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(+)-(*E*)-Chrysanthenyl Acetate: A Molecule with Interesting Biological Properties Contained in the *Anthemis secundiramea* (Asteraceae) Flowers

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Abstract: *Anthemis secundiramea* is a perennial herb native widespread throughout the Mediterranean basin. The oil obtained from the flowers of this plant has antimicrobial properties against gram-positive and -negative bacteria, and inhibits the biofilm formation. The extract of *A. secundiramea* also has antioxidant activity—increasing the activity of different enzymes (SOD, CAT, and GPx). Surprisingly, in the oil extracted from the flowers, there is a single molecule, called (+)-(*E*)-chrysanthenyl acetate: This makes the *A. secundiramea* flowers extract extremely interesting for future topical, cosmetic, and nutraceutical applications.

Keywords: *Anthemis secundiramea*; essential oils; chrysanthenyl acetate; antibacterial and antioxidant activities

1. Introduction

Anthemis secundiramea Biv. subsp. *secundiramea* ('Camomilla costiera') grows on uncultivated sandy or grassy ground near the sea in the Mediterranean region, blooming between April and June.

It belongs to the *Anthemis secundiramea* group (Asteraceae, Anthemideae) that includes six closely related species: *A. secundiramea* Biv., the Sicilian *Anthemis muricata* (DC.) Guss., the North African *Anthemis glareosa* E.A. Durand and Barratte, *Anthemis confusa* Pomel and *Anthemis ubensis* Pomel and *Anthemis urvilleana* (DC.) R. Fern. endemic to Malta [1].

According to Lo Presti and Oberprieler [2], previously cited species belong to a clade also comprise of *Anthemis maritima*, *Anthemis pedunculata*, *Anthemis cupaniana* (from Sicily), and *Anthemis abylaea* (from Morocco). The essential oil of the aerial parts of *Anthemis secundiramea* Biv. Subsp. *Secundiramea* L., collected from a different Sicilian accession, was previously analyzed, and its antibacterial and antifungal activities were assessed [3]. The growing interest in natural products that can be used as an alternative to synthetic chemicals for pharmaceuticals, food additives, and cosmetic industries induced us, in the frame of our previous reports on the Mediterranean *Anthemis* ssp. [4], to investigate the chemical composition, antimicrobial and antioxidant activities of *A. secundiramea* leaves and flowers. In this study, we report the chemical composition the essential oils from leaves and flowers of *A. secundiramea*, growing wild in Sicily and the antimicrobial properties against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, and the antioxidant activity tested measuring

the activities of antioxidant enzymes in polymorphonuclear cells: Superoxide dismutase (SOD); catalase (CAT) and glutathione peroxidase (GPx).

2. Materials and Methods

2.1. Plant Material

Flowers (110 g) and leaves (270 g) *A. secundiramea* Biv. subsp. *secundiramea* were collected at Addaura, Mondello, Palermo, Sicily (38°11′44′′ N; 13°20′22′′ E; 5 m s.l.m.), at the beginning of May 2019. Typical specimen (PAL 2019/68), has been deposited in the Department STEBICEF, Palermo.

2.2. Isolation of the Essential Oil

The fresh samples, ground with an electric grinder, were subjected to water-distillation for 3 h using Clevenger's apparatus [5]. The oils, yellow and pleasant as smell, were dried with anhydrous sodium sulfate, filtered and stored in the freezer at −20 °C, until the time of analysis. The yield (*w*/*w*) was 1.55% (1.70 g) for the flowers and 0.46% (1.24 g) for the leaves.

2.3. Gas Chromatography-Mass Spectrometry Analysis

EO analysis was performed by using a Perkin-Elmer Sigma 115 system, fitted with a fused silica HP-5 MS capillary column (30 m × 0.25 mm i.d. 0.25 µm film thickness). The oven program was as follows: Temperature was initially kept at 40 °C for 5 min, at a rate of 2 °C/min up to 270 °C.

Flame ionization detection (FID) was performed at 280 °C. GC-MS analysis was performed on an Agilent 6850 Ser. II apparatus, fitted with a fused silica DB-5 capillary column (30 m × 0.25 mm), 0.33 µm film thickness, coupled to an Agilent Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V. GC conditions were as given; transfer line temperature, 295 °C.

Kovat's indices (KI) were determined by using retention times of *n*-alkanes (C_8–C_{40}), and the peaks were identified by comparison with mass spectra and their relative retention index with NIST 11, Wiley 9, FFNSC 2, and Adams libraries [6].

