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Chemical Composition, Algicidal, Antimicrobial, and Antioxidant Activities of the Essential Oils of *Taiwania flousiana* Gaussen

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Abstract: *Taiwania flousiana (T. flousiana)* Gaussen is a precious wood in the family Taxodiaceae. This study investigated the chemical components of the essential oil from the stem bark of *T. flousiana* and its algicidal, antifungal, and antioxidant properties. Sixty-nine compounds representing 89.70% of the stem bark essential oil were identified by GC-MS. The essential oil showed strong anti-algae, anti-bacteria, and anti-fungus activities against the tested species, and antioxidant activities. The IC_{50} values of the essential oil against chlorophyll a, chlorophyll b, and the total chlorophyll of *Spirogyra communis* (a species of algae), 24–96 h after the treatment, ranged from 31.77 to 84.92 μg/mL, while the IC_{50} values of butachlor ranged from 40.24 to 58.09 μg/mL. Ultrastructure changes revealed by the transmission electron microscopy indicated that the main algicidal action sites were the chloroplast and cell wall. The essential oil showed antifungal activities on *Rhizoctonia solani* (EC_{50} = 287.94 μg/mL) and *Colletotrichum gloeosporioiles* (EC_{50} = 378.90 μg/mL). It also showed bactericidal activities on *Ralstonia solanacearum* and *Staphylococcus aureus*, with zones of inhibition (ZOIs) being 18.66 and 16.75 mm, respectively at 40 μg/disk. Additionally, the essential oil possessed antioxidant activity estimated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (IC_{50} = 33.51 μg/mL; IC_{50} value of the positive control ascorbic acid was 7.98 μg/mL). Thus, the essential oil of this plant might be used as a possible source of natural bioactive molecules in agrochemical industry as well as in food and cosmetic industries.

Keywords: *Taiwania flousiana* Gaussen; essential oil; algicidal activity; antimicrobial activity; antioxidant activity; algicidal mechanism

1. Introduction

T. flousiana (a synonym of *Taiwania cryptomerioides* Hayata), belonging to the family Taxodiaceae, is endemic to China. *T. flousiana* has been considered as one of the most precious woods in China for its outstanding quality. It has been used in construction industry, furniture industry, and paper industry [1–3]. Thus, the cultivation and plantation of *T. flousiana* have received significant attention [4–8]. The chemical constituents of the extract from the bark of *T. flousiana* have been investigated [9] and there were patents on the use of the chemicals from *T. flousiana* in medicinal industry [10]. Recently, a new chemical isolated from *T. flousiana* with strong herbicidal activity has been patented also [11]. However, it has not been previously investigated chemically for its essential oil.

In the production process of farm produce, various undesirable biotic factors such as algae and microbes can cause great loss of quantity and quality. *Spirogyra* (Zygnemataceae, Zygnematales) is a

genus in the Class Zygnematophyceae (Conjugatophyceae), which is a member of the Infrakingdom Streptophyta. *Spirogyra communis* (Hassall) Kuetzing in the genus is widely distributed in freshwater habitats including flowing water, permanent ponds, and temporary pools and can cause great loss to farm produce [12,13]. Microbes such as *Rhizoctonia solani*, *Fusarium moniliforme* Sheld, and *Fusariun oxysporun*, etc., are common pathogens with a great diversity of host plants and can significantly reduce the quantity and quality of farm produce [14–16]. Normally, synthetic chemicals are extensively used to control them. However, the resistance issues caused by synthetic control agents are very common. Meanwhile, synthetic control agents lack selectivity and are toxic to non-target organisms. As an important part of pesticides, the algicide resistance has been reported. Meanwhile, synthetic algicides such as diuron are toxic and have negative impact on the environment. Similarly, synthetic antimicrobial chemicals have the same problems [17,18]. Therefore, as an alternative to these synthetic control agents, natural compounds and extracts from plants are the main sources [19]. Among them, essential oil has received significant attention [20,21].

Consumers need natural control agents not only in the production process of farm produce, but also in food preservation process. Nowadays, antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods by free radicals. Normally, synthetic antioxidants such as butylated hydroxyanisole have been widely used. However, the toxicity of synthetic antioxidants has been questioned. Thus, the development of natural antioxidants is warmly desired [22]. Plant-derived essential oil has also received significant attention in this field [23]. In this work, we extracted the essential oil of *T. flousiana* and further (i) identified its chemical constituents; (ii) investigated its algicidal, antifungal, antibacterial, and antioxidant activities; (iii) characterized its mechanisms as an algicide.

2. Results

2.1. Chemical Components Identified in the Essential Oil

The major components of the essential oil identified from *T. flousiana* are listed in Table 1. The yield of the essential oil extracted from *T. flousiana* was 0.31% (*v/w*). The GC-MS chromatogram of the essential oil is shown in Figure 1. In total, 69 components were identified and accounted for 89.70% of the total oil composition. The oil composition was dominated by the presence of hexadecanoic acid comprising 27.13% from total, followed by 2-penten-1-ol, 3-methyl-5-[octahydro-4, 5-dimethyl-7a-(1-methylethenyl)-1H-inden-4-yl]-(16.16%), linoleic acid (13.48%), podocarpa-6,8,11,13-tetraen-12-ol, 13-isopropyl-, acetate (7.56%), ferruginol (6.52%), and α-linolenic acid (5.41%). The contents of abietatriene, tetradecanoic acid, pentadecanoic acid ranged from 1.04 to 1.37%. The others were less than 1.00% (Table 1).
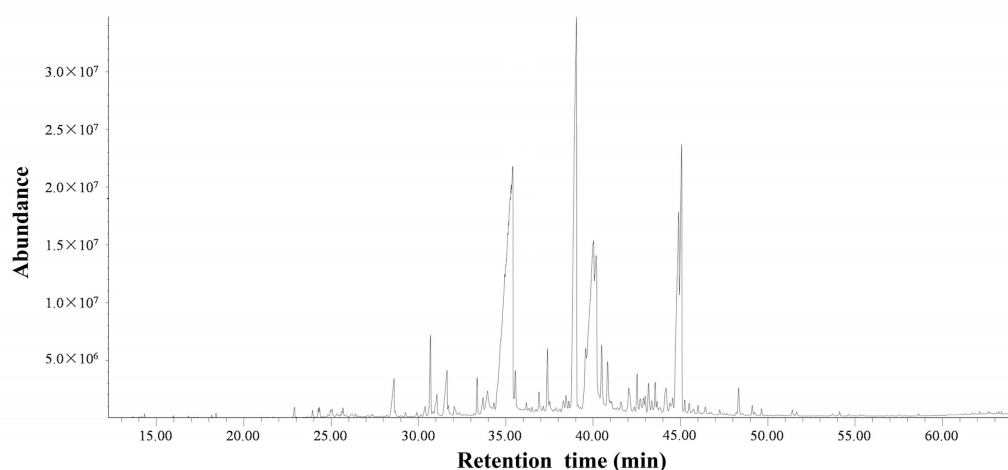

Figure 1. The GC-MS chromatogram of the essential oil.

