimadzu 2010 plus FID, PAL AOC-5000 plus auto sampler and Shimadzu Class-5000 Chromatography Workstation software. Restek Rxi-5MS capillary column (30 mm × 0.25 mm × 0.25 μm) (USA) was used for the analysis. Sample (1 μL, in HPLC grade *n*-hexane) injection was performed in split mode (1:30) at 230 °C. Initial column temperature was 60 °C for 2 min, then increased to 240 °C with a 3 °C/min heating ramp. The final temperature for the oven was held at 250 °C for 4 min. Helium (99.999%) was the carrier gas with 1 mL/min flow rate. MS detection was implemented in electronic impact mode (EI, 70 eV, and scan mode 40-450 *m/z*). Sample was analyzed and mean was reported.

2.6. Identification of volatile constituents

RI values of the volatile components in EO of *C. tinctoria* var. *tinctoria* were determined by Kovats method (Table 1) [39-44]. Volatile compounds of the EO were identified by comparisons of RI values with those reported in the literature RI [45-75] and MS data matching mass spectral libraries (NIST, Wiley7NL, FFNSC1.2, and W9N11).

2.7. Antimicrobial activity assessment (agar-well diffusion method)

All test microorganisms: *Escherichia coli* ATCC35218, *Yersinia pseudotuberculosis* ATCC911, *Pseudomonas aeruginosa* ATCC43288, *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* 709 Roma, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC60193, *Candida tropicalis* ATCC 13803; and *Saccharomyces cerevisiae* RSKK 251 were obtained from the Refik Saydam Institute of Hygiene and Public Health (Ankara, Turkey). The adapted antimicrobial screening test (agar-well diffusion method) was used earlier [76-77]. Each tested microorganism

was suspended in Brain Heart Infusion (BHI) and diluted approximately 10^6 colony-forming units (per mL), which were "flood-inoculated" onto the surface of BHI agar and Sabouraud Dextrose Agar (SDA), then dried. SDA was used for *C. albicans*. Wells (5 mm diameter) were cut from the agar, and the extracts (100 µL, each) were delivered into the wells. The plates were incubated (35 °C, 18 h) and antimicrobial activity was evaluated by measuring the inhibition zone against the test organism. The EO dissolved in *n*-hexane, and other solvent extracts (acetonitrile, methanol, and water solvents) were dissolved in dimethyl sulphoxide to prepare stock solutions (43.500–523.400 µg/mL). *n*-Hexane and dimethyl sulphoxide were used as solvent control with dilution of 1:2. Ampicillin, streptomycin, and fluconazole were used as positive controls at 10 µg/mL, 10 µg/mL, and 5 µg/mL concentrations, respectively (Table 2).

Table 1. Essential oil components from aerial parts of *C. tinctoria* var. *tinctoria.*

Compounds	RI*	RI[a]	(%)[c]
Pentanal	732 [52]	733	0.05
2-Ethyl furan	728 [53]	735	0.08
1-Octene	794 [54]	801	0.76
Hexanal	801 [41]	807	0.97
2*E*-Hexenal	852 [55]	857	0.30
n-Hexanol	863 [41]	868	0.07
1-Nonene	893 [56]	896	0.10
Heptanal	901 [41]	906	0.07
Tricylene	929 [57]	930	0.27
α-Pinene	940 [58]	941	6.35
Camphene	953 [59]	956	10.69
Verbenene	961 [41]	960	0.12
Sabinene	978 [60]	978	0.72
β-Pinene	974 [41]	980	11.26
2-Pentyl furan	990 [61]	993	0.58
Octanal	1003 [56]	1003	0.18
α-Phellandrene	1006 [59]	1008	0.07
α-Terpinene	1018 [62]	1020	0.56
o-Cymene	1022 [41]	1026	0.15
Limonene	1031 [63]	1032	3.92
Eucalyptol	1034 [63]	1036	6.67
Z-*β*-Ocimene	1046 [64]	1047	0.76
γ-Terpinene	1060 [51]	1061	1.06
α-Terpinolene	1086 [41]	1091	0.18
Linalool	1095 [41]	1097	0.59
Nonanal	1100 [41]	1103	0.84
1-Terpineol	1130 [41]	1133	0.08
Verbenol	1130 [41]	1134	0.14
Camphor	1146 [65]	1150	14.90
2*E*-Nonenal	1157 [66]	1159	0.18
Borneol	1169 [67]	1171	18.11
α-Terpineol	1191[68]	1194	0.83
β-Cyclocitral	1220 [31]	1224	0.03
Perillaldehyde	1263 [69]	1268	0.09

Tablo 1. (Continued).

