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Research Article

Essential Oil Constituents of *Tanacetum cilicicum*: Antimicrobial and Phytotoxic Activities

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Aerial parts of *Tanacetum cilicicum* were hydrodistillated for 3 h using Clevenger. Essential oil (EO) yield was 0.4% (v/w). According to the GC/MS analyses, EO of *T. cilicicum* consisted of monoterpenes [α-pinene (2.95 ± 0.19%), sabinene (2.32 ± 0.11%), and limonene (3.17 ± 0.25)], oxygenated monoterpenes [eucalyptol (5.08 ± 0.32%), camphor (3.53 ± 0.27%), linalool (7.01 ± 0.32%), α-terpineol (3.13 ± 0.23%), and borneol (4.21 ± 0.17%)], and sesquiterpenes [sesquisabinene hydrate (6.88 ± 0.41%), nerolidol (4.90 ± 0.33%), α-muurolol (4.57% ± 0.35), spathulanol (2.98 ± 0.12%), juniper camphor (2.68 ± 0.19%), (-)-caryophyllene oxide (2.64 ± 0.19%), 8-hydroxylinalool (2.62 ± 0.15%), and Δ-cadinene (2.48 ± 0.16%)]. In the antimicrobial assay, MIC/MBC values of the EO were the most significant on *B. subtilis* (0.39/0.78 μL/mL) and *B. cereus* (0.78/1.56 μL/mL). The most prominent phytotoxic activities of the EO were observed on *L. sativa*, *L. sativum*, and *P. oleracea*. The results of the present study indicated that EO of *T. cilicicum* includes various medicinally and industrially crucial phytoconstituents that could be in use for industrial applications. The finding of this study is the first report on this species from the East Mediterranean region.

1. Introduction

Essential oils are the secondary metabolites of the aromatic and medicinal plants. In the literature, there are reports on the characterization of the chemical composition of the essential oil as well as some bioactivities of the genus *Tanacetum* (Asteraceae). Those species were *T. annuum* (Granada Alfacar region, Spain) [1], *T. santolinoides* (DC.) Feinbr. and Fertig. (Wadi Elarbaeen, St. Catherine, Sinai Peninsula, Egypt) [2], *T. gracile* (Himalaya-Ganglas, Ladakh region, India) [3], *T. vulgare* L. (Canada, Finland, Slovakia, and Brazil) [4–7], *T. tabrisianum* (Iran) [8], *T. annuum* (Larache region, Northwest of Morrocco) [9], *T. pinnatum* (Lorestan region, Iran) [10], *T. parthenium* (L.) (Kamenica region, Kosova) [11], and *T. parthenium* (Hamedan region, Iran) [12].

In Turkey, various species of the genus *Tanacetum* have been also reported both for the chemical composition and/or antimicrobial, insecticidal, herbicidal, and antioxidant activities as well. Those species were *T. sorbifolium* [13], *T. balsamita* subsp. *balsamita* and *T. chiliophyllum* var. *chiliophyllum* [14], *T. argenteum* subsp. *flabellifolium* [15], *T. aucheranum* and *T. chiliophyllum* [16], *T. argenteum* subsp. *argenteum* [17], *T. zahlbruckneri* [18], *T. chiliophyllum* var. *chiliophyllum* [19], *T. chiliophyllum* var. *monocephalum* [20], *T. heterotomum, T. zahlbrucknei, T. densum* subsp. *amani, T. cadmeum* subsp. *orientale* [21], *T. argenteum* subsp. *argenteum, and T. argenteum* subsp. *canum* [22] and *T. alyssifolium* [23], and *T. cilicicum* [24] (Table 1).

Essential oils of aromatic and medicinal plants have been receiving increasing attention in many industries such as medicine, agriculture, food, and cosmetics [25]. In nature, many plants have been still awaiting for the exploration of their bio-benefits. To date, there has been no previous study indicating the chemical composition as well as antimicrobial and phytotoxic activities of *T. cilicicum* from the East Mediterranean region. Hence, the essential oil composition as well as bioactivities of the aerial parts of *T. cilicicum* could be the first report.

Table 1: Main components in the essential oil of some *Tanacetum* sp.