Table 1. Percent concentration (%) of chemical constituents of *T. flousiana* stem bark essential oils.

No.	Compounds	Molecular Formula	Percentage (%)	RI [a]	RI [b] (Reference)	Methods of Identification
Fatty Acids						
1	Nonanoic acid	$C_9H_{18}O_2$	0.03	1264	2192 [24]	a,c,d
2	Decanoic acid	$C_{10}H_{20}O_2$	0.03	1361	2298 [25]	a,c,d
3	Dodecanoic acid	$C_{12}H_{24}O_2$	0.18	1560	2503 [24]	a,c,d
4	Tridecanoic acid	$C_{13}H_{26}O_2$	0.02	1657	2617 [25]	a,c,d
5	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.10	1764	2670 [25]	a,c,d
6	14-Pentadecenoic acid	$C_{15}H_{28}O_2$	0.52	1848	3181 [26]	a,c,d
7	Pentadecanoic acid	$C_{15}H_{30}O_2$	1.37	1868	2822 [25]	a,c,d
8	Palmitoleic acid	$C_{16}H_{30}O_2$	0.81	1946	2948 [27]	a,c,d
9	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	0.09	1952	-	a,c,d
10	Hexadecanoic acid	$C_{16}H_{32}O_2$	27.13	1992	2931 [25]	a,c,d
11	*cis*-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	0.08	2055	-	a,c,d
12	Heptadecanoic acid	$C_{17}H_{34}O_2$	0.20	2068	2305 [28]	a,c,d
13	Linoleic acid	$C_{18}H_{32}O_2$	13.48	2155	3157 [29]	a,c,d
14	α-Linolenic acid	$C_{18}H_{30}O_2$	5.41	2160	3193 [29]	a,c,d
Monoterpenes						
15	α-Terpineol	$C_{10}H_{18}O$	0.03	1194	1706 [24]	a,c,d
16	Carvacrol	$C_{10}H_{14}O$	0.02	1301	2241 [30]	a,c,d
Sesquiterpenes						
17	(-)-Spathulenol	$C_{15}H_{24}O$	0.09	1599	2144 [25]	a,c,d
18	Widdrol	$C_{15}H_{26}O$	0.12	1611	2179 [31]	a,c,d
19	Cedrol	$C_{15}H_{26}O$	0.13	1614	2093 [32]	a,c,d
20	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol	$C_{15}H_{24}O$	0.05	1631	-	a,c,d
21	8-Cedren-13-ol	$C_{15}H_{24}O$	0.11	1637	2199 [33]	a,c,d
22	γ-Eudesmol	$C_{15}H_{26}O$	0.12	1640	2193 [24]	a,c,d
23	*epi*-α-Muurolol	$C_{15}H_{26}O$	0.06	1650	1621 [34]	a,c,d
24	β-Eudesmol	$C_{15}H_{26}O$	0.06	1660	2257 [24]	a,c,d
25	T-cadinol	$C_{15}H_{26}O$	0.13	1662	2187 [31]	a,c,d
26	Germacra-4(15),5E,10(14)-trien-1β-ol	$C_{15}H_{24}O$	0.05	1667	-	a,c,d
27	Humulenol-II	$C_{15}H_{24}O$	0.09	1681	-	a,c,d
28	α-Bisabolol	$C_{15}H_{26}O$	0.05	1688	2232 [35]	a,c,d
29	Longifolaldehyde	$C_{15}H_{24}O$	0.02	1692	-	a,c,d

Table 1. *Cont.*

No.	Compounds	Molecular Formula	Percentage (%)	RI [a]	RI [b] (Reference)	Methods of Identification
30	β-Acoradienol	$C_{15}H_{24}O$	0.05	1787	-	a,c,d
31	Drimenol	$C_{15}H_{26}O$	0.04	1817	1772 [36]	a,c,d
Diterpenes						
32	Ambrial	$C_{16}H_{26}O$	0.07	1809	-	a,c,d
33	Biformene	$C_{20}H_{32}$	0.32	1937	1907 [37]	a,c,d
34	Cembrene	$C_{20}H_{32}$	0.16	1958	-	a,c,d
35	Manoyl oxide	$C_{20}H_{34}O$	0.64	2000	2376 [31]	a,c,d
36	13-*epi*-Manoyl oxide	$C_{20}H_{34}O$	0.11	2021	2335 [38]	a,c,d
37	Manool	$C_{20}H_{34}O$	0.03	2027	2180 [39]	a,c,d
38	Geranyl linalool	$C_{20}H_{34}O$	0.06	2033	1912 [37]	a,c,d
39	Abietatriene	$C_{20}H_{30}$	1.04	2063	2065 [37]	a,c,d
40	Thunbergol	$C_{20}H_{34}O$	0.02	2076	-	a,c,d
41	Abieta- 7, 13- diene	$C_{20}H_{32}$	0.04	2088	-	a,c,d
42	2-Penten-1-ol, 3-methyl-5-[octahydro-4,5-dimethyl-7a-(1-methylethenyl)-1H-inden-4-yl-	$C_{20}H_{34}O$	16.16	2121	-	a,c,d
43	Sandaracopimarinal	$C_{20}H_{30}O$	0.17	2192	-	a,c,d
44	Larixol	$C_{20}H_{34}O_2$	0.28	2211	-	a,c,d
45	4-*epi*-Dehydroabietol	$C_{20}H_{30}O$	0.77	2227	-	a,c,d
46	Nimbiol	$C_{18}H_{24}O_2$	0.26	2258	-	a,c,d
47	Isopimara-7,15-dien-3-one	$C_{20}H_{30}O$	0.26	2261	-	a,c,d
48	*trans*-Totarol	$C_{20}H_{30}O$	0.19	2286	2280 [32]	a,c,d
49	Dehydroabietal	$C_{20}H_{28}O$	0.78	2305	-	a,c,d
50	Podocarpa-6,8,11,13-tetraen-12-ol, 13-isopropyl-, acetate	$C_{22}H_{30}O_2$	7.56	2332	-	a,c,d
51	Ferruginol	$C_{20}H_{30}O$	6.52	2339	2327 [37]	a,c,d
52	Hinokione	$C_{20}H_{28}O_2$	0.51	2463	-	a,c,d
53	Dronabinol	$C_{21}H_{30}O_2$	0.11	2515	-	a,c,d
Esters						
54	Methyl pentadecanoate	$C_{15}H_{30}O_2$	0.22	1825	2099 [29]	a,c,d
55	Diisobutyl phthalate	$C_{16}H_{22}O_4$	0.14	1870	-	a,c,d
56	Cyperolactone	$C_{15}H_{22}O_2$	0.02	1873	2480 [40]	a,c,d
57	Methyl hexadecanoate	$C_{17}H_{34}O_2$	0.46	1926	2226 [41]	a,c,d

