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The Allelopathic Effects of *Persicaria salicifolia* on the Growth and Antioxidant Enzymes of *Synechocystis pevalekii* and *Tetradesmus bernardii*

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ABSTRACT: In recent years, there has been an increasing focus on the prospect of exploiting macrophytes as an alternative strategy to control undesired algal growth. The present research was conducted to study the effect of different concentrations of an aqueous stem extract of *Persicaria salicifolia* on the growth, some metabolites and antioxidant enzymes of the green microalgae *Synechocystis pevalekii* and *Tetradesmus bernardii*. Chlorophyll *a*, dry weight, total protein, total carbohydrate and proline contents of the tested algae decreased with increasing the crude extract concentrations of *P. salicifolia*. In general, catalase, superoxide dismutase and lipoxygenase activity of *T. bernardii* increased with increasing the aqueous extract of *P. salicifolia*. The identification of phytochemical components of the plant extract by gas chromatograph-mass spectrometry (GC-MS) revealed the presence of various biologically active compounds such as 11-Octadecenoic acid, methyl ester (18.03%) and 9,12-Octadecadienoic acid methyl ester (15.03%) that are capable of inhibiting the growth of *S. pevalekii* and *T. bernardii.* Therefore, *P. salicifolia* may provide a cheap and environmentally friendly alternative for controlling microalgae in aquatic ecosystems.

Keywords: *Persicaria salicifolia*; Phytochemical constituents; *Tetradesmus bernardii*; *Synechocystis pevalekii*; Antioxidant enzymes.

التأثيرات الأليلوباثية لنبات *Persicaria salicifolia* على النمو والانزيمات المضادة للأكسدة لطحلبى *Synechocystis pevalekii* و *Tetradesmus bernardii*

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الملخص: في الآونة الأخيرة، زاد الاهتمام باستغلال النباتات المائية كبديل لمقاومة نمو الطحالب الغير مرغوب فيها. اجريت هذه الدراسة لمعرفة تأثير التركيزات المختلفة من المستخلص المائي لساق نبات *Persicaria salicifolia* على النمو وبعض الانشطة الأيضية وكذلك الانزيمات المضادة للأكسدة لطحلبى *Synechocystis pevalekii* و *Tetradesmus bernardii*. وقد لوحظ نقص فى محتوى الكلوروفيل، الوزن الجاف، البروتينات الكلية، الكربوهيدرات الكلية والبرولين مع زيادة تركيزات المستخلص المائي لساق نبات *P. salicifolia* في كلا الطحالب. بشكل عام زاد نشاط انزيم catalase و Lipoxygenase وكذلك انزيم superoxide dismutase فى طحلب *T. bernardii*. تم تعريف المكونات الكيميائية النباتية لمستخلص النبات قيد الدراسة باستخدام GC-MS وتم التوصل الى وجود مركبات نشطة حيويا مثل 11-Octadecenoic acid, methyl ester (18.03%) and 9,12-Octadecadienoic acid methyl ester (15.03%) والتى من المحتمل ان تكون السبب فى تثبيط نمو طحلبى *S. pevalekii* و*T. bernardii*. وبالتالى يمكن استخدام نبات *P. salicifolia* في التخلص من الطحالب الدقيقة الغير مرغوب فيها والموجودة فى البيئات المائية المختلفة.

الكلمات المفتاحية: *Persicaria salicifolia*، المكونات الكيميائية النباتية، *Synechocystis pevalekii*; *Tetradesmus bernardii*; الانزيمات المضادة للأكسدة.

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1. Introduction

Eutrophication is a widespread problem in aquatic ecosystems due to sewage and surface run-off. Harmful algae can live in eutrophic aquatic ecosystems that are polluted with heavy metals and persistent organic pollutants [1]. They cause negative impacts such as the deterioration of water quality which leads to serious environmental and economic problems [2]. Therefore, it is necessary to explore a new effective and safe way to control the growth of harmful algae. In recent decades, several studies have shown that aquatic macrophytes have the ability to release allelopathic active substances (allelochemicals) into aquatic environments that are capable of inhibiting both cyanobacteria and algae [3].