Tanacetum sp.	EO yield and main components	References
T. sorbifolium	*EO yield*: 0.85% *Main components*: camphor (54.3), pinocarvone (5.1), chrysanthenone (4.7), bornyl acetate (3.9), camphene (3.4), β-pinene (2.8)	[13]
T. argenteum subsp. *flabellifolium*	*EO yield*: 0.36% *Main components*: α-pinene (29.1), (*E*)-sesquilavandulol (15.9), camphor (14.0), (*E*)-lavandulyl acetate (4.9), terpinen-4-ol (3.1), β-caryophyllene (3.1)	[15]
T. aucheranum	*EO yield*: 0.15% *Main components*: 1,8-cineole (23.8), camphor (11.6), terpinen-4-ol (7.2), α-terpineol (6.5), borneol (3.8), (E)-thujone (3.2), epi-α-cadinol (3.1), artemisia ketone (3.0)	[16]
T. chiliophyllum	*EO yield*: 0.22% *Main components*: camphor (17.9), 1,8-cineole (16.6), borneol (15.4), dihydro-α-cyclogeranyl pentanoate (3.0), dihydro-α-cyclogeranyl hexanoate (10.1)	[16]
T. alyssifolium	*Main components*: borneol (35.2), α-thujone (24.6), camphor (12.4), β-eudesmol (6.1), 1,8-cineole (4.8), thymol (4.1), β-thujone (3.3)	[23]
T. balsamita subsp. *balsamita*	*Main components*: trans-chrysanthenol (22.3), chrysanthenyl acetate (19.7), linalool oxide (11.5), 2H-pyran-3(4H)-one (9.7), camphor (7.5), 1,8-cineole (2.7)	[14]
T. chiliophyllum var. *chiliophyllum*	*Main components*: camphor (28.5), 1,8-cineole (17.1), camphene (7.1), isobornyl propionate (5.4), carveol (4.5), benzene, 1-methyl-2 (2.9)	[14]
T. nitens	*Main components*: 1,8-cineole (27.57), α-pinene (4.62), trans-verbenol (4.34), spathulenol (4.14), trans-pinocarveol (4.13), 3-cyclohexzan-1-ol (3.81), α-terpineol (3.68), caryophyllene oxide (3.23) and oplophenon (3.01) benzene, 1-methyl-2 (2.75), δ-cubebene (1.55)	[17]
T. argenteum subsp. *argenteum*	*Main components*: α-pinene (27.86), santolinatriene (8.82), 1,8-cineole (6.82), chrysanthenone (3.83), cadina-1,4-dien (3.52) β-pinene (3.16), spathulenol (2.83), borneol (2.68), cis-sabinenehydrate (2.66), trans-verbenol (2.52), trans-pinocarveol (2.40), α-terpineol (2.41)	[17]
T. tabrisianum	*EO yield of the flower part*: 0.16% *Main components*: 1,8-cineole (17.6), hexadecanoic acid (10.3), borneol (6.9), decanoic acid (5.8), trans-linalooloxide acetate (5.3)	[18]
T. zahlbruckneri	*EO yield of the stem part*: 0.10% *Main components*: 1,8-cineole (22.5), hexadecanoic acid (8.0), trans-linalooloxide acetate (4.0), borneol (3.0)	[18]
T. chiliophyllum var. *monocephalum*	*EO yield of the flower part*: 0.06% *Main components*: camphor (17.3), 1,8-cineole (8.3), unknown I (6.6), (*E*)-β-ionone cubenol unknown III (5.2), camphene (3.4), marsupellol unknown IV (3.0), borneol (2.9)	[20]
T. chiliophyllum var. *monocephalum*	*EO yield of the stem part*: 0.05% *Main components*: camphor (10.4), (*E*)-β-ionone unknown I (10.4), cubenol unknown II (9.2), marsupellol unknown IV (7.4), hexadecanoic acid (3.5), (*E*)-nerolidol (3.2), phytol (2.8)	[20]
T. chiliophyllum var. *monocephalum*	*EO yield of the root part*: < %0.01 *Main components*: hexadecanoic acid (37.5), cubenol unknown III (8.7), alismol (6.3), geranyl isovalerate (5.3), (*E*)-nerolidol (3.3), marsupellol unknown IV (3.1), (*E*)-β-ionone unknown I (2.6)	[20]
T. chiliophyllum var. *chiliophyllum* (A)	*EO yield of the flower and stem part*: 0.1 and 0.2% *Main components*: camphor (32.5, 36.2), 1,8-cineole (1.6, 16.1), chamazulene (9.2, 2.9), pinocarvone (3.2, 2.4), hotrienol (2.7, 0.3), borneol (2.7, 2.8), chrysanthenyl isovalerate I (2.0, 3.0), chrysanthenyl isovalerate II (2.1, 2.8), β-eudesmol (4.7, 1.1)	[19]
T. chiliophyllum var. *chiliophyllum* (B)	*EO yield of the flower and stem part*: 0.6 and 0.1% *Main components*: 1,8-cineole (12, 18.4), terpinen-4-ol (10.3, 9.0), hexadecanoic acid (4.2, 7.6), (E)-sesquilavandulol (5.8, 1.6), α-thujone (3.0, 1.2), trans-chrysanthenyl acetate (3.5, 2.8), α-eudesmol (3.4, 1.4)	[19]
T. chiliophyllum var. *chiliophyllum* (C)	*Main components*: 1,8-cineole (22.1, 28.9), α-pinene (5.3, 1.5), terpinen-4-ol (6.5, 5.6), p-cymene (4.2, 4.3), trans-chrysanthenyl acetate (3.7, 2.0), pinocarvone (1.8, 2.8), (E)-sesquilavandulol (3.6, 0.0)	[19]

Table 1: Continued.

Tanacetum sp.	EO yield and main components	References
T. heterotomum	*Main components*: α-pinene (3.4), camphene (1.2), β-pinene (4.5), 1,8-cineole (17.9), α-terpinolene (1.2), cis-sabinenehydrate (1.7), camphor (22.4), pinocarvone (1.2), borneol (18.8), α-terpineol (1.3), α-copaene (1.3), β-selinene (1.4), caryophyllene oxide (1.1), hexadecanoic acid (1.6)	[21]
T. zahlbrucknei	α-Thujene (1.2), α-pinene (1.0), camphene (2.3), 1,8-cineole (1.3), bicyclo (3.1) hexan-3-one (2.1), trans-chrysanthemol (1.7), camphor (3.8), borneol (21.3), 3-cyclohexen-1-ol (2.4), α-terpineol (1.9), bicyclo (2.2) heptan-2-ol (1.1), thymol (2.7), germacrene D (21.4), spathulenol (16.2), aromadendrene (1.2)	[21]
T. densum subsp. *amani*	α-Pinene (6.7), camphene (1.5), β-pinene (8.9), 1,8-cineole (16.7), cyclohexene (1.2), cis-sabinenehydrate (1.3), 4-acetyl-1-methylcyclohexan (1.2), camphor (26.8), pinocarvone (2.4), cis-chrysanthemol (1.3), borneol (3.8), α-copaene (2.6), β-bourbenene (1.4), trans-β-farnesene (1.2), germacrene D (3.9), spathulenol (1.4), β-bisabolene (1.1)	[21]
T. cadmeum subsp. *orientale*	*Main components*: α-pinene (7.6), camphene (5.4), β-pinene (1.8), α-phellandrene (1.1), limonene (1.2), 1,8-cineole (19.6), trans-chrysanthemol (3.1), camphor (17.2), pinocarvone (1.8), 2-cyclohexen-1-ol (1.7), borneol (5.3), α-copaene (2.8), β-elemene (1.9), β-cubebene (1.3), isoledene (1.5), muurolene (1.6), epi-bicyclosesquiphellandrene (1.7), α-cadinol (1.3), aromadendrene (1.3)	[21]
T. argenteum subsp. *argenteum*	EO yield: 0.32% *Main components*: α-pinene (67.9), β-pinene (4.8), α-phellandrene (2.8), limonene (2.0), 1,8-cineole (2.2), *p*-cymene (2.1), β-caryophyllene (1.5), germacrene D (2.2), δ-cadinene (1.6), caryophyllene oxide (1.1), T-cadinol (1.4), α-cadinol (1.6)	[22]
T. argenteum subsp. *canum*	EO yield: 0.35% *Main components*: α-pinene (53.6), α-thujene (1.1), camphene (1.1), β-pinene (1.0), α-phellandrene (2.5), 1,8-cineole (14.8), *p*-cymene (2.1), *trans*-sabinene hydrate (2.9), camphor (4.7), terpinen-4-ol (2.1), *trans*-pinocarveol (1.2), *trans*-verbenol (1.0), α-terpineol (1.2), spathulenol (1.2)	[22]
T. cilicicum	*Main components*: bicyclo (31.1), hept-2-en-4-ol (21.92), camphor (15.56), 1,8-cineole (13.45)	[24]