Table 1. *Cont.*

No.	Compounds	Molecular Formula	Percentage (%)	RI [a]	RI [b] (Reference)	Methods of Identification
58	Methyl linoleate	$C_{19}H_{34}O_2$	0.35	2099	2490 [42]	a,c,d
59	Methyl linolenate	$C_{19}H_{32}O_2$	0.17	2105	2478 [42]	a,c,d
Phenols						
60	2-Allyl-4-methylphenol	$C_{10}H_{12}O$	0.05	1373	-	a,c,d
61	2,2-Methylene-bis(4-methyl-6-tert-butylphenol)	$C_{23}H_{32}O_2$	0.10	2421	-	a,c,d
Alkanes						
62	Docosane	$C_{22}H_{46}$	0.07	2200	2196 [34]	a,c,d
63	Pentacosane	$C_{25}H_{52}$	0.05	2499	2500 [25]	a,c,d
64	Heptacosane	$C_{27}H_{56}$	0.06	2698	2700 [25]	a,c,d
65	Nonacosane	$C_{29}H_{60}$	0.03	2895	2900 [25]	a,c,d
Others						
66	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	$C_{14}H_{22}O_3$	0.02	1740	-	a,c,d
67	*cis*-9-Hexadecenal	$C_{16}H_{30}O$	0.03	1750	-	a,c,d
68	1-Hexadecanol	$C_{16}H_{34}O$	0.17	1881	2384 [27]	a,c,d
69	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	0.03	2041	-	a,c,d
	Total		89.70			

a Kováts retention indices (RI) calculated from the retention time in relation to those of a series of C7–C30 n-alkanes on a HP-5 column. b Kováts retention indices (RI) on HP-Innowax column from literature. c Values compared with [43]. d The mass spectra of authentic reference compounds where possible and by reference to NIST 17 database. "-" Kováts retention indices (RI) on HP-Innowax column was not found from the literature.

2.2. Algicidal Activity and Algicidal Mechanism of Action of T. flousiana Essential Oil

Algicidal activity of the essential oil extracted from stem bark of *T. flousiana* on *S. communis* was tested for the first time. The algicidal effects of *T. flousiana* essential oil on *S. communis* were dose-dependent at the concentrations from 12.5 to 200 µg/mL 24 to 72 h after the treatment.

The IC_{50} values of the essential oil on the inhibition of chlorophyll a ranged from 40.64 to 90.10 µg/mL 24–96 h after the treatment. As a contrast, those of butachlor ranged from 36.60 to 55.28 µg/mL. The IC_{50} values of the essential oil on the inhibition of chlorophyll b ranged from 53.39 to 106.91 µg/mL 24–96 h after the treatment. As a contrast, those of butachlor ranged from 47.29 to 79.12 µg/mL. Specially, 48 h after the treatment, The IC_{50} values of the essential oil was 47.49 µg/mL, while that of butachlor was 62.95 µg/mL, indicating that the essential oil showed a better algicidal effect at 48 h after the treatment based on the inhibition of chlorophyll b. The IC_{50} values of the essential oil on the inhibition of the total chlorophyll ranged from 31.77 to 84.92 µg/mL 24–96 h after the treatment. As a contrast, those of butachlor ranged from 40.24 to 58.09 µg/mL. Specially, 72 h after the treatment, the IC_{50} values of the essential oils was 31.77 µg/mL, while that of butachlor was 40.91 µg/mL, suggesting that the essential oil showed a better algicidal effect at 48 h after the treatment based on the inhibition of chlorophyll b.

In summary, the algicidal activity of the essential oil was comparable to or even better than that of butachlor (Table 2).

Table 2. IC_{50} of *T. flousiana* essential oil and butachlor on the content of chlorophyll a, chlorophyll b, and total chlorophyll of *S. communis*.

Pigment	Treatment	Time (h)	Regression Equation	r	IC_{50} (µg/mL)	95%CL (µg/mL)
Chlorophyll a	Essential oil	24	y = 1.2251 + 1.9312x	0.9327	90.10	74.01–109.68
		48	y = −0.0821 + 3.0372x	0.9896	47.13	42.09–52.76
		72	y = 1.6596 + 2.0762x	0.9437	40.64	34.98–47.21
		96	y = 2.6742 + 1.3534x	0.9536	52.29	42.40–64.50
	Butachlor	24	y = 0.7963 + 2.4091x	0.9459	55.58	48.57–63.60
		48	y = 0.9345 + 2.5763x	0.9794	37.85	33.08–43.30
		72	y = 1.5056 + 2.2331x	0.9946	36.71	31.66–42.58
		96	y = 1.4714 + 2.2568x	0.9174	36.60	31.49–42.54
Chlorophyll b	Essential oil	24	y = 0.7342 + 2.1024x	0.9438	106.91	86.86–131.58
		48	y = −1.2225 + 3.7115x	0.9759	47.49	42.73–52.77
		72	y = 1.9149 + 1.7859x	0.9560	53.39	45.22–63.05
		96	y = 2.7763 + 1.2659x	0.9584	57.09	45.60–71.47
	Butachlor	24	y = 0.6332 + 2.3004x	0.9186	79.12	67.10–93.00
		48	y = −0.0687 + 2.8175x	0.9467	62.95	55.74–71.10
		72	y = 1.5328 + 2.0981x	0.9424	44.93	38.67–52.20
		96	y = 1.8578 + 1.8762x	0.9530	47.29	40.24–55.57
Total chlorophyll	Essential oil	24	y = 1.2976 + 1.9193x	0.9309	84.92	70.19–102.75
		48	y = −0.0821 + 3.0372x	0.9623	46.62	41.67–52.15
		72	y = 1.6945 + 2.2008x	0.9200	31.77	27.16–37.17
		96	y = 2.7412 + 1.3035x	0.9482	54.05	43.48–67.18
	Butachlor	24	y = 0.6609 + 2.4596x	0.9436	58.09	50.76–66.49
		48	y = 0.7453 + 2.6025x	0.9719	43.14	37.87–49.14
		72	y = 1.7028 + 2.0456x	0.9566	40.91	35.07–47.74
		96	y = 1.7113 + 2.0495x	0.9256	40.24	34.43–47.04