Allelochemicals are produced by the secondary metabolism of plants and microorganisms, and they can have a positive or negative impact on the growth, survival, and reproduction of other organisms. Additionally, they can disrupt the structure and dynamics of ecosystems by influencing the growth and development of nearby plants and microorganisms [4]. Macrophyte allelochemicals belong to various chemical classes such as sulfur compounds, polyphenols, oxygenated fatty acids and polyacetylenes [5].

Because allelopathy is species-specific, it serves as a major mechanism for controlling the growth of cyanobacterial populations in aquatic environments. But, a number of variables (aquatic plant species characteristics, the physiochemical characteristics of substances that are allelopathically active, the area and density of water body overgrowth, hydrochemical and hydrological regimes, light intensity, temperature, etc.) affect the allelopathic activity of macrophytes and may impact the effect of allelochemicals on algal communities [3]. Zuo *et al*. [6] reported that many co-existing aquatic plants have synergistic effects on algal inhibition. Additionally, *Ceratophyllum demersum* has a strong inhibitory impact on the strain of *Microcystis aeruginosa* that produces microcystin, according to Amorim *et al*. [7].

Persicaria salicifolia is one of seven *Persicaria* species present in Egypt [8] that grows in the Nile Delta as a helophyte and geophyte. It is a hydrophyte found on the riverbanks, shores of lakes, drains and canals [9]. The aim of this study was to examine the effect of *P. salicifolia* on the growth and production of metabolites and antioxidant enzymes of *Synechocystis pevalekii* and *Tetradesmus bernardii* along with identifying the phytochemical components of *P. salicifolia* using GC/MS.

2. Materials and methods

Plant collection and preparation:

Fresh stems of *Persicaria salicifolia* Willed were collected from Arab El-Madabegh, (Assiut- Egypt) at latitude of 27°, 10’, 12.4" N and latitude of 31°, 9’, 23.3" E. The plant materials were thoroughly washed in tap water and rinsed with distilled water. The stems were air dried for 15 days, crushed and pulverized into a fine powder, stored in a glass container, and protected from sunlight until use.

Preparation of plant aqueous extracts:

Aqueous extracts of plants were prepared according to Krishnaiah *et al*. [10] by mixing about 1g of powdered sample in 50 ml of distilled water for 24h at room temperature. The extracts were filtered using Whatman No. 1 filter paper (pore size 11 µm) and stored in the dark at 4 °C before they were used for experiments.

Qualitative analysis of phytochemical compounds

The stem aqueous extracts were analyzed phytochemically for the detection of alkaloids, saponins, phenols, tannins, flavonoids, terpenoids and glycosides, according to the method of Evans [11], and Edeoga *et al*. [12].

Quantitative analysis of the aqueous extract:

The phytochemical compounds of *Persicaria salicifolia* were extracted by water and analyzed using gas chromatography-mass spectrometer (GC-MS), (Agilent Technologies, Palo Alto, CA, USA), Model 7890A-5975B [Column, DB 5 ms, Agilent form (30 m × 250 µm × 0.25 µm)]. The column was initially maintained for 2 min at 40 °C, and then, the temperature was increased to 50 °C at a rate of 4 °C/min and held for 3 min, then increased to 150 °C at a rate of 10 °C/min and held for 3 min, then increased to 220 °C at a rate of 10 °C/min and held for 6 min, finally increased to 280 °C at a rate of 15 °C/min and held for 10 min. Helium (purity 99.999%) was used as the carrier gas with a flow rate of 0.5 ml/min for 10.9 min, then 1 ml/min per min to 1 ml/min for 30 min. Electron impact ionization mass spectrometry (EI-MS; Agilent 7890B Single Quad 5977A MSD, USA) was used to detect the m/z value of the separated phytochemical compound. The mass spectrum data of each peak of the chromatogram were compared with the Willey 9 and NIST library for the identification of the phytochemical compounds.