Bicycloelemene	1330 [70]	1336	0.06
Neryl acetate	1359 [41]	1361	0.12
α-Copaene	1382 [71]	1381	0.31
β-Caryophyllene	1417 [41]	1420	1.31
Neryl acetone	1434 [41]	1438	2.41
α-Humulene	1452 [41]	1458	0.43
Valencene	1489 [72]	1486	6.37
β-Bisabolene	1505 [41]	1510	2.24
β-Curcumene	1514 [41]	1519	0.04
Spathulenol	1577 [41]	1581	1.40
Guaiol	1601 [41]	1604	2.53
γ-Eudesmol	1635 [73]	1640	0.08
Cubenol	1645 [41]	1649	0.61
α-Bisabolol	1685 [41]	1689	0.01
Pentadecanal	1710 [74]	1710	0.04
Palmitic acid	1966 [75]	1962	0.00
Tricosane	2300 [41]	2296	0.15
Chemical classes	%[c]	NC[d]	
Monoterpenes	36.11	13	
Oxygenated Monoterpen	23.22	7	
Sesquiterpenes	10.75	7	
Oxygenated Sesquiterpen	4.62	5	
Terpene related	20.51	2	
Aldehyde	2.69	9	
Alcohol	0.07	1	
Aliphatic hydrocarbons	1.00	3	
Aromatic hydrocarbons	0.65	2	
Esters	0.12	1	
Acids	0.01	1	
Total	99.79	51	

*Literature RI values; [a] Retention index calculated from retention times relative to that of *n*-alkane series (C_6-C_{30}); [b]HD: Hydrodistillation; [e]%: Percentages obtained by FID peak-area normalization; [d]NC: Number of compounds.

2.8. Agar dilution minimum inhibitory concentration (MIC) assay

After the antimicrobial properties of the EO, *n*-hexane, acetonitrile, methanol, and water extracts of *C. tinctoria* var. *tinctoria* were investigated quantitatively (Table 2), MIC values (µg/mL) were determined [76-77]. The antibacterial and antifungal assays were carried out in Mueller–Hinton broth (MH) (pH 7.3) and buffered yeast nitrogen base (Difco, Detroit, MI) (pH 7.0), respectively. The microdilution test plates were incubated (35 °C, 18 h). Brain heart infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis*, and incubated at 35 °C (48–72 h). The MIC was the lowest concentration that showed no growth. Ampicillin (10 mg/mL), streptomycin (10 mg/mL), and fluconazole (5 mg/mL) were used as standard, respectively. *n*-Hexane and dimethyl sulphoxide were used as solvent control with dilution of 1:10 [39, 76-77].

2.9. Lipase inhibitory effect assay (PPL)

Lipase inhibitory assay for the EO and solvent extracts (*n*-hexane, acetonitrile, methanol, and water) of *C. tinctoria* var. *tinctoria* were studied with the modified method using *p*-nitrophenyl butyrate (*p*-NPB) as substrate [39]. All extracts (25, 100,

Table 2. Antimicrobial activity of the EO and solvent extracts of *C. tinctoria* var. *tinctoria.*