2.3. The Effect of Light on the Algicidal Activity of T. flousiana Essential Oil in S. communis

Further study revealed that light could affect the algicidal activity of *T. flousiana* essential oil in *S. communis*. 96 h after the essential oil treatment with light, the IC_{50} values of the essential oil

ranged from 71.58 to 87.89 µg/mL, which were much lower than the values without light ranging from 1156.28 to 1229.24 µg/mL (Table 3). This result indicated that some active ingredients of the essential oil were photo-activated.

Table 3. IC_{50} *T. flousiana* essential oil and Butachlor on the content of chlorophyll a, chlorophyll b, and total chlorophyll of *S. communis* with or without light (96 h).

| Treatment		Regression Equation	r	IC_{50} (µg/mL)	95%CL (µg/mL)
With Light	chlorophyll a	y = −1.9294 + 3.7360x	0.9062	71.58	57.66–88.84
	chlorophyll b	y = −1.5076 + 3.3476x	0.9310	87.89	67.42–114.59
	total chlorophyll	y = −2.0191 + 3.6402x	0.9473	84.77	65.94–108.97
Without Light	chlorophyll a	y = 2.1333 + 0.9279x	0.9814	1229.24	121.24–12463.23
	chlorophyll b	y = 1.6774 + 1.0713x	0.9071	1156.28	156.28–10205.66
	total chlorophyll	y = 2.1286 + 0.9302x	0.9328	1221.58	120.76–12357.62

2.4. Antifungal Activities of T. flousiana Essential Oil

The effects of the essential oil of T. flousiana on Rhizoctonia solani Kuhn, Colletotrichum gloeosporioiles, Fusarium moniliforme Sheld, Thanatephorus cucumeris (Frank) Donk., Fusariun oxysporun f. sp. cubense, and Didymella bryoniae (Auersw.) Rehm. mycelial growth are listed in Table 4. The antifungal activity estimated by the EC_{50} value of the oil indicated a high variation of EC_{50} values among the fungal species (Table 4). The lowest EC_{50} value was observed against R. solani (287.94 µg/mL), while the highest was detected against D. bryoniae (3162.34 µg/mL). Generally, the oil showed better inhibitory activities on R. solani (287.94 µg/mL) and C. gloeosporioiles (378.90 µg/mL). The oil possessed activities on F. moniliforme, T. cucumeris, and F. oxysporun f. sp. cubense and the EC_{50} values were 923.03, 623.36, and 809.07 µg/mL, respectively.

Table 4. The antifungal activities of the essential oil of *T. flousiana* (EC_{50}).

Fungus	Time (h)	Regression Equation	r	EC_{50} (µg/mL)	95%CL(µg/mL)
Rhizoctonia solani	24	y = 1.8819 + 1.2679x	0.9876	287.94	195.23–424.68
Colletotrichum gloeosporioiles	48	y = 2.8088 + 0.8498x	0.9261	378.90	205.68–698.17
Fusarium moniliforme	72	y = 2.4418 + 0.8627x	0.9865	923.03	529.24–1609.80
Thanatephorus cucumeris	72	y = 1.4164 + 1.2822x	0.9716	623.36	453.38–857.06
Fusariun oxysporun	72	y = 1.5112 + 1.1997x	0.9754	809.07	545.56–1199.86
Didymella bryoniae	72	y = 1.5316 + 0.9910x	0.9122	3162.34	1187.37–8422.33

2.5. Antibacterial Activities of T. flousiana Essential Oil

The in vitro antibacterial activities of *T. flousiana* essential oil, against four species of microorganisms were estimated by measuring the diameter of inhibition zone and varied by the sample types and bacteria strains. The *T. flousiana* essential oil showed obvious activity against *Ralstonia solanacearum* Yabuuhi et al. (ATCC 11696) and *Staphylococcus aureus*, S. *aureus* (ATCC 25923) strains. The growth of the two bacteria species was inhibited by the essential oil in a dose-dependent manner under the exposure of increasing concentrations (0, 5, 10, 20, 30, and 40 µg/disk). At 40 µg/disk, the diameters of the inhibition zone (ZOI, mm) caused by the essential oil to *R. solanacearum* and *S. aureus* were 18.66 and 16.75 mm, respectively. However, the essential oil had not exhibited significant growth inhibition against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC 8739) and *Bacillus subtilis* (Ehrenberg) Cohn. (ATCC 23857), with the ZOIs being 7.23 and 7.91 mm, respectively, at 40 µg/disk (Figure 2).
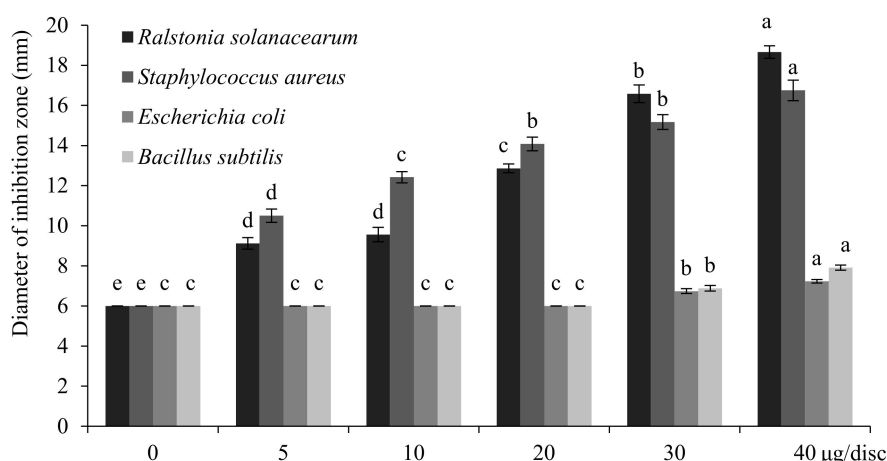
Figure 2. Antibacterial activity of *T. flousiana* essential oil estimated by diameter of inhibition zone. Diameter of inhibition zone includes diameter of discs (6 mm). Bacteria were cultured for 12 h at 37 °C. Different letters represent values that differed significantly in the Duncan's multiple range test ($p < 0.05$).