Algae and growth conditions:

The cyanobacterial alga *Synechocystis pevalekii* Ercegovic, and green alga *Tetradesmus bernardii* (Smith) Wynne. were isolated from the stem of *Persicaria salicifolia* and *Echinochloa stagnina*, respectively, and the algae were identified according to the Algaebase website (www.algaebase.org). The Rippka and Herdman [13] modified medium was used for the growth of *S. pevalekii*, while *T. bernardii* was grown in Bold's basal medium [14], and incubated at 28-30 °C under continuous light intensity of 48 $\mu mol.m^{-2}s^{-1;}$ for 7 days in case of *S. pevalekii* and 10 days for *T. bernardii*. The algae were cultivated in 300 mL Erlenmeyer flasks with 150 mL of the culture medium. The microalgal suspension was calibrated to an absorbance of 0.1 at an optical density of 680 nm. Using either 0.1 M NaOH or 0.1 M HCl, the media's pH were adjusted to 7.5. The culture media were treated with high (40%), medium (20%) and low (10%) concentrations (after preliminary experiments) of aqueous stem extract and control (without plant extract). The cultures were harvested towards the end of the exponential phase. Three replicates were set up for each treatment. The growth of cultures was monitored by the determination of dry weight. Pigment content (Chl. *a*) was daily estimated spectrophotometrically using Unico UV-2100 spectrophotometer (United States) according to Metzner *et al*. [15]. The maximum growth rate (μ), and generation time were calculated by determination of chlorophyll *a* and using the following formula:

$$\mu\ (h^{-1}) = (LnA_2\text{-}LnA_1)/\ (t_2\text{-}t_1) \quad (1)$$

$$G\ (h) = \ln 2/\mu \quad (2)$$

where A_2 and A_1 represent the chlorophyll *a* concentration at times t_2 and t_1, respectively; t_2-t_1= the time elapsed in hours between two determinations of chlorophyll *a* concentration.

Determination of total carbohydrate, protein, lipid, free amino acids and proline contents:

Total carbohydrate contents of the algal extract were determined by the Anthrone sulfuric acid method according to Badour [16]. Total proteins, free amino acids, and proline content were spectrophotometrically measured using the method described by Lowry *et al*. [17], Lee and Takahashi [18], and Bates *et al*. [19], respectively. Total lipids were determined by the sulfophosphovanilin method (SPV) described by Drevon and Schmitt [20].

Assay of enzyme activity
Preparation of enzyme extract

Fifty ml of algal cultures were centrifuged at 5000 rpm, and the pellet was homogenized in 5 ml of 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM ethylenediamine tetraacetic acid (EDTA) and 0.1 g polyvinylpyrrolidone (PVP) by combination of freezing and sonication. The homogenate was centrifuged at 18000 rpm for 10 min. at 4 °C and the supernatants were collected and used for the assays of catalase, ascorbate peroxidase, lipoxygenase, and superoxide dismutase. All colorimetric measurements (including enzyme activities) were made at 20 °C using Unico UV-2100 spectrophotometer (United States). The specific activity was expressed as units/mg protein.

Assay of catalase activity (CAT)

Catalase activity was assayed by following the consumption of H_2O_2 for 1 min. as described by Aebi [21], and Matsumura *et al.* [22].

Assay lipoxygenase activity (LOX)

Lipoxygenase activity was estimated according to the method of Minguez-Mosquera *et al*. [23].

Assay superoxide dismutase (SOD)

The activity of superoxide dismutase was assayed by following the autoxidation of epinephrine as described by Misra and Fridovich [24].

Statistical analysis

All data obtained were subjected to one-way analysis variance (ANOVA), using the SPSS statistical package (SPSS Inc., Chicago, Ill., USA). For comparison of the means, the Duncan' multiple range tests ($p \leq 0.05$) were used.

3. Results

Qualitative analysis of phytochemical constituents for the *Persicaria salicifolia*

Qualitative screening of phytochemical components such as alkaloids, glycosides, saponins, phenols, tannins, flavonoids and terpenoids in the stem of *Persicaria salicifolia* are presented in Table 1. Medically active chemicals were detected in an aqueous extract obtained from the stem of *Persicaria salicifolia*. It was observed that the aqueous extract included moderate levels of alkaloids and saponins, but low levels of glycosides, phenols, and tannins. The higher amounts of flavonoids and terpenoids were recorded in the aqueous extract of *P. salicifolia*.