2. Experimental

2.1. Plant Material and Essential Oil Distillation. Aerial parts of *T. cilicicum* were collected from Bağlıca Plateau (Amanos Mountains, Hatay-İskenderun, 36°34′48,52″N, 36°16′05,87″E). Essential oil (EO) from air-dried aerial parts of *T. cilicicum* was obtained with Clevenger for 3 h distillation period, which was followed by calculating the oil yield (v/w). A total of 100 g plant material was used during each distillation cycle. The blue coloured EO was dried with anhydrous sodium sulphate to remove the water from the distillate and then preserved in amber vials at +4°C for further analyses.

2.2. Analyses of the EO of T. cilicicum Using Gas Chromatography and Mass Spectrometry (GC/MS). The characterization of the compounds in the EO was obtained with Gas Chromatography and Mass Spectrometry. The conditions used during the analyses were described in Table 2 [26]. n-Alkane mixture was used to determine and calculate the linear retention index (Kovats Indices) of each compound in the EO under the same temperature programme used for the analyses [27]. Mass spectra of the EO constituents were compared with those of the references documented in NIST (National Institute of Standards and Technology, 2013, Gaithersburg, MD, USA) and Wiley database. Tentative identification as well as the retention indices of the compounds in this assay was compared with those of NIST. In addition, quantitative analyses of the EO constituents (% area) as average of 3 repeated analytical assays were carried out with the measurement of the peak space normalization.

Table 2: GC/MS conditions.

System	Shimadzu QP 2010 Plus (Shimadzu, Kyoto, Japan) GC
Colon	TRB-Wax (Teknokroma, Barcelona, Spain) fused silica capillary column (60 m × 0.25 mm i.d. and film thickness, 0.25 μm)
Temperature programme	40°C/5 min, 3°C/1 min, 240°C/15 min (total running: 86 min)
Injector	AOC-20i/20s autosampler
Injection volume	1 μl (1 part of EO/100 part of n-hexane, v/v)
Carrier gas	Helium (flow rate, 1 mL/min)
Detector	MS-QP 2010 series mass-selective detector
Split ratio	1/50
Electron energy	70 eV
Mass spectra	35–450 m/z
Scanning rate	1 scan/s

2.3. Antimicrobial Assays and Test Microorganisms. Six Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Bacillus cereus* EU, *Enterococcus faecalis* ATCC 29212, *Enterococcus casseliflavus* ATCC 700327, *Staphylococcus aureus* ATCC 29213, and *Staphylococcus aureus* ATCC BAA 977), four Gram-negative bacteria (*Enterobacter hormaechei* ATCC 700323, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922), and two yeast species (*Candida parapsilosis* ATCC 22019 and *Candida albicans* ATCC 14053) were used in the assays.

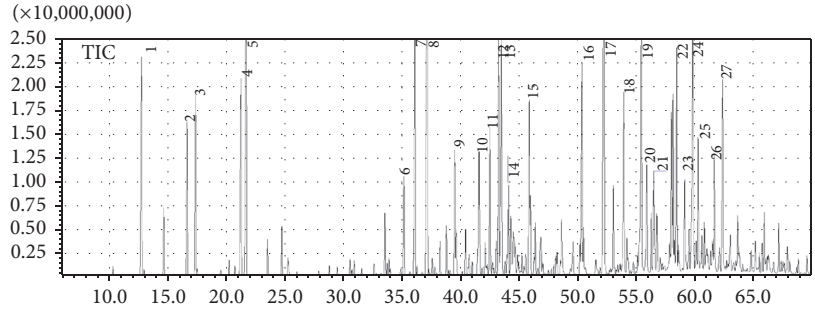

Figure 1: GC/MS chromatogram of the EO from *T. cilicium*. The peaks in the chromatogram belong to the main compounds which are more than 1% of the EO chemical composition: (1) α-pinene, (2) β-pinene, (3) sabinene, (4) limonene, (5) eucalyptol, (6) α-copaene, (7) camphor, (8) linalool, (9) 4-terpineol, (10) alloaromadendrene, (11) trans-verbenol, (12) α-terpineol, (13) borneol, (14) germacrene D, (15) Δ-cadinene, (16) spathulanol, (17) sesquisabinene hydrate, (18) (-)-caryophyllene oxide, (19) nerolidol, (20) cis-caryophyllene, (21) α-cedrene, (22) 8-hydroxylinalool, (23) spathulenol, (24) α-muurolol, (25) α-cadinol, (26) epiglobulol, and (27) juniper camphor (the peak numbering given here is not in accordance with peak numbers in Table 3).

2.4. Disc Diffusion Assay. The turbidity of the freshly bacterial culture grown on Nutrient Agar medium (37°C/ 24 h) and Sabouraud Dextrose Agar was adjusted to 0.5 McFarland Unit. 100 μL of the suspension of each microorganism in sterile saline water was distributed on Mueller Hinton Agar for bacterial cultures and Sabouraud Dextrose Agar for fungal cultures. After spreading homogenously by drigalski spatula, a sterile antibiotic assay disc was placed on each medium. EO of *T. cilicicum* (15 μL) was pipetted onto each disc. The petri dish was then sealed with parafilm in order to retain the EO in the glass petri atmosphere. Throughout the assay, controls were (I) only test medium in a petri plate, (II) essential oil impregnated assay disc on an uninoculated medium, and (III) test culture plated on appropriate medium. All treatments were maintained at 35.5°C for 24 h [28]. The diameters of the zones around the discs were measured as mm. Trimethoprim/Sulfamethoxazole (SXT5), Vancomycin (VA30), Teicoplanin (TEC30), and Nystatin (100 IU) were used as the standard antibiotics during the disc diffusion assay.