2.6. Antioxidant Activity

Essential oils have been proposed as potential substitutes for synthetic antioxidants in food preservation because of their antioxidant activity [44]. In this work, the antioxidant activity of *T. flousiana* oil was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay. In this assay, the antioxidant reacts with the stable free radical 2,2-diphenyl-1-picrylhydrazyl with a deep violet color and produces 2,2-diphenyl-1-picrylhydrazine with no color [45]. The free radical scavenging activity is usually expressed either as percentage of DPPH inhibition or by the antioxidant consumption for a 50% DPPH reduction (IC_{50}). The amount of essential oil needed to decrease the initial DPPH by 50% (IC_{50}) is a parameter widely used to measure the antioxidant activity. The lower the IC_{50} value, the more potent the antioxidant is. In our results, positive control ascorbic acid had the strongest antioxidant activity with IC_{50} value of 7.98 μg/mL. The IC_{50} value of the essential oil of *T. flousiana* was 33.51 μg/mL (Table 5), indicating it scavenged the free radical DPPH.

Table 5. IC_{50} values of *T. flousiana* essential oil and ascorbic acid measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Treatment	Regression Eq	IC_{50} (μg/mL)	r	95% CL (μg/mL)
Essential oil	$y = 1.6712 + 2.1903x$	33.51	0.9766	26.15–42.95
Ascorbic acid	$y = 3.5608 + 1.6001x$	7.98	0.9808	5.76–11.04

All treatments were performed in triplicates and repeated at least three times. IC_{50}: concentration (μg/mL) for a 50% inhibition.

2.7. Effects of Essential Oil on Algal Internal Structure

We found that the chloroplast disintegration and the shrink of plane transverse cell walls became more apparent with the increase of the essential oil concentration. With the treatment of 50 μg/mL, the chloroplast started to disintegrate. Meanwhile the structure of the chloroplast shrank. Severe damage of cell structure was observed at higher essential oil concentrations (100–200 μg/mL). In these treatments, plasmolysis occurred and damage to basic cell structure was severe, as revealed by the fact that the chloroplast disintegration became more apparent, and as well by damage to the plasma membrane and was accompanied by increased cell wall opacity. As a contrast, intact cell wall and normally distributed chloroplast were present in the control cells (Figure 3).
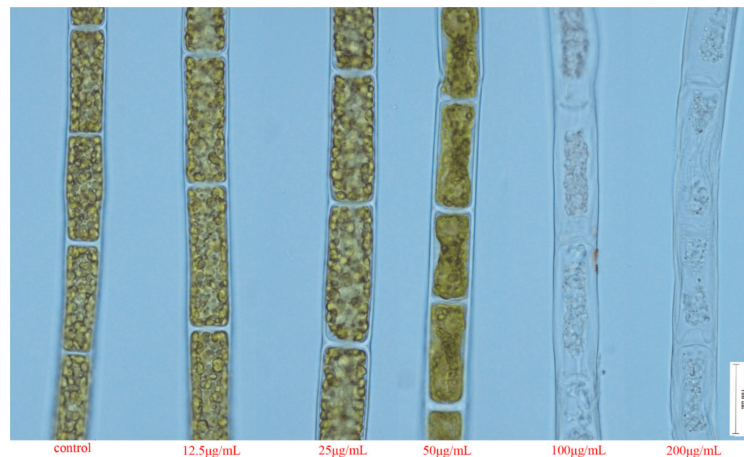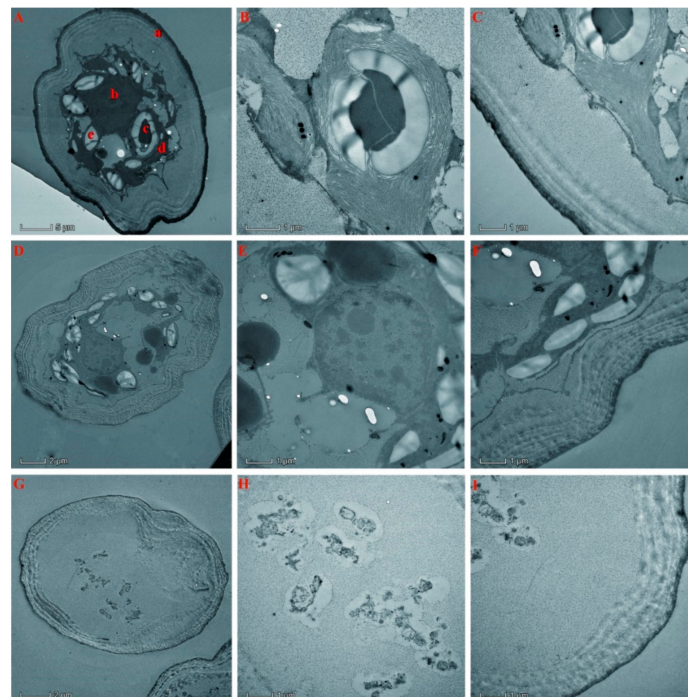
Figure 3. Effect of the essential oil of *T. flousiana* on the morphology of *S. communis*. *S. communis* was treated with the essential oil at different concentrations for 96 h, the morphological changes were imaged with microscope (Nikon Eclipse Ti) at a magnification of 10×.

2.8. Alteration of the Cell Ultrastructure of S. communis by the Essential Oil of T. flousiana

The treated *S. communis* cells were analyzed by transmission electron microscope (TEM). Specifically, it was found that essential oil at 200 μg/mL could damage the cell wall and the chloroplast, leaving the cells vacuolated, with only some organelle remained in the cell (Figure 4G–I). This result indicated that the main action site of the essential oil of *T. flousiana* might be the cell wall and chloroplast. It was noteworthy that the chloroplast is an important target of the essential oil of *T. flousiana*, this finding further supported our result that the light contributed to the algicidal activity of the oils presented in Table 3.