2.4. (+)-Trans-Chrysanthenyl Acetate (1)

$[\alpha]_D^{30}$ + 54.3 °C (*c* 1.13, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 5.37 (1H, m, H-3), 5.00 (1H, t, J = 5.4 Hz, H-7), 2.52 (1H, ddd, $^3J_{1,7}$ = 5.4, $^4J_{1,5}$ = 5.4, $^4J_{1,3}$ = 1.3 Hz, H-1), 2.34 (1H, m, H-5), 2.10 (2H, m, H-4a, H-4b), 2.00 (3H, s, Ac), 1.62 (3H, dt, $^4J_{3.10}$ = 1.9, $^5J_{4,10}$ = 1.9 Hz, Me-10), 1.26 (3H, s, Me-8), 0.91 (3H, s, Me-9).

2.5. Antimicrobial Activity Assays

The presence of antimicrobial molecules in the essential oil extracted from *A. secundiramea* leaves and flowers was detected using Kirby-Bauer assay [7,8] against *Escherichia coli DH5α*, *P. aeruginosa PAOI*, or *S. aureus ATCC 6538P* strains.

Another method to evaluate the antimicrobial activity involved the *E. coli DH5a, P. aeruginosa PAOI*, or *S. aureus ATCC 6538P* strains cell viability counting [9]. Bacterial cells were incubated with both essential oils at 50, 100, 250, and 500 µg/mL concentration. Each experiment was performed in triplicate, and the reported result was an average of three independent experiments. (p value was <0.05).

2.6. Antibiofilm Activity Assay

Crystal Violet dye was used to evaluate the biofilm formation of *P. aeruginosa PAOI.* A 96 wells plate was prepared in which each well contained a final volume of 200 µL; BM2 culture medium was used, the negative control contained only bacterial cells and medium, the other samples contained cells and essential oil [25, 50, 100 µg/mL]. The plate was incubated at 37 °C for 36 h. Plates were air-dried for 45 min, and each well was stained with 200 µl of 1% crystal violet solution for 45 min. The quantitative analysis of biofilm production was performed by adding 200 µL ethanol-acetone

solution (4:1) to destain the wells. The OD of the crystal violet present in the destaining solution was measured at 570 nm by spectrophotometric reading, carried out with a Multiskan microplate reader (Thermo Electron Corporation) [10]. The biofilm formation percentage was calculated by dividing the OD values of samples treated with oils and untreated samples.

2.7. Eukaryotic Cell Culture

HaCat (human keratinocytes) cells are spontaneously transformed aneuploid immortal keratinocyte cell line from adult human skin, widely used in scientific research. These cells were maintained in Dulbecco Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2. The essential oil extracted from *A. secundiramea* leaves and flowers were added in a complete growth medium for the cytotoxicity assay [11,12].

2.8. Blood Collection and Polymorphonuclear Leukocytes (PMN) Isolation

Peripheral blood was collected from three healthy fasting donors between 07.30 and 08.30 a.m. Samples were withdrawn by K_3EDTA vacutainers (Becton Dickinson, Plymouth, UK). PMNs were isolated using a discontinuous gradient, as reported in Harbeck et al. [13]. The blood was centrifuged for 30 min at 200× *g* at room temperature. The PMN layer, banded at the interface of the two employed Percoll densities, was collected and washed twice in PBS. The purity of isolated PMNs (evaluated on May Grunwald Giemsa stained cytocentrifuged smears) and cell viability (checked with the trypan blue dye exclusion test) both ranged between 90% and 95%.

2.9. Antioxidant Enzymes Measured PMN Cells

The activities of the enzymes SOD, CAT, and GPx in PMN cells were evaluated by using the commercial kits (BioAssay System, San Diego, CA, USA). The activity of enzymes was expressed in U/L [14].

2.10. Statistical Analysis

The effect of leaf and flowers essential oil extracts of *A. secundiramea* on activities of antioxidant enzymes in polymorphonuclear cells were examined by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post-hoc test ($p < 0.05$).

3. Results and Discussions

3.1. Essential Oil Composition Analysis

Hydrodistillation of *A. secundiramea* Biv. subsp. *secundiramea* leaves (L) gave a pale-yellow oil, (yield (0.46%). Only four components were recognized, representing 99.2% of the total composition. The metabolites are listed in Table 1 according to their retention indices on an HP-5MS column. All the four compounds were chrysantenyl derivatives with (*E*)-chrysanthenyl acetate (Figure 1) (91.5%) as main compounds, followed by chrysanthenone (4.2%), (*E*)-chrysanthenol (3.3%), and a small amount of (*Z*)-chrysanthenol (0.2%). Quite amazingly, hydrodistillation of *A. secundiramea* Biv. subsp. *secundiramea* flowers (F) gave an oil (yield 1.55%) containing only (*E*)-chrysanthenyl acetate (100.0%). The positive optical rotation of the oil determined its absolute configuration as (+)-(*E*)-chrysanthenyl acetate, whose synthesis starting from (+)-verbenone has been previously described [15]. The chromatograms of the two oils (L and F) are reported in Figure 2, whereas Figure 3 shows the ^{1}H-NMR spectrum of the oil from flowers.