Quantitative analysis of the aqueous extract of the *Persicaria salicifolia*

The GC-MS phytochemical study of *P. salicifolia* revealed the presence of twenty components in the extract. 11-Octadecenoic acid, methyl ester (18.03%), 9, 12-Octadecadienoic acid methyl ester (15.03%) and Linoleic acid (7.73%) were the main compounds in the extracts of *P. salicifolia*, while the compounds present in small amounts were 1-Methyl-4-[nitromethyl]-4-piperidinol (0.08%), 1-(3,5-Dimethyl-1-adamantanoyl) semicarbazide (0.17%) and 2-Amino-1-(O-methoxyphenyl) propane (0.27%), (Table 2, Figure 1).

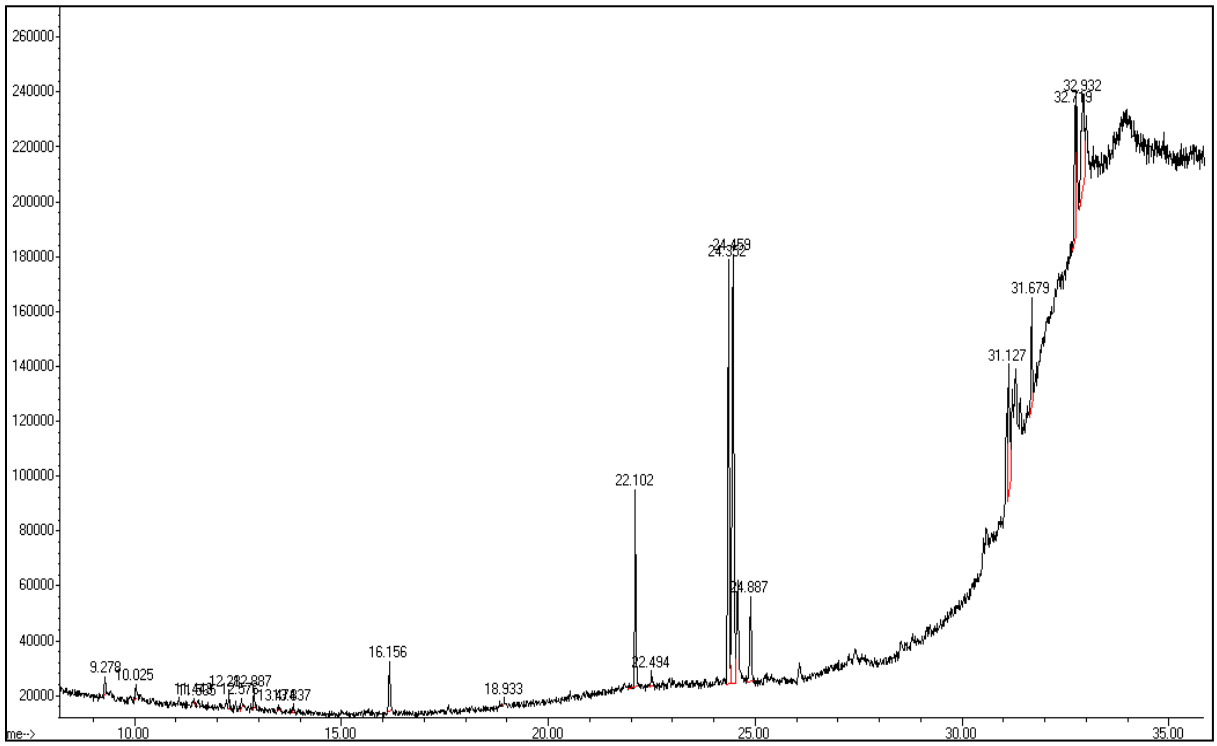

Figure 1. A typical chromatogram of the bioactive compounds present in the aqueous extract of *Persicaria salicifolia*.

Table 1. Qualitative analysis of some phytochemical constituents of the aqueous stem extract of *Persicaria salicifolia*.