Figure 4. Effect of the essential oil of *T. flousiana* on the ultrastructure of *S. communis*. *S. communis* was treated with the essential oil at different concentrations (50 and 200 μg/mL) for 96 h and the ultrastructure changes were assessed by TEM). The control cells (**A–C**), treatment with the essential oil of *T. flousiana* (**D–F**) treated with the essential oil of *T. flousiana* at 50 μg/mL and (**G–I**) treated with the essential oil of *T. flousiana* at 200 μg/mL). a: cell wall; b: nucleus; c: chloroplast; d: thylakoid; e: starch grain.

3. Discussion

In 2001, research interests in the components of the essential oil of *T. cryptomerioides* were reported for α-cadinol, ferruginol, and cedrol isolated from the essential oils of sapwood and heartwood of *Taiwania cryptomerioides* Hayata [46], and a-cadinol, T-muurolol, ferruginol, and T-cadinol obtained from *T. cryptomerioides* heartwood [47]. Further study in 2012 identified 35 compounds from the twig essential oil of *T. cryptomerioides*, of which cadinol (45.9%), ferruginol (18.9%), and β-eudesmol (10.8%) were the major compounds [48]. In our study, 69 components were identified from the bark of *T. flousiana* and accounted for 89.70% of the total oil composition. The main components were hexadecanoic acid (27.13%), 2-penten-1-ol, 3-methyl-5-[octahydro -4,5-dimethyl-7a-(1-methylethenyl)-1H-inden-4-yl]-(16.16%), linoleic acid (13.48%), podocarpa- 6,8,11,13-tetraen-12-ol, 13-isopropyl-, acetate (7.56%), ferruginol (6.52%), and α-linolenic acid (5.41%). The contents of abietatriene, tetradecanoic acid, pentadecanoic acid ranged from 1.04 to 1.37%. The others were less than 1.00%. The diterpene ferruginol was also one of the major components of bark essential oil of *T. flousiana*, cedrol and β-eudesmol were also present in the bark essential oil of *T. flousiana*, but with quite less contents. The contents of ferruginol, cedrol, and β-eudesmol were 6.52%, 0.13%, and 0.06%, respectively. The differences of our results from those in the literatures were probably because of the different parts of the test material and its collection locations.

Further, we found that *T. flousiana* essential oil possessed inhibition of chlorophyll content on the *S. communis*, which is a species of algae. Harmful algae blooms have increased globally and many researchers are focusing on the development of the effective control agents. Plant-derived chemicals are important sources of selective and biodegradable algicides [49]. Recently, algicidal polyphenolic *p*-hydroxybenzoic acid, coumarin, and fatty acids have been isolated from different plants [50–52]. Wang et al. [53] reported the algicidal activities of essential oils from six plant species, namely, *Potamogeton cristatus*, *Potamogeton maackianus*, *Potamogeton lucens*, *Vallisneria spinulosa*, *Ceratophyllum demersum*, and *Hydrilla verticillata*. The inhibition rates of essential oils on *M. aeruginosa* were 30.2–41.7% at a concentration of 50.0 μg/mL. Phenolic and fatty acids were found to be the algicidal chemicals [54]. Normally, phenolic and fatty acids were the common components of essential oils, which provide useful information for further study of these chemicals in control of the submerged weeds. Notably, we found that light contributed to the algicidal activity of *T. flousiana* essential oil in *S. communis*. This indicated that some active ingredients of the essential oil were photo-activated, which deserves further study.

In 2017, Chen et al. reported that phytochemicals (ferruginol, T-cadinol, alpha-cadinol, and T-muurolol) of *T. cryptomerioides* heartwood had the potential to be used as environmentally benign antifungal agents against brown root rot fungus *Phellinus noxius* in place of synthetic or inorganic fungicides. Their results showed that ferruginol, T-cadinol, alpha-cadinol, and T-muurolol were found to exhibit excellent antifungal activities against *P. noxius*, with IC_{50} values 16.9, 25.8, 33.8 and 50.6 μg/mL, respectively [55]. In our study, the content of ferruginol in *T. flousiana* essential oil was 3.94% and the oil also showed antifungal activity. Thus, the antifungal activity of the active ingredient ferruginol deserves more attention.

In 2002, Wang et al. demonstrated that ferruginol exhibited the strongest antioxidant activity among the diterpenes isolated from *T. cryptomerioides* heartwood [56]. In 2012, Ho et al. also reported that *T. cryptomerioides* twig essential oil showed antioxidant activity against DPPH. The IC_{50} of the DPPH free radical scavenging capability of the essential oil was 90.80 μg/mL and ferruginol (IC_{50} = 48.0 μg/mL) was identified to be the main active ingredient for the free radical scavenging [57]. In our study, the content of ferruginol was 3.94% in *T. flousiana* oil while the IC_{50} value of the essential oil was 33.51 μg/mL. As a contrast, in the report of Ho et al. the content of ferruginol in *T. cryptomerioides* twig oil was 18.9% while the IC_{50} value of the essential oils was 90.80 μg/mL [57]. In general, the antioxidant activity of essential oils is the product of additive, synergistic, and/or antagonistic effects from a complex mixture of several classes of compounds. These suggested that other components of the *T. flousiana* essential oil may possess the antioxidant activity. The biological functions of each component of the essential oil need to be further investigated.

Additionally, our results indicated that the main action site of the essential oil of *T. flousiana* might be the cell wall and chloroplast. Mechanism study plays a very important role in the development of new algicidal chemicals. Further mechanism studies should focus on cell wall and chloroplast.

4. Material and Methods

4.1. Plant Material

Whole *T. flousiana* plant was collected from Enshi autonomous prefecture in central China in July, 2019 (Enshi, Hubei province, China). The plant was further identified as *T. flousiana* (a synonym of *Taiwania cryptomerioides* Hayata) and a voucher specimen was deposited in the Key Laboratory of Natural Pesticides & Chemical Biology, Ministry of Education, South China Agricultural University, China. The plant material was air-dried for up to 3 weeks at the ambient temperature and 24 h in a 50–60 °C incubator prior to pulverization.