2.5. Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC) of the EO from T. cilicicum. Two-fold concentrations of the EO ranging from 0.0485 to 200.0 μL/mL in Mueller Hinton Broth + 0.5% Tween 80 for bacteria and Sabouraud Dextrose Broth + 0.5% Tween 80 for yeast were prepared before assay. Each tube received 100 μL of microbial suspension. The following were used to check the test results for comparison: (I) medium including different essential oil concentrations dissolved in 0.5% Tween 80: MHB or SDB + Tween 80 + different essential oil concentrations, (II) MHB or SDB + Tween 80 + test strain, and MHB or SDB + Tween 80, and (III) only MHB or SDB. All treatments were incubated at 37°C for 24 h. Tube without any visible growth was treated as Minimum Inhibitory Concentration of the test EO in concern. The broth in test tubes was plated onto appropriate media to determine MBC/MFC [29]. In the broth dilution assay, commercially available antibiotics such as Ampicillin (A6140, Sigma) were used in Mueller Hinton Broth for bacteria, and Clotrimazole (CG019, Sigma) was tested in Sabouraud Dextrose Broth for yeast species.

2.6. Phytotoxicity Assay. Seeds of *Lactuca sativa* (lettuce), *Lepidium sativum* (cress), and *P. oleracea* (common purslane) were used in the direct-contact assay of the essential oil [30, 31]. Two round filter papers were placed into each glass petri dish (90 mm in diameter) and then sterilized at 121°C for 15 min. Afterwards, sterilized seeds with aqueous sodium hypochlorite solution (1.5%, v/v) for 10 min were aseptically placed onto double layered membranes [32]. EO of *T. cilicicum* was dissolved in 0.5% Tween 80 at the concentrations from 0.062 to 2.0 mg/mL. A total of 10 mL from each concentration was aseptically placed to lower layer of the filter paper by using a sterile glass pipette. Throughout the assay, control did include only distilled water. All petri plates were sealed with parafilm. Treated petri dishes as well as the control groups were maintained for 7 days at 24°C, 12/12 h, 1.500 lux light/dark period, and about 80% relative humidity. At the final day of maintenance, germinated seeds were counted and then recorded. The length of seedling growths was measured as mm. Glyphosate N-(phosphonomethyl) glycine was tested as the commercial herbicide.

2.7. Statistical Analysis. Assessment of the test results was carried out using SPSS17 programme. Variance analyses (ANOVA) of the results of the test parameters were performed. Tukey Multiple Comparative test was employed to determine the differences in test parameters. The mean and standard deviations of the results were calculated. The statistical differences were indicated as a superscript in the mean values. All assays in this study were carried out in triplicate.

3. Results and Discussion

3.1. Essential Oil Composition of T. cilicicum. As shown in Table 3 and Figure 1, EO of the aerial parts from *T. cilicicum* consisted of mainly (%) linalool (7.01 ± 0.32), sesquisabinene hydrate (6.88 ± 0.41), eucalyptol (5.08 ± 0.32), nerolidol (4.90

Table 3: Essential oil composition of *T. cilicicum*.

Number	Retention index	Compound	%
1	1010	α-Pinene	2.95 ± 0.19
2	1015	α-Thujene	0.08 ± 0.01
3	1052	Camphene	0.73 ± 0.03
4	1095	β-Pinene	1.89 ± 0.10
5	1109	Sabinene	2,32 ± 0,11
6	1113	Verbenene	0,08 ± 0,01
7	1154	β-Myrcene	0,07 ± 0,01
8	1168	α-Terpinene	0,19 ± 0,02
9	1188	Limonene	3,17 ± 0,25
10	1198	Eucalyptol	5,08 ± 0,32
11	1235	γ-Terpinene	0,36 ± 0,02
12	1260	para-Cymene	0.54 ± 0.03
13	1286	Amyl isovalerate	0.06 ± 0.01
14	1345	3-Methyl-3-butenyl isovalerate	0.11 ± 0.01
15	1383	Nonanal	0.23 ± 0.01
16	1429	trans-Linalool oxide (fur.)	0.12 ± 0.01
17	1450	trans-Sabinene hydrate	0.80 ± 0.02
18	1455	Menthone	0.16 ± 0.03
19	1487	α-Copaene	1.24 ± 0.09
20	1509	Camphor	3.53 ± 0.27
21	1513	Benzaldehyde	0.13 ± 0.01
22	1533	Linalool	7.01 ± 0.32
23	1545	4-Acetyl-1-methyl-1-cyclohexene	0.29 ± 0.01
24	1550	trans-p-Mentha-2-en-1-ol	0.13 ± 0.01
25	1560	Pinocarvone	0.40 ± 0.02
26	1574	Bornyl acetate	0.52 ± 0.07
27	1590	4-Terpineol	1.45 ± 0.09
28	1593	Terpendiol I	0.64 ± 0.04
29	1614	trans-p-Mentha-2.8-dien-1-ol	0.52 ± 0.03
30	1621	Myrtenal	0.25 ± 0.01
31	1639	Pulegone	0.33 ± 0.07
32	1643	Alloaromadendrene	2.00 ± 0.16
33	1655	Isoledene	0.48 ± 0.03
34	1666	trans-Verbenol	2.06 ± 0.11
35	1671	cis-Citral	0.18 ± 0.02
36	1684	α-Terpineol	3.13 ± 0.23
37	1690	Borneol	4.21 ± 0.17
38	1706	Germacrene D	1.26 ± 0.09
39	1718	α-Muurolene	0.94 ± 0.06
40	1726	Carvone	0.28 ± 0.01
41	1729	Bicyclogermacrene	0.20 ± 0.02
42	1737	Verbenol	0.21 ± 0.04
43	1746	Neryl acetate	0.30 ± 0.03
44	1754	Δ-Cadinene	2.48 ± 0.16
45	1756	γ-Cadinene	0.81 ± 0.07
46	1764	1.7.7-Trimethylbicyclo[2.2.1]hept-5-en-2-ol	0.27 ± 0.06
47	1767	Ar-Curcumene	0.61 ± 0.05
48	1780	Myrtenol	0.76 ± 0.04
49	1785	Isocarveol	0.42 ± 0.06
50	1821	trans-Carveol	0.32 ± 0.02
51	1828	cis-Calamenene	0.13 ± 0.01
52	1831	Geraniol	0.84 ± 0.07

Table 3: Continued.