Phytochemicals	Presence
Alkaloids	++
Glycosides	+
Saponins	++
Phenols	+
Tannins	+
Flavonoids	+++
Terpenoids	+++

Weak (+), moderate (++) and highly (+++) presence

Table 2. Phytocomponents identified in the aqueous extract of *Persicaria salicifolia* by GC-MS analysis.

Name of compounds	(%)
11-Octadecenoic acid, methyl ester	18.03
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	15.03
Linoleic Acid	7.73
9,17-Octadecadienal	5.81
Hexadecanoic acid methyl ester	4.85
Phenylallyl cinnamate	3.6
Methyl octadecanoate	3.14
2,3-Dihydroxypropyl elaidate	3.05
2,4-bis(1,1-dimethylethyl) Phenol	2.01
1-(2-methoxyphenyl)propan-2-amine	1.17
Pentacosane	0.8

4-Methoxyamphetamine;	0.51
2-[6'-Bromobenzo[1,3]dioxol-5'-yl)ethyl 4-nitrobenzenesulfonate	0.5
3-Methyl-3,5--(cyanoethyl)tetrahydro-4-thiopyranone	0.43
Hentriacontane	0.42
Dibutyl benzene-1,2-dicarboxylate	0.41
2-Amino-1-(O-methoxyphenyl)propane	0.27
1-(3,5-Dimethyl-1-adamantanoyl)semicarbazide	0.17
1-Methyl-4-[nitromethyl]-4-piperidinol	0.08

Effect of aqueous stem extracts of *P. salicifolia* on the growth and some metabolites of *S. pevalekii* and *T. bernardii*

In this experiment, the cultures of *S. pevalekii* and *T. bernardii* were grown under different concentrations of the aqueous extract of *P. salicifolia*. The data in Table 3 and Figure 2 (A, B) show that the chl. *a* and dry weight of *S. pevalekii* decreased at the high conc. (40%) of the aqueous extract of *P. salicifolia*, but the low and medium concentrations have a non-significant effects ($p \geq 0.5$). On the other hand, the chl. *a* and dry weight of *T. bernardii* were significantly decreased with increasing the aqueous extract of *P. salicifolia* ($p \leq 0.05$; Table 3).

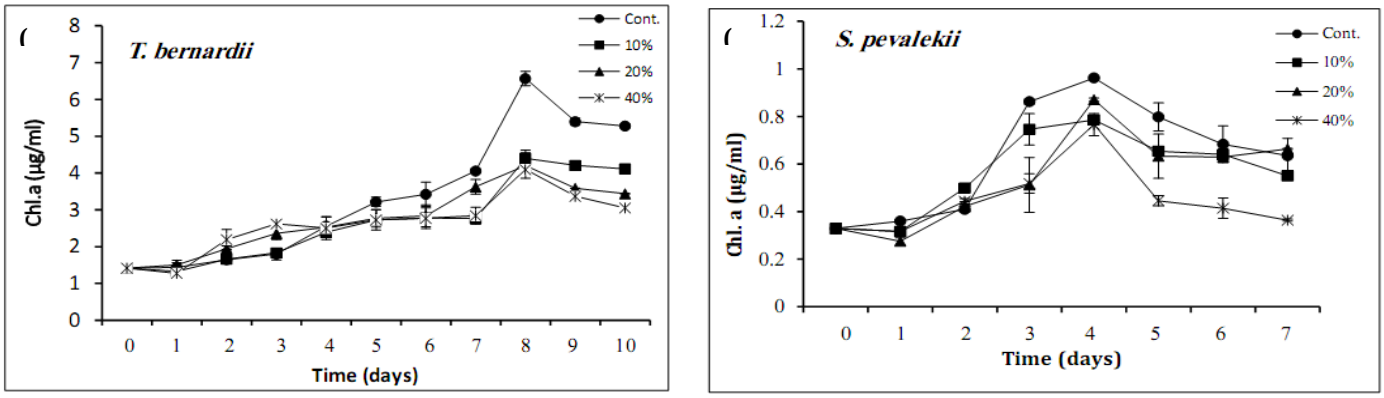

Figure 2. Growth curves of (A) *S. pevalekii* and (B) *T. bernardii* grown at various concentrations of aqueous stem extracts of *Persicaria salicifolia*.