Table 1. Composition of essential oils from *A. secundiramea* Biv. ssp. *secundiramea* (Asteraceae).

K_i [a]	K_i [b]	Components	Ident.[c]	F %	L %
1114	1684	(*Z*)-Chrysanthenol	1,2		0.2
1126	1521	Chrysanthenone	1,2		4.2
1163	1764	(*E*)-Chrysanthenol	1,2		3.3
1236	1585	(*E*)-Chrysanthenyl acetate	1,2	100.0	91.5
		Total		**100.0**	**99.2**

[a] Retention index on an HP-5MS column; [b] Retention index on a DB-5 column; [c] Identification, 1 = comparison of retention index; 2 = comparison of mass spectra with MS libraries identification.

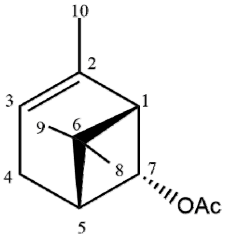
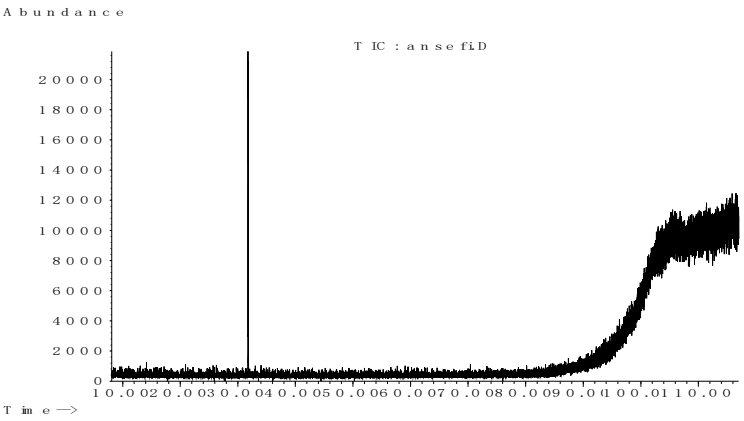


Figure 1. Structure of (+)-(*E*)-chrysanthenyl acetate.

F



L

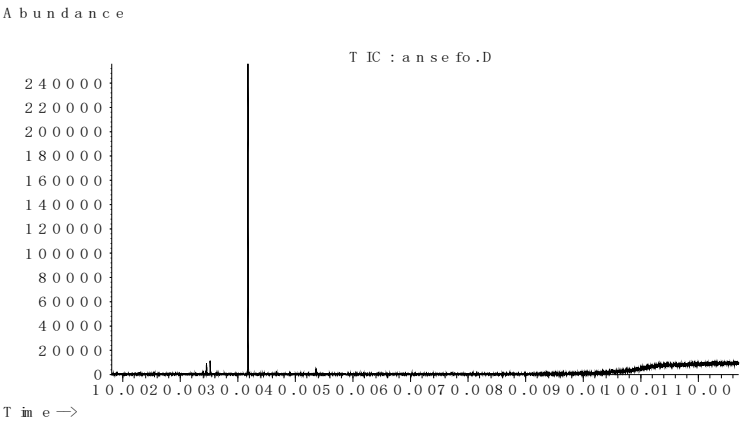


Figure 2. Chromatograms of the essential oils of *A. secundirramea* flowers (**F**) and leaves (**L**).