Number	Retention index	Compound	%
53	1859	Benzyl isovalerate	0.57 ± 0.03
54	1874	trans-p-Mentha-1(7).8-dien-2-ol	0.36 ± 0.03
55	1879	Spathulanol	2.98 ± 0.12
56	1883	benzyl-Isovalerate	0.52 ± 0.04
57	1912	α-Calacorene	0.22 ± 0.06
58	1923	Palustrol	0.13 ± 0.02
59	1932	Sesquisabinene hydrate	6.88 ± 0.41
60	1981	(-)-Caryophyllene oxide	2.64 ± 0.19
61	1989	Benzyl tiglate	0.51 ± 0.04
62	2020	Tridecanal	0.38 ± 0.03
63	2026	Nerolidol	4.90 ± 0.33
64	2041	cis-Caryophyllene	1.58 ± 0.09
65	2053	(-)-Cedreanol	0.70 ± 0.04
66	2059	α-Cedrene	1.21 ± 0.08
67	2067	Elemol	1.00 ± 0.09
68	2120	8-Hydroxylinalool	2.62 ± 0.15
69	2141	Spathulenol	1.21 ± 0.07
70	2153	Eugenol	0.54 ± 0.04
71	2163	α-Muurolol	4.57 ± 0.35
72	2171	Cubenol	0.35 ± 0.02
73	2177	α-Cadinol	1.51 ± 0.13
74	2193	ar-Turmerol	0.47 ± 0.03
75	2221	Epiglobulol	1.58 ± 0.11
76	2245	Juniper camphor	2.68 ± 0.19
77	2289	Isoaromadendrene epoxide	0.69 ± 0.04
78	2294	Tricosane	0.13 ± 0.01
79	2327	α-Santalol	0.34 ± 0.02
80	2407	Nerolidol	0.56 ± 0.04
81	2494	Pentacosane	0.21 ± 0.02
82	2570	1-Octadecanol	0.47 ± 0.03
83	2597	Phytol	0.19 ± 0.02
Total			100.00

± 0.33), α-muurolol (4.57 ± 0.35), borneol (4.21 ± 0.17), camphor (3.53 ± 0.27), limonene (3.17 ± 0.25), α-terpineol (3.13 ± 0.23), spathulanol (2.98 ± 0.12), α-pinene (2.95 ± 0.19), juniper camphor (2.68 ± 0.19), (-)-caryophyllene oxide (2.64 ± 0.19), 8-hydroxylinalool (2.62 ± 0.15), Δ-cadinene (2.48 ± 0.16), sabinene (2.32 ± 0.11), trans-verbenol (2.06 ± 0.11), and alloaromadendrene (2.0 ± 0.16). β-Pinene (1.89 ± 0.10), cis-caryophyllene (1.58 ± 0.09), epiglobulol (1.58 ± 0.11), α-cadinol (1.51 ± 0.13), 4-terpineol (1.45 ± 0.09), germacrene D (1.26 ± 0.09), α-copaene (1.24 ± 0.09), α-cedrene (1.21 ± 0.08), and spathulenol (1.21 ± 0.07) were other significant compounds in the EO of *T. cilicicum*. EO yield of the aerial parts of *T. cilicicum* was 0.4% (v/w). In previous reports, essential oil yield and chemical constituents of *Tanacetum* sp. were reported in different species belonging to genus *Tanacetum* (Table 1). It seemed that yield and composition differed from previous reports owing to the species differences belonging to this genus.

3.2. Antimicrobial Activity Results of the EO of T. cilicicum. Antibiotics raise serious concerns in relation to antimicrobial resistance problems for all group of organisms. Therefore, natural sources of the environments have been intensively studied by many scientists to combat their undesirable effects to the health of living organisms and their environments as well [33–35]. In this study, EO of *T. cilicicum* was tested on twelve microorganisms, with two methods: disc diffusion and macrobroth dilution. In addition, standard antibiotics were used for comparison during the assays. As shown in Table 4, the results of the disc diffusion assay indicated that the most sensitive microorganisms were *B. subtilis* and *S. aureus* 29213. This was followed by *B. cereus* = *S. aureus* BAA > *E. faecalis* > *E. casseliflavus*. The susceptibilities of the Gram-negative bacteria towards the essential oil were less than Gram-positive bacteria. The most susceptible Gram-negative bacterium was *E. hormaechei* > *E. coli* > *K. pneumonia* = *P. aeruginosa*. In addition, *C. parapsilosis* was also more susceptible than that of *C. albicans*.

Table 4: Antimicrobial activities of the EO from *T. cilicicum* using disc diffusion assay.

Microorganisms	Source	EO (15 μL)	SXT5	VA30	TEC30	NS 100 IU
		Inhibition zones (mm)				
B. subtilis	ATCC 6633	19.33 ± 0.57^{aB}	34.66 ± 0.57^{aA}	19.33 ± 0.57^{cB}	15.33 ± 0.57^{eC}	—
B. cereus	EU (food isolate)	16.00 ± 0.00^{bC}	23.33 ± 1.15^{eA}	20.00 ± 0.00^{bcB}	22.33 ± 0.57^{aA}	—
E. faecalis	ATCC 29212	15.67 ± 0.57^{bcC}	25.00 ± 1.00^{deA}	21.00 ± 1.00^{bcB}	17.66 ± 0.57^{dC}	—
E. casseliflavus	ATCC 700327	14.33 ± 0.57^{cD}	26.66 ± 0.57^{dA}	24.66 ± 0.57^{aB}	21.00 ± 1.00^{abC}	—
S. aureus	ATCC 29213	19.66 ± 0.57^{aB}	32.00 ± 0.00^{bA}	21.66 ± 1.52^{bB}	20.00 ± 1.00^{bcB}	—
S. aureus	ATCC BAA977	16.33 ± 0.57^{bD}	33.00 ± 0.00^{abA}	20.00 ± 0.00^{bcB}	18.66 ± 0.57^{cdC}	—
K. pneumoniae	ATCC 700603	10.33 ± 0.57^{eB}	18.00 ± 1.00^{fA}	0.00 ± 0.00^{dC}	0.00 ± 0.00^{fC}	—
E. hormaechei	ATCC 700323	12.00 ± 0.00^{dB}	26.00 ± 0.00^{dA}	0.00 ± 0.00^{dC}	0.00 ± 0.00^{fC}	—
P. aeruginosa	ATCC 27853	10.00 ± 0.00^{eA}	0.00 ± 0.00^{gB}	0.00 ± 0.00^{dB}	0.00 ± 0.00^{fB}	—
E. coli	ATCC 25922	10.66 ± 0.57^{deB}	28.66 ± 0.57^{cA}	0.00 ± 0.00^{dC}	0.00 ± 0.00^{fC}	—
C. parapsilosis	ATCC 22019	29.00 ± 0.00^{aA}	—	—	—	16.00 ± 1.00^{bB}
C. albicans	ATCC 14053	16.33 ± 0.57^{bB}	—	—	—	19.66 ± 1.15^{aA}

EO: essential oil; SXT5: Trimethoprim/Sulfamethoxazole; VA30: Vancomycin; TEC30: Teicoplanin; NS 100 IU: Nystatin. The small case letters as the superscript in the columns and capital letters as the superscript in the columns showed the statistical differences in each column and row, respectively ($p < 0.05$). — indicates not tested.