The maximum growth rate and minimum generation time of *S. pevalekii* were 0.031 h^{-1} and 22.35 h, respectively and were recorded for the control cultures. On the other hand, the maximum growth rate and minimum generation time of *T. bernardii* were recorded for cultures incubated with 40% concentration of the *P. salicifolia* extract and were found to be 0.023 h^{-1} and 30.71 h, respectively (Table 3). Generally, total carbohydrate, total protein and proline contents significantly decreased ($p \leq 0.05$) with increasing the concentration of aqueous extract of *P. salicifolia* in *S. pevalekii* and *T. bernardii* ($p \leq 0.05$), while the amino acid contents in the studied algae increased with the increase of the aqueous extract. The maximum total lipid contents were recorded at 10% concentration of aqueous plant extract in both of *S. pevalekii* and *T. bernardii* (59.32 and 204.33 mg/g dry wt., respectively) (Table 3).

Effect of aqueous stem extracts of *P. salicifolia* on the antioxidant enzymes activity of *S. pevalekii and T. bernardii*

Antioxidant enzymes activity (CAT, LOX and SOD) were measured in *S. pevalekii* and *T. bernardii* under various concentrations of aqueous stem extract of *P. salicifolia*, as shown in Figure 3. The highest catalase activity was recorded in *S. pevalekii* at 20% conc. (0.17 µmol min^{-1} mg^{-1} protein), while the activity of CAT in *T. bernardii* was significantly increased ($p \leq 0.05$) with increasing concentration of aqueous extract of *P. salicifolia*.

Generally, the activity of LOX in *S. pevalekii* was significantly increased at low and medium conc. of the aqueous extract of *P. salicifolia* (60.88 and 61.47 µmol min^{-1} mg^{-1} protein, respectively) ($p \leq 0.05$). Additionally, by increasing the concentration of *P. salicifolia* in comparison to control culture, a significant increase in the LOX activity of *T. bernardii* was observed ($p \leq 0.05$).

In *S. pevalekii*, superoxide dismutase (SOD) activity did not decrease significantly at 10% and 20% conc. of aqueous plant extract ($p \geq 0.05$), but was significantly decreased ($p \leq 0.05$) at 40% conc. On the other hand, as the plant extract concentrations increased, SOD activity in *T. bernardii* was observed to increase significantly ($p \leq 0.05$) (Figure 3).

Table 3. Effect of different concentrations of *P. salicifolia* aqueous extracts on the growth and some metabolites of *Synechocystis pevalekii and Tetradesmus bernardii.*

		Chl.a (µg/ml)	D.W. (mg/ml)	μ_{max} (h^{-1})	G(h)	T.C.	T.P.	Amino acids	Total lipids	proline
						(mg/g dry wt.)				
S. pevalekii	**Cont.**	0.64 ±0.02^{b}	0.50 ±0.03^{a}	0.031	22.35	15.27 ±0.51^{d}	56.14 ±3.48^{b}	6.75 ±0.16^{b}	51.6 ±1.08^{b}	2.74 ±0.15^{c}
	10%	0.72 ±0.01^{c}	0.8 ±0.06^{b}	0.019	36.29	12.68 ±0.29^{c}	22.06 ±0.98^{a}	3.56 ±0.29^{a}	59.32 ±0.52^{c}	0.59 ±0.06^{a}
	20%	0.66 ±0.00^{b}	0.70 ±0.06^{b}	0.022	31.38	10.43 ±0.06^{b}	17.84 ±0.77^{a}	10.71 ±0.61^{c}	52.90 ±0.88^{b}	0.59 ±0.04^{a}
	40%	0.36 ±0.00^{a}	0.40 ±0.01^{a}	0.016	42.50	5.93 ±0.16^{a}	17.13 ±0.57^{a}	18.16 ±0.77^{d}	32.49 ±0.39^{a}	2.07 ±0.09^{b}
T. bernardii	**Cont.**	5.28 ±0.06^{d}	0.32 ±0.01^{b}	0.020	34.53	35.17 ±1.04^{bc}	96.40 ±3.34^{d}	6.19 ±0.30^{a}	116.55 ±5.56^{b}	2.32 ±0.15^{c}
	10%	4.11 ±0.02^{c}	0.31 ±0.01^{ab}	0.019	35.87	36.17 ±0.80^{c}	37.58 ±2.58^{b}	9.67 ±1.40^{b}	204.34 ±9.63^{c}	0.51 ±0.02^{a}
	20%	3.44 ±0.01^{b}	0.30 ±0.01^{ab}	0.011	64.76	32.43 ±1.51^{ab}	52.90 ±1.30^{c}	9.74 ±0.79^{b}	84.14 ±5.20^{a}	0.53 ±0.02^{a}
	40%	3.05 ±0.12^{a}	0.29 ±0.00^{a}	0.023	30.71	29.11 ±0.53^{a}	28.23 ±0.81^{a}	9.71 ±0.18^{b}	95.67 ±1.83^{a}	0.87 ±0.07^{b}