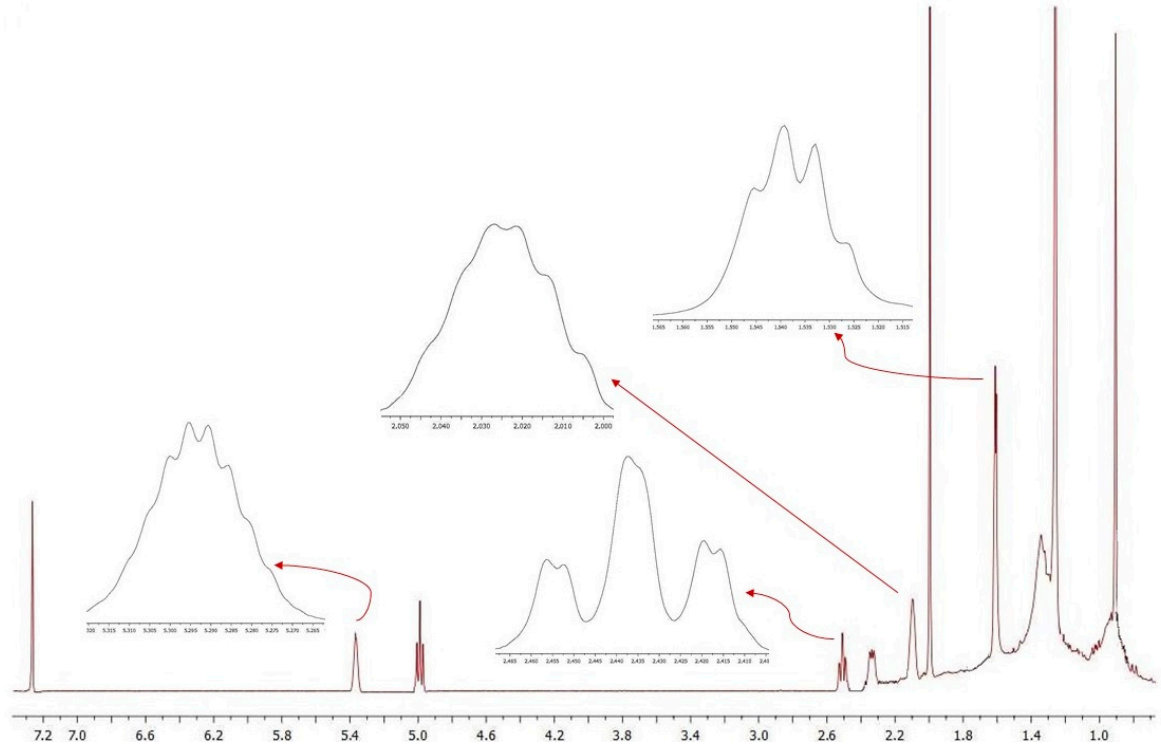


Figure 3. ^{1}H-NMR spectrum of F containing only (*E*)-chrysanthenyl acetate.

Some interesting considerations can be made by comparing our results with those reported in the literature for *A. maritima*, *A. cupaniana*, and *A. secundirramea*, belonging to the same clade.

A component analysis on the essential oil of six accessions from Corsica and twelve accessions from Sardinia of *A. maritima* divided them into two groups. In the first one, comprising the populations of Corsica and west Sardinia, 6-methylhept-5-en-2-one was identified as the main component. Several chrysanthenyl derivatives were also present, but (*E*)-chrysanthenyl acetate was totally absent. On the other hand, the populations from east Sardinia, belonging to the second group, were characterized by the high quantity of (*E*)-chrysanthenyl acetate and other chrysanthenyl derivatives [16]. Subsequent investigations on six accessions of *A. maritima* from Tuscany [17] and of five population collected on the Adriatic coast of Italy [18] confirmed the high variability of the occurrence of (*E*)-chrysanthenyl acetate ranging from 0 to 55.6% and from 0 to 28.1%, respectively.

On the other hands, the analysis of the oil isolated from the aerial parts of *A. cupaniana* showed a very poor content of chrysanthenyl derivatives with (*E*)-chrysanthenyl acetate completely absent [4]. Quite recently, the analysis of the essential oil of the aerial parts *A. secundiramea*, collected in the western part of Sicily, showed the presence of several irregular oxygenated monoterpenes with (*Z*)-chrysanthenyl acetate (9.9%) and (*E*)-chrysanthenyl acetate (7.7%) among the main compounds [3]. Other species of *Anthemis* showing a good occurrence of (*E*)-chrysanthenyl acetate were *A. cretica* ssp. *messanensis* from Sicily (28.8-24.2%) [19] and *A. montana* from Serbia (11.3%) [20].

In Table 2, the occurrence of (*E*)-chrysanthenyl acetate in different species is reported. (*E*)-chrysanthenyl acetate, apart from *Anthemis* ssp., is present in many other species belonging to the Asteraceae family, and among them, the taxa that contain larger amount (>20%) of this metabolite are *Achillea crithmifolia*, *Achillea millefolium*, *Chrysanthemum shiwogiku*, *Tanacetum parthenium*, *Tanacetum polycephalum*, and *Tanacetum vulgare*. Other species containing a high quantity of this monoterpene are *Allium neapolitanum* (Alliaceae Family), *Ferulago pauciradiata* (Apiaceae Family), *Lamium amplexicalule* (Lamiaceae Family), and *Zieria cytisoides* (Rutaceae Family).

Table 2. The occurrence of (*E*)-chrysanthenyl acetate.