Sample extracts	Const. (µg/mL)		Microorganisms, inhibition zone (mm), and minimal inhibition concentration (MIC, µg/mL)									
			Gram (–)			Gram (+)			No Gr.	Fungi		
			Ec.	*Yp.*	*Pa.*	*Ef.*	*Sa.*	*Bc.*	*Ms.*	*Ca.*	*Ct.*	*Sc.*
EO	59600	mm	-	8	-	-	6	10	15	8	8	10
		MIC	-	2980	-	-	2980	1490	372.5	2980	2980	1490
n-Hexane	72200	mm	-	-	-	-	6	-	-			
		MIC	-	-	-	-	3610	-	-	-	-	-
Acetonitrile	43900	mm	-	-	-	-	14	15	12	6	6	6
		MIC	-	-	-	-	274	274	548	2195	2195	2195
Methanol	52340	mm	-	-	-	-	14	12	12			
		MIC	-	-	-	-	3271	6542	6542	-	-	-
Water	43500	mm	-	-	-	-	-	-	10			
		MIC	-	-	-	-	-	-	1087	-	-	-
Amp.	10	mm	10	10	18	10	10	15				
		MIC	10	18	128	35	10	15				
Strep.	10	mm							35			
		MIC							4			
Flu	5	mm								25	25	25
		MIC								<8	<8	<8

Ec*: Escherichia coli*, Yp: *Yersinia pseudotuberculosis*, Pa: *Pseudomonas aeruginosa*, Sa: *Staphylococcus aureus*, Ef: *Enterococcus faecalis*, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis*, Ca: *Candida albicans*, Sc: *Saccharomyces cerevisiae*, Ct: *Candida tropicalis*, Amp.: Ampicillin, Strep.: Streptomycin, Flu.: Fluconazole, (-): no activity of test concentrations

200, and 400 µg/mL concentrations) were dissolved with buffer solution (0.1 M Tris-HCl, pH = 8.0) and 0.1% DMSO. Orlistat was used as positive control and prepared as 6.25, 12.5, 25, 50, and 100 µg/mL concentration solutions. The experimental method was designed with A, B, C, and D wells; A: 90 enzyme solution [(Crude porcine PL type II) - (200 units/mL)], 5 µL substrate solution (10 mM p-NPB in acetonitrile); 5 µL buffer solution (0.1 M Tris-HCl buffer, pH = 8.0); B: 90 µL enzyme solution, 10 µL buffer solution; C: 90 µL enzyme, 5 µL sample solution, 5 µL substrate solution; and D: 90 µL enzyme solution], 5 µL sample solution, 5 µL buffer solution. The plates were incubated at 37 °C (15 min) then substrate solution (10 mM *p*-NPB in acetonitrile) was added to each related well which were incubated again at 37 °C (15 min). The absorbance of the solutions was observed at 405 nm in a 96-well microplate using a SpetrostarNano-BMG LABTECH spectrophotometer. Experiments were carried out in triplicate. Results were stated as mean ± standard deviation (SD). Statistical significance level was considered $p < 0.05$ [78]. The percentage of PPL inhibition was calculated by the following equation: PPL inhibition (%) = [[(A – B) – (C – D)] / (A–B)] × 100. Finally, IC_{50} values for the PPL were calculated graphically [39].

3. Results and discussion

3.1. EO composition of *C. tinctoria* var. *tinctoria*

Volatile components in the EO of the *C. tinctoria* var. *tinctoria* were analyzed by GC-FID/MS using Rxi-5MS capillary column. Identification of the volatile constituents in EO was made by comparison of RI and MS data with literature [39-75]. The chemical profile of volatiles, the percentage content, and calculated retention indices of the constituents of the taxon are presented in Table 1. Borneol (18.11%), camphor (14.90%), ***β***-pinene (11.26%), camphene (10.69%), eucalyptol (6.67%), valencene (6.37%), and ***α***-pinene (6.35%) were the major compounds in the EO of *C. tinctoria* var. *tinctoria* (Table 1). Monoterpenes (36.11%) and oxygenated monoterpenes (23.22%) were the main constituents of the EO obtained from aerial parts of *C. tinctoria* var. *tinctoria*., and sesquiterpenes (10.75%) and oxygenated sesquiterpenes (4.62 %) were the second major components in the EO of *C. tinctoria* var. *tinctoria*.