Table 5: Minimum Inhibitor Concentration and Minimum Bactericidal/Fungicidal Concentration values of *T. cilicicum* essential oil.

Microorganisms		EO (μL/mL)		AMP (μg/mL)		CLO (μg/mL)	
		MIC	MBC/MFC	MIC	MBC	MIC	MFC
B. subtilis	ATCC 6633	0.39 ± 0.00^{a}	0.78 ± 0.00^{a}	15.62 ± 0.00^{d}	31.25 ± 0.00^{a}	—	—
B. cereus	EU (food isolate)	0.78 ± 0.00^{a}	1.56 ± 0.00^{a}	1000 ± 0.00^{g}	$>1000 \pm 0.00^{d}$	—	—
E. faecalis	ATCC 29212	6.25 ± 0.00^{b}	12.50 ± 0.00^{c}	0.48 ± 0.00^{a}	125.0 ± 0.00^{b}	—	—
E. casseliflavus	ATCC 700327	6.25 ± 0.00^{b}	12.50 ± 0.00^{c}	0.48 ± 0.00^{a}	31.25 ± 0.00^{a}	—	—
S. aureus	ATCC 29213	6.25 ± 0.00^{b}	6.25 ± 0.00^{b}	31.25 ± 0.00^{e}	250.0 ± 0.00^{c}	—	—
S. aureus	ATCC BAA977	5.20 ± 1.80^{b}	6.25 ± 0.00^{b}	125.0 ± 0.00^{f}	250.0 ± 0.00^{c}	—	—
K. pneumoniae	ATCC 700603	10.41 ± 3.60^{c}	50.00 ± 0.00^{e}	$>1000 \pm 0.00^{g}$	$>1000 \pm 0.00^{d}$	—	—
E. hormaechei	ATCC 700323	6.25 ± 0.00^{b}	25.00 ± 0.00^{d}	7.81 ± 0.00^{c}	41.66 ± 8.04^{a}	—	—
P. aeruginosa	ATCC 27853	12.5 ± 0.00^{c}	50.00 ± 0.00^{e}	$>1000 \pm 0.00^{g}$	$>1000 \pm 0.00^{d}$	—	—
E. coli	ATCC 25922	6.25 ± 0.00^{b}	6.25 ± 0.00^{b}	3.90 ± 0.00^{b}	125.0 ± 0.00^{b}	—	—
C. parapsilosis	ATCC 22019	3.12 ± 0.00^{ab}	12.5 ± 0.00^{c}	—	—	300 ± 0.00^{a}	$>300 \pm 0.00^{a}$
C. albicans	ATCC 14053	1.56 ± 0.00^{a}	6.25 ± 0.00^{b}	—	—	$>300 \pm 0.00^{a}$	$>300 \pm 0.00^{a}$

In each column, small case letters as the superscript in the columns showed the statistical differences ($p < 0.05$). — indicates not tested.
MIC: Minimum Inhibitory Concentration; MFC: Minimum Fungicidal Concentration; AMP: Ampicillin; CLO: Clotrimazole.

As shown in Table 5, according to MIC/MBC (μL/mL) assays, it was found that the most potent activity was observed on *B. subtilis* (0.39/0.78) and *B. cereus* (0.78/1.56). Higher inhibitory and cidal values of the EO from *T. cilicicum* were observed for other tested microorganisms. This was statistically the same for *S. aureus* BAA (5.20/6.25) = *E. casseliflavus* (6.25/12.5) = *S. aureus* 29213 (6.25/6.25) = *E. hormaechei* (6.25/25.00) = *E. coli* (6.25/6.25) = *E. faecalis* (6.25/12.5). Less activities were determined for two Gram-positive bacteria and the activity was statistically the same for *K. pneumoniae* (10.41/50.00) and *P. aeruginosa* (12.5/50.00). MIC and MFC values (μL/mL) of *T. cilicicum* were 1.56/6.25 for *C. albicans* and 3.12/12.5 for *C. parapsilosis*.

In previous studies, EO of *Tanacetum* sp. was assayed on various microorganisms. The EO from *T. balsamita* subsp. *balsamita* and *T. chiliophyllum* var. *chiliophyllum* revealed inhibitory zones on the growth of *B. subtilis* ATCC 6633 (13-17 mm), *S. aureus* ATCC 6538 P (17–15), *E. coli* ATCC 25922 (15-16 mm), *C. glabrata* ATCC 66032 (15–18 mm), and *C. tropicalis* ATCC 13803 (14–17 mm) [14]. In another study, EO from the flowers and stems of *T. chiliophyllum* var. *chiliophyllum* grown in three different localities showed MIC values (μg/mL) as follows: *S. aureus* ATCC 6538 (250/500, 150/500, and 250/>500), meticillin resistant *S. aureus* (clinical isolate) (500/125, 750/500, and 250/250), *S. epidermidis* ATCC 12228 (250/250, 150/500, and 500/31.2), *B. cereus* NRRL B-3711 (250/125, 150/500, and >500/125), *B. subtilis* NRRL B-4378 (500/125, 375/500, and 500/62.5), *E. coli* NRRL B-3008 (>500/62.5, >150/500, and >500/62.5), *P. aeruginosa* ATCC 27853 (500/250, 150/500, and 500/31.25), and *E. aerogenes* NRRL 3567 (500/500, >150/>500, and >500/62.5) [19]. EO from the flowers and stems of *T. chiliophyllum* var. *monocephalum* had MIC values (μg/mL): *S. aureus* ATCC 6538 (>500, 1000), meticillin resistant *S. aureus* (125, >500),

B. cereus NRRL B-3711 (62.5, 1000), *B. subtilis* NRRL B-4378 (250, >1000), *E. coli* NRRL B-3008 (>500, >1000), *P. aeruginosa* ATCC 27853 (500, 1000), and *E. aerogenes* NRRL 3567 (>500, >1000) [20]. MIC values (μg/mL) of the EO from *T. argenteum* subsp. *flabellifolium* were as follows: *E. coli* ATCC 25922 (250), *S. aureus* ATCC 6538 (125), *P. aeruginosa* ATCC 27853 (125), *E. aerogenes* NRRL 3567 (125), and *C. albicans* O.G.U (125) [15].