Taxa	Origin	*trans*-chrysanthenyl Acetate	Ref.
Alliaceae Family			
Allium neapolitanum	Sicily	28.1	[21]
Apiaceae Family			
Ferulago pauciradiata	Turkey	24.9	[22]
Asteraceae Family			
Achillea crithmifolia	Bulgaria	21.9-0	[23]
Achillea crithmifolia	Serbia	18.8	[24]
Achillea millefolium	Portugal	15.8	[25]
Achillea millefolium	Serbia	21.3-5.8	[26]
Achillea wilhelmsii	Iran	8.5	[27]
Anacyclus clavatus	Tunisia	12.3	[28]
Anacyclus monanthos subsp. *cyrtolepidioides*	Algeria	9.8	[29]
Anthemis cretica ssp. *messanensis*	Sicily	28.8-24.2	[19]
Anthemis cretica ssp. *columnae*	Sicily	0.3-0.1	[19]
Anthemis maritima	Corsica, West Sardinia	0.7-0	[16]
Anthemis maritima	Sardinia	68.5-12.6	[16]
Anthemis maritima	Adriatic Coast, Italy	28.1-0	[18]
Anthemis maritima	Tuscany, Italy	55.6-0	[17]
Anthemis montana	Serbia	11.3	[20]
Anthemis secundiramea	West Sicily	7.7	[3]
Anthemis xylopoda	Turkey	5.5-3.8 *	[30]
Artemisia absinthium	Lithuania	11.6-0	[31]
Bubonium graveolens	Algeria	18.7-2.8	[32]
Chrysanthemum coronarium	Greece	13.2-7.8	[33]
Chrysanthemum coronarium	Tunisia	12.8	[34]
Chrysanthemum coronarium	Italy	6.7-5.5	[35]
Chrysanthemum japonense var. *debile*	Japan	3.8	[36]
Chrysanthemum shiwogiku	Japan	23.2	[37]
Tanacetum cadmeum ssp. *orientale*	Turkey	8.5-0	[38]
Tanacetum chiliophillum	Iran	10.5-5.5	[39]
Tanacetum kotshyi	Iran	2.1	[39]
Tanacetum parthenium	Denmark	15.7	[40]
Tanacetum parthenium	cultivated	27.3-22.4	[41]
Tanacetum parthenium	Belgium	23.5	[42]
Tanacetum parthenium	Turkey	22.1-0	[43]
Tanacetum persicum	Iran	2.1	[39]
Tanacetum polycephalum	Iran	20.0-3.5	[39]
Tanacetum polycephalum subsp. *farsicum*	Iran	24.7	[44]
Tanacetum santoniloides	Egypt	13.2	[45]
Tanacetum vulgare	Estonia	30.7-0	[46]
Tanacetum vulgare	Poland	18.4	[47]
Tanacetum vulgare	Belgium	78.3-0	[42]
Tanacetum vulgare	Serbia	41.4	[48]

Table 2. *Cont.*

Taxa	Origin	*trans*-chrysanthenyl Acetate	Ref.
Tanacetum vulgare	Hungary	17.3-3.5	[49]
Tanacetum vulgare	Poland	6.5	[50]
Bryophytes			
Gackstroemia magellanica	Chie	high	[51]
Lamiaceae Family			
Lagochilus gypsaceus	Uzbekistan	7.1	[52]
Lagochilus inebrians	Uzbekistan	9.4	[52]
Lagochilus setulosus	Uzbekistan		[52]
Lamium amplexicalule	Italy	52.5-37.8	[53]
Rutaceae Family			
Zieria cytisoides	Australia	47.6	[54]

* Undetermined isomer.

3.2. Effect of *A. secundiramea* Essential Oils on Bacterial Survival

Essential oils extracted from *A. secundiramea* leaves and flowers were tested against two gram-negative and one positive strains: *E. coli DH5α, P. aeruginosa PAOI*, and *S. aureus ATCC 6538P*, respectively. *E. coli* represents a model strain to test antimicrobial activity, while *P. aeruginosa* and *S. aureus* are opportunistic pathogens. In particular, some bacterial strains of species *P. aeruginosa* can cause infections in patients suffering from a disorder of the skin barrier [55]. *S. aureus* is an important human pathogen that is responsible for most of the bacterial skin and soft tissue infections in humans too. This strain can also become more invasive and cause life-threatening infections, such as bacteremia, pneumonia, abscesses of various organs, meningitis, osteomyelitis, endocarditis, and sepsis. These infections represent a major public health threat because of their considerable number and spread [56]. As shown in Figure 4A, the oil extracted from flowers was able to inhibit the three strains: *E. coli*, *P. aeruginosa*, and *S. aureus* growth, forming an inhibition halo. Figure 4B reports a quantitative analysis of inhibition halos (about 60, 35, and 38 AU/mL, respectively), almost comparable to the antibiotic control. We used ampicillin to inhibit *E. coli* and *S. aureus* cells growth, and colistin, a polymyxin that acts on the bacterial membrane of gram-negative microorganisms, used as a positive control in the *P. aeruginosa* experiment. The essential oil extracted from the *A. secundiramea* leaves seems to be less efficient than that from flowers.