4.2. Isolation of Essential Oil

The air-dried stem bark powder (250 g) of *T. flousiana* was subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil was dried with anhydrous sodium sulphate. The yield (v/w, dry weight basis) was calculated as volume (mL) of extracted essential oil per 250 g of plant material. Then, the essential oil was stored in hermetically sealed dark-glass at 4 °C until further analysis.

4.3. Analysis of the Essential Oil

Analysis of the essential oils was carried out with an Agilent Technologies 7693A Gas Chromatograph with 5977B Mass Spectrometer. A HP-5 MS capillary column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies Inc., Santa Clara, USA) was employed. Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 mL/min, split ratio: 15:1. Oven temperature was programmed as follows: 40 °C initially rising to 150 °C at a rate of 6 °C/min; rising to 270 °C at a rate of 3 °C/min; rising to 300 °C at a rate of 10 °C/min and held for 3 min. The injector and detector were held at 325 °C. The mixtures of the normal alkane of C_7–C_{30} (1000 μg/mL) and EO samples dissolved in hexane of 0.8 μL were injected and all samples were filtered through a 0.22 μm organic phase filter. The Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of 30–550 AMU in full scan mode. The MS (Agilent Technologies Inc., Santa Clara, USA) transfer line was set at 250 °C.

4.4. Identification of the Essential Oil Chemical Constituents

The essential oil constituents identification was carried out by comparing their recorded mass spectra with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST 17 database) or with authentic compounds and confirmed by comparison of their retention index with authentic compounds reported in the literature [58]. The relative percent of each component in essential oil was counted by the area normalization method. The retention index was defined by the following:

$$RI = 100n + 100(t_x - t_n)/(t_{n+1} - t_n) \tag{1}$$

where t_n, t_{n+1}, and t_x were net retention times [59].

Identification of the individual components was based on: (i) Comparison with the mass spectra of authentic reference compounds possible and by reference to NIST 17 database, and Adams terpene library [43]; (ii) comparison of their retention indices (RI) on a HP-5, calculated relative to the retention times of a series of C-7 to C-30 *n*-alkanes, with linear interpolation, with those of authentic compounds or literature data [43].

4.5. Evaluation of the Algicidal Activity with Light

S. communis was collected from the Southern China Botanical Garden, Chinese Academy of Sciences. *S. communis* was incubated in a modified Bold basal medium composed of $NaNO_3$ (250 mg/L), K_2HPO_4 (75 mg/L), $CaCl_2·H_2O$ (25 mg/L), $MgSO_4·7H_2O$ (75 mg/L), NaCl (25 mg/L), KH_2PO_4 (175 mg/L), $Na_2EDTA·2H_2O$ (4.5 g/L), $FeCl_3·6H_2O$ (0.582 g/L), $MnCl_2·4H_2O$ (0.246 g/L), $ZnCl_2$ (0.030 g/L), $Na_2MoO4·2H_2O$ (0.024 g/L), $CoCl_2·6H_2O$ (0.012 g/L), vitamin B1 (1.1 g/L), vitamin B6 (0.025 g/L), and vitamin B12 (0.135 g/L) [60].

The alga was cultured in 500 mL of sterilized culture medium in 1000 mL conical flasks under an irradiance of 4000 lux, 12 h light/12 h dark (12:12), at 25 ± 1 °C for 5 days. Then the 10 mL of the algal cultures (0.1 g) was transferred to a 6-well plate. The stock solution of the essential oil was prepared in acetone. The final concentrations of the essential oil in the test solution were 12.5, 25, 50, 100, and 200 μg/mL, respectively. Acetone in the test solution was lower than 0.2% (v/v). The commercial herbicide, butachlor (12.5, 25, 50, 100, and 200 μg/mL), was used as a control. The plates were sealed with polyethylene wrapping film and incubated in a growth chamber at 25 ± 1 °C, RH 50–60%, and a photoperiod of 12:12. Each treatment has three replicates. All experiments were repeated at least three times.

The algicidal activity was determined by the chlorophyll content as described by Dere et al. [61] with some modifications. Specifically, after being dried with absorbent paper, *S. communis* in each replicate was grounded in 2 mL of 80% acetone. Then the mixture was transferred into a centrifugal tube (2 mL) followed by centrifugation for 10 min at 4000 rpm. The supernatant was transferred into a tube and diluted with acetone to 4 mL. Then 2 mL of the solution was mixed with 80% acetone to make a final volume of 10 mL and the absorbances were recorded at 663 nm and 645 nm. Acetone (80%) alone was used for the blank control. The amount of the pigments was calculated according to the following formulas:

$$C_a = 12.7 \times A_{663} - 2.69 \times A_{645} \tag{2}$$

$$C_b = 22.9 \times A_{645} - 4.68 \times A_{663} \tag{3}$$

$$C_{ab} = 8.02 \times A_{663} + 20.21 \times A_{645} \tag{4}$$

The content of the chlorophyll was calculated according to the following formula:
The chlorophyll content (mg/g) = (Chlorophyll concentration × the volume of the tested

$$\text{solution} \times \text{the dilution factor})/\text{the sample mass}) \times 100 \tag{5}$$

The inhibition rate of chlorophyll was calculated according to the following formula:

$$\text{The inhibition rate of chlorophyll (\%)} = (([C] - [S])/[C]) \times 100 \tag{6}$$

where [C] means the chlorophyll content of control and [S] means the chlorophyll content of sample.

4.6. Evaluation of the Algicidal Activity without Light

The same method as described above in 4.5 was followed except the plates were incubated under a photoperiod of 24:0 (dark:light). The inhibition rate of chlorophyll was evaluated 96 h after the treatment.

4.7. Morphological Changes of S. communis Treated with the Essential Oil of T. flousiana

The morphological changes of *S. communis* treated with the essential oil of *T. flousiana* for 24, 48, and 72 h were evaluated with light microscopy (Leica DMLB2, Leica Microsystems, Wetzlar, Germany). Transmission electron microscopy (TEM) (FEI Tecnai 12, FEI company, Hillsboro, USA) evaluation was further performed in order to examine the effect of the essential oil of *T. flousiana* (at 12.5, 25, 50, 100 and

200 µg/mL) on the ultra-structure of *S. communis*. The TEM samples were processed as previously described by Houot et al. [62]. Samples were examined by TEM (FEI Tecnai 12, FEI company, Hillsboro, USA).