In another study, the EO of *T. aucheranum* and *T. chiliophyllum* revealed various level of antimicrobial activities on test microorganisms except *P. cichorii* and *B. subtilis* ATCC 6633 using the disc diffusion and broth dilution assay. Inhibitory zones (as mm) and MIC values (μL/mL) of the essential oils from *T. aucheranum* and *T. chiliophyllum* were as follows, respectively: *P. chlororaphis* (13.5 mm, 166.7) (13.3 mm, 500.0), *P. syringae* pv. *syringae* (7.8 mm, 500.0), (7.5 mm, 500.0), *B. coagulans* (11.8 mm, 166.7), (10.2 mm, 166.7), *E. faecalis* ATCC 29122 (9.0 mm, 1000.0), (0.0, 0.0), *S. aureus* ATCC 29213 (9.5 mm, 1000,0), (11.5 mm, 1000.0), *E. intermedius* (10.7 mm, 166.7), (10.2 mm, 54.4), *E. coli* (9.8 mm, 166.7), (8.8 mm, 500.0), *P. aeruginosa* ATCC 27859 (19.8 mm, 166.7), (16.5 mm, 500.0), *P. aeruginosa* ATCC 9027 (11.0 mm, 1000.0), (12.0 mm, 1000.0), and *K. trevisanii* (9.3 mm, 500.0), (8.2 mm, 166.7) [16]. The differences of the results of the present study when compared to the previous reports could be attributed to the chemical differences in the species assayed.

3.3. Phytotoxicity Assay of T. cilicicum. As shown in Table 6, EO of *T. cilicicum* as well as the herbicide (glyphosate (N-(phosphonomethyl) glycine) at 0.062, 0.125, 0.25, 0.50, 1.0, and 2.0 mg/mL were assayed on the seeds of *L. sativa*, *L. sativum,* and *P. oleracea*. Effects of test substances were dependent on the dose activity. EO at 0.25 mg/mL and the lower concentrations did not reveal any inhibitory effects on the seeds of *L. sativa*, *L. sativum,* and *P. oleracea*. Higher concentrations ranging from 0.50 to 2.0 mg/mL had inhibitory effects on the growth of *L. sativa*, *L. sativum,* and *P. oleracea*. Compared to the control, 0.50, 1.0, and 2.0 mg/mL doses of the EO inhibited the seed germination by 87.97 and 100% in *L. sativa*, by 55, 86, and 92% in *L. sativum*, and by 19, 50, and 89% in *P. oleracea*, respectively.

None of the doses of the herbicide revealed any inhibitory effects on the seed germination of *P. oleracea*. As the EO, lower concentrations of the herbicide ranging from 0.062 to 0.25 mg/mL had no inhibitory effect on the seed germination of *L. sativa* and *L. sativum*. At 0.50 mg/mL and higher doses, herbicide inhibited the seed germination by 10, 10, and 20% in *L. sativa* and by 13, 13, and 13% in *L. sativum*, respectively. It appeared that the inhibitory effects of the essential oil on the germination of test seeds seem to be more noteworthy than those of the herbicide.

3.4. Effects on the Seedling Growth. In the agriculture, herbicides have been utilised in the fields to overcome undesirable attack of various organisms to crops. Detrimental effects of these chemicals are not questionable anymore because of long term negative effects on the soil structure and community [36, 37]. Natural compounds are very attractive constituents to overcome the negative effects of the synthetic chemicals. In this study, inhibitory effects at the lowest dose of the EO (0.0625 mg/mL) were not observed on the radicle growth of *L. sativa* seeds. Two-fold increase onwards showed decreasing effects on the radicle. The most significant decreasing effects were 95% at 1.0 mg/mL and 100% at 2.0 mg/mL, respectively. Plumules of *L. sativa* were also inhibited significantly by 85% at 0.50 mg/mL; however, a complete inhibition was observed at 1.0 and 2.0 mg/mL. Herbicide in this assay was not as effective as the EO at the same doses (Table 6). A complete inhibition was not observed on neither the length of radicle nor the plumule of *L. sativa*. Herbicide seemed to have more inhibitory effects on the radicle growth of *L. sativa* rather than the plumule growth at 0.0625–2.0 mg/mL. It appeared that herbicide was not found to be as effective as the essential oil.

EO revealed significant inhibitory effects on radicle growth of *L. sativum* by 93% at 0.50 mg/mL, 98% at 1.0 mg/mL, and 99% at 2.0 mg/mL. It was also found to be very active on the plumule growth of *L. sativum* by 91% at 0.50 mg/mL onwards. A complete inhibition of the plumule growth of *L. sativum* was observed at 1.0 and 2.0 mg/mL. It seemed that EO seemed to be more effective on the plumule growth of *L. sativum*. Compared to the EO, herbicide revealed similar inhibitory effects on the radicle growth, whereas inhibition on both the radicle and the plumule was not effective as the EO.

EO of *T. cilicicum* inhibited the growth of *P. oleracea* at all doses; however, the most significant inhibitory effects on the radicle growth of *P. oleracea* were observed at 0.5 mg/mL onwards. At 0.50, 1.0, and 2.0 mg/mL, inhibitions of the radicle were 95%, 97%, and 99%, respectively. The plumule growth of *P. oleracea* was also inhibited by 94% at 0.50 mg/mL; however, a total inhibition was observed at 1.0 and 2.0 mg/mL of the EO doses. The present findings indicated that effects of the EO were more prominent on the plumule growth. Unlike EO, different doses of the herbicide had no inhibitory effect on neither the radicle nor the plumule growth of *P. oleracea*; however, inhibitory effects of the herbicide were more prominent on radicle growth rather than plumule growth of *P. oleracea*.