In the literature, the essential oil analyses of *C. tinctoria* plant at different times and regions showed differences in their main components and ratios like borneol (16.0%) and spatulenol (16.0%) [79]; 1,8-cineole (7.9%) and β-pinene (7.3 %) [80]; α-eudesmol (10.2 %), and γ-cadinol (8.7%) [81]; and 1,8-cincole (7.9%), and β - pinene (7.3 %) [82]. These results showed the variation for the EO of *C. tinctoria* species in the literature.

A literature review has revealed that phytochemical analysis of *C. tinctoria* var. *tinctoria* had shown lipophilic extract, and GC-MS analysis was mentioned that content was rich in saturated fatty acids [83]. Isolation of 3-glucoside, and 3-rutinoside of patuletin from acetone extract of the leaves of *A. tinctoria* var. *subtinctoria* was reported [84].

In a study, the total flavonoid and phenolic contents, antimicrobial, the antioxidant, antibiofilm activities, and anticholinesterase of *A. stiparum* subsp. *sabulicola* aerial parts methanolic extract and essential oil were reported and 72 constituents (99.02%) were exhibited, and major compounds were determined as germacrene D, *t*-cadinol, camphor, spathulenol, and isoamyl salicylate with percentage of 11.13%, 11.01%, 6.73%, 6.50%, 6.45%, respectively [82]. The volatile constituents of the EOs obtained from the aerial parts of *A. cretica* subsp. *messanensis* (Brullo) Giardina & Raimondo, from the aerial parts of the rock-grown form and the cultivated of *A. arvensis* L. subsp. *arvensis*, and from flowers and leaves of *A. cretica* subsp. *columnae* (Ten.) Frezén were reported.

Torreyol (85.4 %) from *A. arvensis* subsp. *arvensis*, (*E*)-chrysanthenyl acetate (28.8%) from *A. cretica* subsp. *messanensis*, and 18-cineole (13.3% and 12.2%) from both flower and leaf oils of *A. cretica* subsp. *columnae* were reported as main constituents [34]. The essential oil analysis of *A. fungosa* and its antioxidant (IC_{50} = 3000 ± 8.3 µg/mL, compared with the standard, quercetin, with an IC_{50} of 33.3 ± 1.3 µg/mL) and antimicrobial activities against nine microorganisms (six bacteria and three fungi) were found to have strong antimicrobial activity (MIC = 32 µg/mL) against *K. bacteria*, *S. epide*rmidis, and all of the tested fungi [85]. The hepatoprotective and antiinflammatory activities of the methanolic extract of *A. scrobicularis* herbs were reported; *A. scrobicularis* was toxicologically safe when orally taken and possessed very important hepatoprotective and antiinflammatory activities and it had the potential to be used in inflammatory and hepatic diseases [86]. As for the antimicrobial and antioxidant activities of methanol extracts of *A. cretica* subsp. *argaea* and *A.* fumariifolia, they showed 59.10% and 55.41% inhibition against linoleic acid oxidation, respectively. The study demonstrated that the methanol extracts of *A. fumariifolia* and *A. cretica* subsp. *argaea* had strong antibacterial activity against many tested bacteria (14 mm and 15 mm inhibition zone against *B. cereus*) [87].

The literature comparison of this study showed that similar compounds were found at different rates. However, more terpene components (74.70%) were characterized in this work. In addition, borneol was detected as major compound in the EO of the *C. tinctoria* var. *tinctoria* that may be used as taxonomical marker for the future classification of the *C. tinctoria* var. *tinctoria*. The variations in the volatile organic compounds on aerial parts of *C. tinctoria* var. *tinctoria* with other species may be due to environmental and analysis conditions. Thus, it could be pointed out that qualitative and quantitative results of this study were quite different from the previous reports.