A more sensitive method, shown in Figure 5, to calculate the efficiency of antimicrobial activity uses fixed concentration (500 µg/mL). Figure S1 shows this at different concentrations (50, 100, and 250 µg/mL, which confirms that *A. secundiramea* flowers and leaves extracts have antimicrobial activity. The oil extracted from the flowers has a stronger antimicrobial activity than the one extracted from the leaves against the three bacterial strains tested. The antimicrobial activity of *A. secundiramea* oil is probably due to a single molecule, the (*E*)-chrysanthenyl acetate, which is present in a pure form in the extract of flowers. Recent studies report that chrysanthenyl acetate, has been suggested as the active component. Chrysanthenyl acetate inhibits prostaglandin synthetase and might have analgesic properties [57]. Other studies suggest that compound analog chrysanthenyl acetate, may contribute to the antimigraine activity, due to its prostaglandin inhibition [58]. The antimicrobial activity of (*E*)-chrysanthenyl acetate, to the best of our current knowledge, is not well described in the literature. In general, this compound can be found in different plants and species of *Anthemis*, but never in pure form, as in the case of *A. secundiramea* flowers [59]. This aspect is very promising, as with the extraction from the flowers, it is very easy to obtain, and the yield is very high, already making the oil available in good quantities for different applications. Both oil extracts are not cytotoxic against eukryotic cells (HaCat line) tested for three different times (24, 48, and 72 h) at two different concentrations (100 and 500 µg/mL), and the data is shown in Figure S2 (see Supplementary Materials).

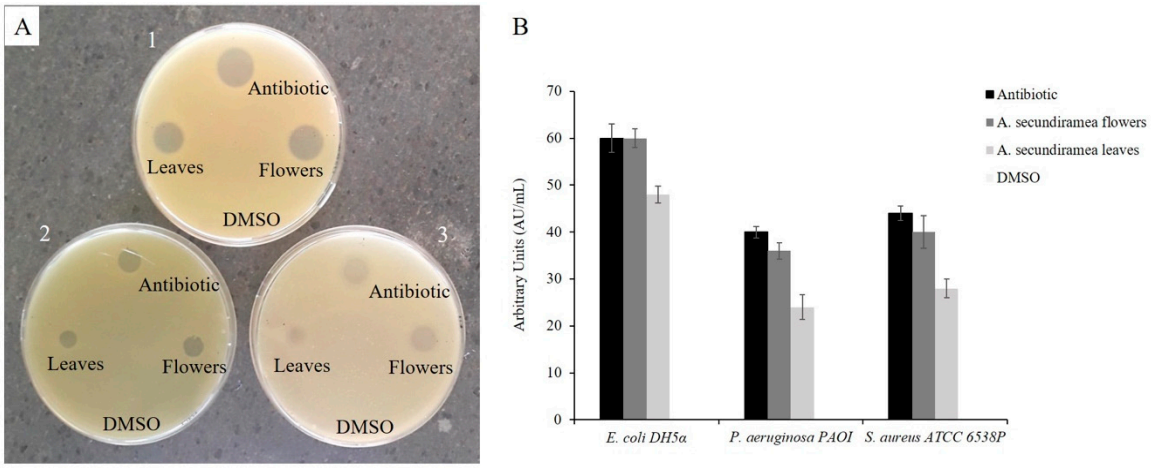


Figure 4. (**A**) Inhibition halo of *A. secundiramea* flowers and leaves essential oils against *E. coli DH5α* (1), *P. aeruginosa PAOI* (2) and *S. aureus ATCC 6538P* (3). Positive and negative control is represented by antibiotics (2 Colistine, 1 and 3 Ampicillin), and dimethyl sulfoxide (DMSO 2%), respectively. (**B**) The inhibition halos, shown in panel A, are expressed in AU/mL Values are expressed as the average of three different experiments; standard deviations were always less than 10%.