In a previous study, the bioherbicidal potential of two species of the genus *Tanacetum* has been reported by Salamci et al. (2007), who found that EO of *T. aucheranum* and *T. chiliophyllum* at the dose of 30 μL per petri receiving 50 test seeds completely inhibited the seed germination, radicle, and plumule growth of *A. retroflexus*, *C. album,* and *R. crispus*. The findings of the previous and the present results indicated that *Tanacetum* species has active bioherbical constituents and deserves to be studied in more detail in advance.

4. Conclusion

In the present study, EO of *T. cilicicum* includes significant amount of antimicrobial compounds against a wide range of microorganisms and therefore, it could be used in food, agricultural, and pharmaceutical applications and so forth. Furthermore, significant phytoconstituents of the EO from *T.*

Table 6: Effects of the essential oil from *T. cilicicum* on the seed germination (%), radicle, and plumule growth of *L. sativa, L. sativum,* and *P. oleracea*.

	Lactuca sativa			*Lepidium sativum*			*Portulaca oleracea*		
	SG (%)	RL (mm) (mean ± SD)	PL (mm) (mean ± SD)	SG (%)	RL (mm) (mean ± SD)	PL (mm) (mean ± SD)	SG (%)	RL (mm) (mean ± SD)	PL (mm) (mean ± SD)
Control	100.0 ± 0.00^{a*}	40.73 ± 1.04^{a}	27.33 ± 1.77^{a}	100.0 ± 0.00^{a}	50.86 ± 3.14^{a}	39.33 ± 1.54^{a}	100.0 ± 0.00^{a}	43.36 ± 1.20^{a}	20.13 ± 1.88^{a}
EO (mg/mL)									
0.062	100.0 ± 0.00^{a}	40.70 ± 1.16^{a}	20.33 ± 1.30^{b}	100.0 ± 0.00^{a}	49.73 ± 2.31^{a}	30.90 ± 2.80^{b}	100.0 ± 0.00^{a}	33.36 ± 1.64^{b}	16.20 ± 3.10^{ab}
0.125	100.0 ± 0.00^{a}	33.30 ± 1.63^{b}	17.83 ± 1.27^{b}	100.0 ± 0.00^{a}	39.73 ± 1.08^{b}	24.23 ± 1.90^{c}	100.0 ± 0.00^{a}	31.40 ± 1.58^{b}	15.63 ± 2.64^{ab}
0.25	100.0 ± 0.00^{a}	29.16 ± 1.86^{c}	14.16 ± 1.49^{c}	100.0 ± 0.00^{a}	34.16 ± 1.52^{c}	21.73 ± 2.36^{c}	100.0 ± 0.00^{a}	20.33 ± 2.59^{c}	11.76 ± 2.52^{b}
0.50	13.33 ± 2.00^{b}	10.75 ± 1.59^{d}	04.00 ± 0.81^{d}	45.53 ± 1.15^{b}	07.44 ± 1.82^{d}	09.61 ± 2.00^{d}	81.10 ± 1.65^{b}	02.33 ± 0.84^{d}	01.25 ± 0.44^{c}
1.00	03.00 ± 0.00^{c}	02.00 ± 0.00^{e}	00.00 ± 0.00^{e}	14.43 ± 2.88^{c}	02.00 ± 0.74^{e}	00.00 ± 0.00^{e}	50.00 ± 3.56^{c}	01.09 ± 0.29^{d}	00.00 ± 0.00^{c}
2.00	00.00 ± 0.00^{d}	00.00 ± 0.00^{e}	00.00 ± 0.00^{e}	07.76 ± 0.57^{d}	01.00 ± 0.00^{e}	00.00 ± 0.00^{e}	11.10 ± 1.73^{d}	01.00 ± 0.00^{d}	00.00 ± 0.00^{c}
Herbicide (mg/mL)									
0.062	100.0 ± 0.00^{a}	07.23 ± 1.40^{b}	26.13 ± 1.25^{a}	100.0 ± 5.01^{a}	33.33 ± 1.14^{b}	22.00 ± 1.57^{b}	100.0 ± 0.00^{a}	11.00 ± 2.82^{b}	14.63 ± 1.80^{b}
0.125	100.0 ± 0.00^{a}	05.56 ± 1.04^{bc}	24.36 ± 1.55^{ab}	100.0 ± 4.35^{a}	29.06 ± 1.06^{bc}	20.53 ± 1.52^{b}	100.0 ± 0.00^{a}	07.96 ± 1,71^{c}	10.50 ± 1.35^{c}
0.25	100.0 ± 0.00^{a}	04.80 ± 0.84^{c}	20.10 ± 1.96^{b}	100.0 ± 1.52^{a}	26.20 ± 2.63^{c}	18.88 ± 1.99^{b}	100.0 ± 0.00^{a}	07.26 ± 1.30^{c}	08.11 ± 0.96^{cd}
0.50	90.00 ± 0.57^{a}	04.74 ± 0.81^{c}	10.59 ± 2.76^{c}	86.66 ± 1.52^{b}	06.85 ± 1.26^{d}	04.83 ± 1.62^{c}	100.0 ± 0.00^{a}	05.60 ± 1.29^{cd}	07.70 ± 1.27^{cd}
1.00	90.00 ± 1.52^{a}	04.20 ± 0.95^{c}	08.37 ± 1.84^{cd}	86.66 ± 1.52^{b}	05.86 ± 0.89^{d}	04.00 ± 0.90^{c}	100.0 ± 0.00^{a}	04.28 ± 0.71^{d}	05.05 ± 1.25^{de}
2.00	80.00 ± 2.04^{b}	03.85 ± 0.74^{c}	05.35 ± 1.53^{d}	86.66 ± 0.57^{b}	05.00 ± 1.08^{d}	03.63 ± 1.21^{c}	100.0 ± 0.00^{a}	04.00 ± 0.69^{d}	03.73 ± 0.73^{e}

SG: seed germination; RL radicle length; PL plumule length; SD: standard deviation. *In the same column, different letters showed significant statistical differences for the applied doses of the essential oil ($p < 0.05$).

cilicicum could be suggested for use in the various formulation of biopesticides. However, further studies are required for isolating the bioactive constituents in the EO and testing alone and/or their synergistic, antagonistic relationships as well as determining their toxic effects on test organisms before potential industrial benefits.

Competing Interests

The authors declare that they have no competing interests.

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