4.8. Determination of Antimicrobial Effects of the Essential Oils on Mycelial Growth

Fungi including *R. solani*, *C. gloeosporioiles*, *F. moniliforme*, *T. cucumeris*, *F. oxysporun* f. sp. *cubense*, and *D. bryoniae* were obtained from the Department of Plant Pathology, South China Agricultural University. In vitro antifungal assays were conducted according to the method of Boubaker et al. [63], with slight modifications. Briefly, sterile molten potato-dextrose-agar (PDA) supplemented with essential oil of *T. flousiana*, at final concentrations of 62.5, 125, 250, 500, and 1000 µg/mL, was poured into Petri plates (6-cm-diameter). All tests were performed in PDA supplemented with 0.5% (*v/v*) DMSO to enhance oil solubility. Afterwards, plates were inoculated with fungal cultures with 5-mm diameter agar disks from one-week-old cultures, with mycelia surface facing down. The agar plates were then incubated at 25 °C for 2 days. Plates with medium supplemented with 0.5% DMSO only was used as the control. The antifungal activity was expressed as percent of mycelial radial growth inhibition and calculated according to the following formula: MGI (%) = ((C − T)/C) × 100, where C and T represent mycelial growth diameter in control and EO treated plates, respectively. Each treatment has three replicates. All experiments were repeated at least three times.

The in vitro antibacterial activity of the essential oil from *T. flousiana* was carried out by using filter paper disc diffusion assay [64,65]. Two Gram-positive bacteria *S. aureus* and *B. subtilis* and two Gram-negative bacteria *E. coli* and *R. solanacearum,* provided by the Department of Plant Pathology, South China Agricultural University, were tested. Typically, 500 µl of a suspension of the tested microorganisms (approx. 10^6 colony-forming units (CFU)/mL) was spread with a sterile cotton swab on the surface of Mueller-Hinton agar (MHA) plates at 37 °C and allowed to dry for 10 min. A stock solution of the essential oil was prepared by dissolving 20 mg in 1 mL of DMSO. Then the stock solution was diluted with 0.1% aqueous Tween solution to get series solution of 4000, 3000, 2000, 1000, and 500 µg/mL. Each sterile filter paper disc (6 mm in diameter Whatman disks) was impregnated with 10 µL solutions, respectively. The Petri dishes were kept at 4 °C for 2 h to allow the diffusion of the oil, and further incubated at 37 °C for 24 h. Activity was expressed as percent of zone of inhibition (ZOI, mm). The net zone of inhibition was determined by subtracting the disc diameter (i.e., 6.0 mm) from the total zone of inhibition shown by the test disc in terms of clear zone around the disc. The control was the aqueous solution of 0.1% Tween + 8% DMSO. Each treatment had three replicates. All experiments were repeated at least three times.

4.9. Determination of Antioxidant Activity

The method of El-Gawad [66] was used. The antioxidant activity of *T. flousiana* essential oil was measured in terms of radical scavenging activity, using the stable radical DPPH (Sigma-Aldrich, Darmstadt, Germany) [48]. A reaction mixture of 1 mL of a hexane solution of the essential oil with different concentrations (5, 10, 20, 40, and 80 µg/mL) and equal volume of the methanolic solution of 0.3 mM DPPH was prepared, mixed well and incubated under dark condition for 15 min at room temperature. Ascorbic acid (5, 10, 20, 40, and 80 µg/mL) was used as the reference. The decrease in absorbance at 517 nm was determined using a spectrophotometer (UV-8500PC, Shanghai, China). The IC_{50} (the amount of sample necessary to decrease the absorbance of DPPH by 50%) was calculated graphically. Each treatment has three replicates. All experiments were repeated at least three times. The percent of the inhibition of the DPPH radical was calculated as following:

$$\text{The inhibition (\%)} = 1 − (\text{Absorbance of sample/Absorbance of control}) \times 100 \qquad (7)$$

4.10. Statistical Analysis

Statistical analysis (ANOVA) by means of the IBM SPSS Statistics, Version 19.0 (International Business Machines Corporation, New York, USA) was applied to the data to determine the differences

($p < 0.05$). Then, the estimation of the median effective concentration (EC_{50}), median inhibition concentration (IC_{50}), and their 95% confidence limits were obtained. All quantitative data were presented as the mean ± SD of at least three independent experiments using the Duncan's multiple range test or Student's *t* test for group differences. A $p < 0.05$ was considered as statistically significant.

5. Conclusions

Current investigation highlights the detailed chemical composition of EO extracted from *T. flousiana* and their bioactive potential. Sixty-nine compounds representing 89.70% of the stem bark essential oil of *T. flousiana* were identified. The main components were hexadecanoic acid (27.13%), 2-penten-1-ol, 3-methyl-5-[octahydro-4,5-dimethyl-7a-(1-methylethenyl)-1H-inden-4-yl]- (16.16%), linoleic acid (13.48%), podocarpa-6,8,11,13-tetraen-12-ol, 13-isopropyl-, acetate (7.56%), ferruginol (6.52%), and α-linolenic acid (5.41%).

The algicidal activity of the essential oil on *S. communis* was comparable to that of butachlor. The IC_{50} values of the essential oil ranged from 31.77 to 84.92 μg/mL, while the IC_{50} values of butachlor ranged from 40.24 to 58.09 μg/mL. Thus the essential oil from *T. flousiana* could be considered as a potential algicidal substitute of synthetic ones. In addition, ultrastructure changes revealed by the transmission electron microscopy indicated that the main action sites of the essential oil of *T. flousiana* on *S. communis* cell were the chloroplast and cell wall.

The EO showed antifungal activities on *Rhizoctonia solani* ($EC_{50} = 287.94$ μg/mL) and *Colletotrichum gloeosporioiles* ($EC_{50} = 378.90$ μg/mL). Meanwhile, The EO also showed bactericidal activities against *Ralstonia solanacearum* and *Staphylococcus aureus*, with ZOIs being 18.66 and 16.75 mm, respectively at 40 μg/disk.

The level of antioxidant activity estimated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method showed that the essential oil of *T. flousiana* demonstrated obvious antioxidant activity with IC_{50} value of 33.51 μg/mL. While the IC_{50} value of the positive control ascorbic acid was 7.98 μg/mL. Thus, the essential oil of this plant could be used as a potential source of natural bioactive molecules in agrochemical industry as well as in food and cosmetic industries.

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Sample Availability: Samples of the compounds are available from the authors.