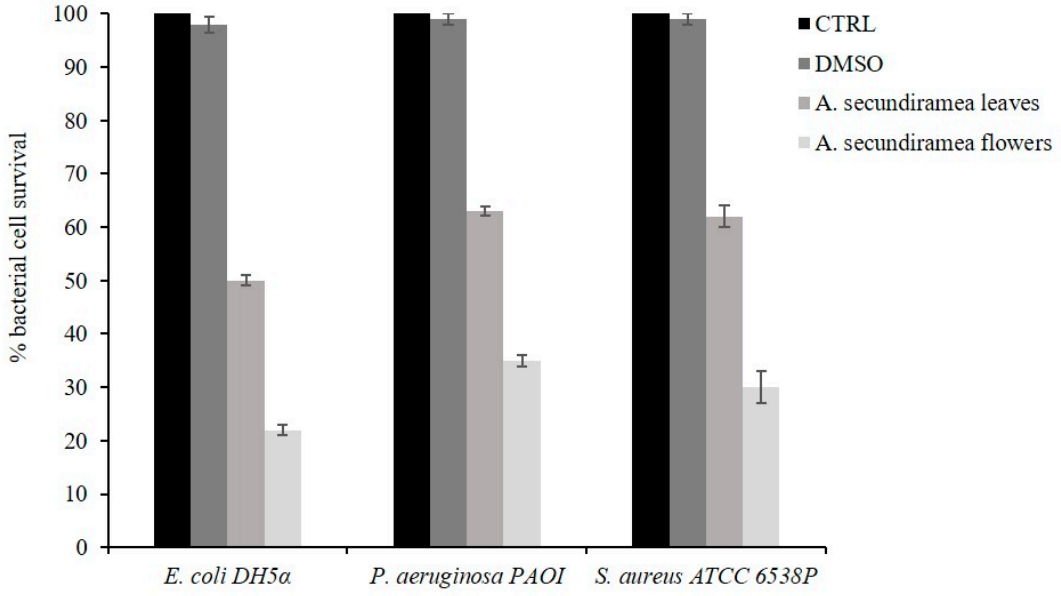


Figure 5. Antibacterial activity of *A. secundiramea* essential oils from flowers and leaves, evaluated by colony count assay, against *E. coli DH5α*, *P. aeruginosa PAOI*, and *S. aureus* ATCC 6538P at a fixed concentration of 500 μg/mL. Untreated cells represented control because negative controls are bacterial cells with dimethyl sulfoxide (DMSO 2%). Each bar is the average of three different experiments. p value is <0.05.

3.3. Effect of A. secundiramea Essential Oils on Biofilm Formation

Biofilm formation is a quorum-sensing regulated differentiation system used by different bacteria on plant surfaces [60], but also in the human body environment, as a protection against other microorganisms and antibacterial substances [61]. To study the leaf and flower oil effect on bacterial biofilm formation, we used *P. aeruginosa PAOI*, a biofilm producer, as the indicator strain. Bacterial cells were grown with and without the addition of leaf and flower oil at different concentrations (25, 50, and 100 μg/mL), not inhibiting the planktonic growth. As shown in Figure 6, a progressive increase of

A. secundiramea oil concentration corresponds to a decrease of biofilm formation. In particular, 100 µg/mL of flowers extract oil inhibited the biofilm formation for about 65%. To exclude any antimicrobial effect on planktonic growth, we followed the bacterial growth curve with and without both extracts at maximum concentration, used in the previous experiment. In this condition, PAOI growth rate did not change when compared to the untreated cells (data not shown). Biofilm formation of microorganisms causes persistent tissue and foreign body infections resistant to treatment with antimicrobial agents, such as *Staphylococcus epidermidis*, *P. aeruginosa*, *S. aureus*, and *E. coli* [62]. Our oil, containing a single compound that possesses antibiofilm activity, represents an important resource to counteract the biofilm formation.

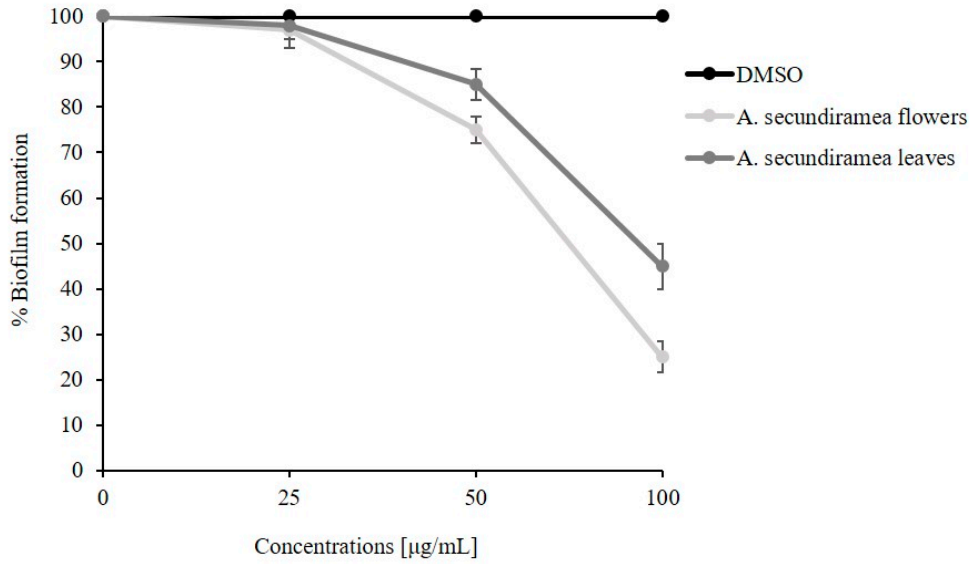


Figure 6. Colorimeter assay to evaluate the formation of *P. aeruginosa PAOI* biofilm with different oil concentrations (0, 25, 50, 100 µg/mL). The negative control is represented by cells treated with the DMSO (2%), and the positive control is represented by untreated cells. Values are expressed as the average of three different experiments; standard deviations were always less than 10%.