3.1. Antimicrobial activity of EO and solvent extracts

The antimicrobial properties of the EO, *n*-hexane, acetonitrile, methanol, and aqueous extracts of *C. tinctoria* var. *tinctoria* were tested by an in vitro agar-well diffusion method using *P. aeruginosa*, *Y. pseudotuberculosis*, *E. coli*, *E. faecalis*, *B. cereus*, *S. aureus*, *M. smegmatis*, *C. tropicalis*, *S. cerevisiae*, and *C. albicans* (Table 2). After the inhibition diameters were observed in mm, the MIC values (µg/mL) were calculated [76-77] (Table 2). EO of *C. tinctoria* var. *tinctoria* was the most active against *M. smegmatis* with 15 mm inhibition (MIC 372.5 µg/mL). The acetonitrile extract of *C. tinctoria* var. *tinctoria* showed the best zone diameters as 14 mm, 15 mm, and 12 mm against *S. aureus*, *B. cereus*, and *M. smegmatis* with MIC values of 274 µg/mL, 274 µg/mL, and 548 µg/mL, respectively. EO and solvent extracts were more active for the gram-positive bacteria, while EO extract was only active for the gram-negative *Y. pseudotuberculosis*. These antimicrobial activities indicate the presence of active components in these extracts. None of the extracts were active against *E. coli*, *P. aeruginosa*, and *E. faecalis*. *n*-Hexane, methanol, and water extracts of *C. tinctoria* var. *tinctoria* were not active against tested fungi. No correlation was observed between solvent polarities and antimicrobial activity.

In the previous antimicrobial evaluation of *Anthemis species*; essential oils of *A. pseudocotula A. pectinata* var. *pectinata* and *A. dipsacea* were reported against eight microorganisms but did not affect the growth of *E. faecalis*, *Enterobacter cloacae*, *Salmonella thyphimurium*, and *Staphylococcus epidermidis*. When compared with standard antibiotics such as ceftazidime, sulbactam, ampicillin, and nystatin; the essential oils of *A. pseudocotula*, *A. pectinata* var. *pectinata*, and *A. dipsacea* at a concentration of 20 µL/disc had inhibitory effect on *E. coli*, *P. aeruginosa*, and *S. aureus* [19]. EO of *A. cretica* subsp. *messanensis* showed quite a good antimicrobial activity towards *E. coli*, *Streptococcus faecalis*, and *S. epidermidis* with MIC values of 25 µg/mL, 25 µg/mL, and 12.5 µg/mL, respectively [34]. Antimicrobial activities of aerial parts methanolic extract and essential oil of *A. stiparum* subsp. *sabulicola* were reported, and methanol extract displayed better antimicrobial activity than EO of *A.* stiparum subsp. *sabulicola*, being active against *S. aureus* and *Bacillus subtilis*, with MIC of 1.56 mg/mL [82]. Methanol extract of *A. fumariifolia and A. cretica* subsp. *argaea* were reported against 13 bacteria and two yeasts. Test results showed that the methanol extract had great potential of antibacterial activity against many bacteria which were tested. The inhibition zones for bacterial strains were found to be in the range of 6–14 mm and 7–15 mm, respectively. Nevertheless, they had no inhibitory effect on *S. cerevisiae* and *C. albicans* [87].

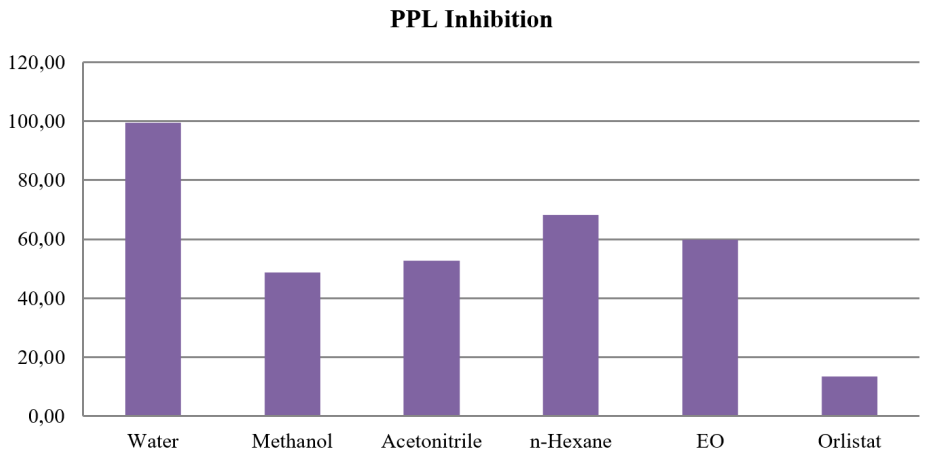

Figure. Porcine pancreatic lipase (PPL) inhibitory effect assay of essential oil and the solvent extracts (water, methanol, acetonitrile, and *n*-hexane) of *C. tinctoria* var. *tinctoria*. PPL inhibitory (IC50 = µg/mL ± SD) (Values are mean ± SD, n = 3), *p ≤ 0.05

3.2. Lipase enzyme activity of EO and solvent extracts

The literature has shown that some of the common natural sources with lipase inhibitors contain active ingredients including polyphenols, flavonoids, terpenoids, and other active ingredients [35]. Terpenoids were the major constituents of the EO; thus, we investigated the lipase activity of EO and solvent extracts of *C. tinctoria* var. *tinctoria*. Essential oil and solvent extracts of *C. tinctoria* var. *tinctoria* were evaluated for lipase enzyme inhibition activities compared with orlistat as positive control (IC_{50}: 13.49 ± 1.2262 µg/mL). The highest activity was found in the methanol extract (IC_{50}: 48.73 ± 2.8265 µg/mL). Afterwards, the best activities were determined with IC_{50} values of 52.60 ± 3.7526 µg/mL, 59.80 ± 4.3285 µg/mL, and 68.28 ± 3.1215 µg/mL in acetonitrile, essential oil, and *n*-hexane, respectively (Figure). The lowest activity was found in the water extract (IC_{50}: 99.5 ± 5.5678). No correlation was observed between solvent polarities and lipase activity. In another study, enzyme inhibitions of ethyl acetate, methanol, and water extracts of *A. chia* L. flowers were reported. MeOH extract of it showed the highest activity in tyrosinase inhibitory and α-amylase activity with 290.22 mg kojic acid equivalents (KAEs)/g extract and 413.66 mg acarbose equivalents (ACEs)/g extract, respectively [88]. In a study, key enzyme inhibitory potentials for the ethyl acetate, methanol, and aqueous extracts obtained from aerial parts of *A. cretica* subsp. *tenuiloba* and *A.* tinctoria var. *pallida* were mentioned. Ethyl acetate and methanol extracts showed potent activity against AChE with the highest activity observed for methanol extract (3.28 ± 0.43 mg GALAE/g) of *A.* tinctoria var. *pallida* and ethyl acetate extract of *A. cretica* subsp. *tenuiloba* (4.68 ± 0.21 mg GALAE/g). In case of BChE inhibitions of extracts; ethyl acetate extract of *A.* tinctoria var. *pallida* (3.48 ± 0.21 mg GALAE/g) and ethyl acetate (2.51 ± 0.34 mg GALAE/g), and methanol (1.15 ± 0.05 mg GALAE/g) extract of *A. cretica* subsp. *tenuiloba* were found to be more promising. Furthermore, enzyme inhibitory effects against α-glucosidase and tyrosinase were given, as well [89].

4. Conclusion

The composition of the EO obtained from aerial part of *C. tinctoria* var. *tinctoria* characterized and lipase enzyme and antimicrobial activities for the EO and solvent extracts were investigated for the first time. Monoterpenes were the main chemical class in the EO. Borneol (18.1%), camphor (14.9%), and **β**-pinene (11.3%) were the major components in the EO of *C. tinctoria* var. *tinctoria*. The EO showed the best activity against *M. smegmatis* (372.5 µg/µL MIC value). The acetonitrile extract was the most active against the *S. aureus* and *B. cereus* (274 µg/mL MIC value). The best activity for the lipase enzyme inhibitory of EO and solvent extracts (*n*-hexane, acetonitrile, methanol, and water) was found to be methanol extract with 48.73 µg/mL IC_{50} value. Therefore, the overall results of observed lipase enzyme and antimicrobial activities suggest that EO and solvent extracts of *C. tinctoria* var. *tinctoria* could be promising for pharmaceutical and other industrial applications. In a further study, Bio-guided activity isolation and purification could be carried out on *C. tinctoria* var. *tinctoria* for the bioavailability.

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