3.4. A. secundiramea Essential Oils Antioxidant Activity

Antioxidative enzymes status was evaluated by SOD, CAT, and GPx activities in PMN cells treated to flowers and leaf essential oil extracts of *A. secundiramea*. Figure 7 shows that both the flower and leaf essential oil extract causes an increase in the activity of antioxidant enzymes in PMN cells compared to control (samples-not treated) and in particular, the activity of SOD, CAT, and GPX enzymes is greater in PMN cells treated with the extract of flower essential oils compared to the extract of essential oil of leaves. A direct effect of the antioxidant activity has been studied in *A. kotschyana* [63] in which the antioxidant activity of *A. kotschyana* was determined by analyzing DPPH free radical scavenging of its water and ethanol extracts, demonstrating a remarkable correlation between radical scavenging potential and concentration detected for standards (BHA, BHT, and ascorbic acid) and the plant extracts. In particular, the extracts and standards had decreasing absorbance with increasing concentration, which means they scavenged more radicals. A study of *Chrysanthemum* [64] essential oils also has shown a direct action of chrysanthenyl acetate on ROS production. According to our best knowledge, there are no papers that report the activity of chrysanthenyl acetate on antioxidant enzymes. Some articles report that essential oils also containing chrysanthenyl acetate cause an increase in enzymes, such as SOD and GPX [65,66]. On the other hand, a previous document on essential oils extracted from *Mentha* x *piperita* and *Mentha arvensis* L. not containing chrysanthenyl acetate led to an increase in the activity of antioxidant enzymes, such as CAT, GST, POX, and SOD [67]. The data presented here show that in addition to the direct action on ROS, the essential oils of *A. secundiramea*,

and in particular, the chrysanthenyl acetate exerts an indirect antioxidant action increasing the activity of antioxidant enzymes.

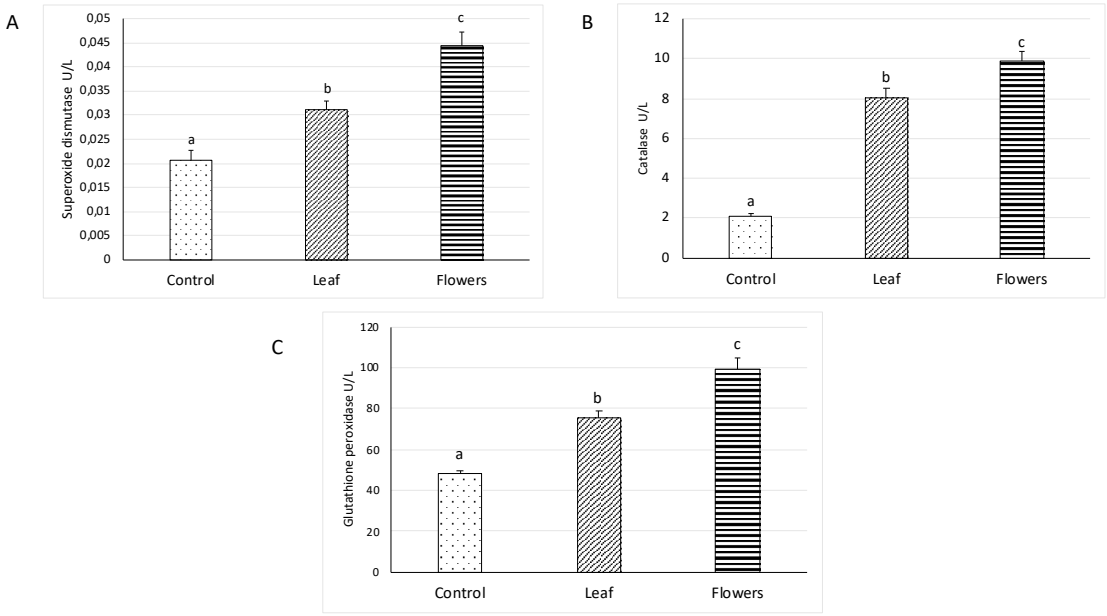


Figure 7. Effect of leaf and flowers essential oil extracts of *A. secundiramea* on activities of antioxidant enzymes in polymorphonuclear cells. (**A**) Superoxide dismutase; (**B**) catalase; (**C**) glutathione peroxidase. Data are presented as mean and standard error, and were analyzed with the paired *t*-test. * $p < 0.001$.

4. Conclusions

In this study, we can conclude that the essential oil of *A. secundiramea* has relatively good antibacterial activity against both gram-negative and positive strains, and is non-toxic for eukaryotic cells at the applied concentration. The oil obtained from the flowers also has a good antibiofilm activity and exceptional purity—which is importance within this field. In addition, antioxidative enzyme status was evaluated by SOD, CAT, and GPx activities in PMN cells treated with flowers and leaves essential oil extracts of *A. secundiramea*. The activity of antioxidant enzymes is greater in PMN cells treated with the extract of flowers essential oils compared to the extract of essential oil of leaves. The set of effects could be extremely interesting regarding the possible use of the *A. secundiramea* essential oils in nutraceutical products and cosmetics.

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