Cont. =Control, μ_{max}= maximum growth rate, G= generation time, D.W= dry weight, T.P. = total proteins, T.C. = total carbohydrates. The data are given as averages of three replicates ± std. error. Values followed by the different letters are significantly different at $p \leq 0.05$.

4. Discussion

Plants produce secondary metabolites to protect themselves from herbivores by regulating defense signaling pathways and performing defensive functions [25]. The most bioactive compounds such as alkaloids, saponins, flavonoids and terpenoids were found in aqueous extract for stem of *Persicaria salicifolia*. Phytochemical constituents such as tannins, alkaloids, flavonoids, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve as a defense mechanism against invasion by many microorganisms, insects and other herbivores [26]. Alkaloid, saponins and tannins of *Senna siamea* leaf extracts can be responsible for antibacterial activity [27] and the inhibition of cyanobacterial growth [28]. In addition, certain aquatic plants such as *Nuphar lutea* have the ability to produce alkaloids, such as resorcin, which is very active against both phytoplankton and zooplankton [29]. 11-Octadecenoic acid, methyl ester, 9, 12-Octadecadienoic acid methyl ester and Linoelaidic acid had the highest concentrations among compounds identified in the extracts of *P. salicifolia*, with concentrations ranging between 18.03%, 15.03% and 7.73%, respectively. These compounds may be responsible for the observed effect on the growth of *S. pevalekii* and *T. bernardii*. According to Rukshana *et al*. [30], hexadecanoic acid, methyl ester in leaf extract of *Pergularia daemia* has antibacterial properties. Zhang *et al*. [31] also stated that allelopathic compounds such as linoleic acid can inhibit the growth of *Microcystis aeruginosa*, *Chlorella pyrenoidosa*, and *Scenedesmus obliquus*.

Aquatic macrophytes can control the growth of algae via inhibition or stimulation by releasing allelopathic substances [32]. The chl. *a* and dry weight of *S. pevalekii* in this investigation decreased at low conc. (40%) of *P. salicifolia* crude extract, and they were reduced significantly at all crude extract concentrations. These results are consistent with those of Chia *et al*. [33] who found that the chlorophyll *a* and dry weight of *Scenedesmus quadricauda* decreased with increasing the aqueous crude extract of *Azadirachta indica*. Fawzy *et al*. [34] also reported that the growth of *Cyanosarcina fontana* decreased with an increasing the concentration of the root extract of *Verbesina encelioides*.

The current study showed that the total carbohydrates, total proteins and proline contents were significantly decreased by increasing the concentrations of aqueous extract of *P. salicifolia* in *S. pevalekii* and *T. bernardii*. However, the amino acid content was increased with increasing the aqueous plant extract in the tested algae. The maximum total lipid contents of *S. pevalekii* and *T. bernardii* were recorded at 10% of aqueous extract. Fawzy *et al*. [34] revealed that the total carbohydrates, total proteins, and total lipids were significantly decreased by increasing the concentrations of the root extract of some medicinal plants. The ample evidence indicated that manipulation of algal growth media could also achieve improvements in lipid biosynthesis [35].

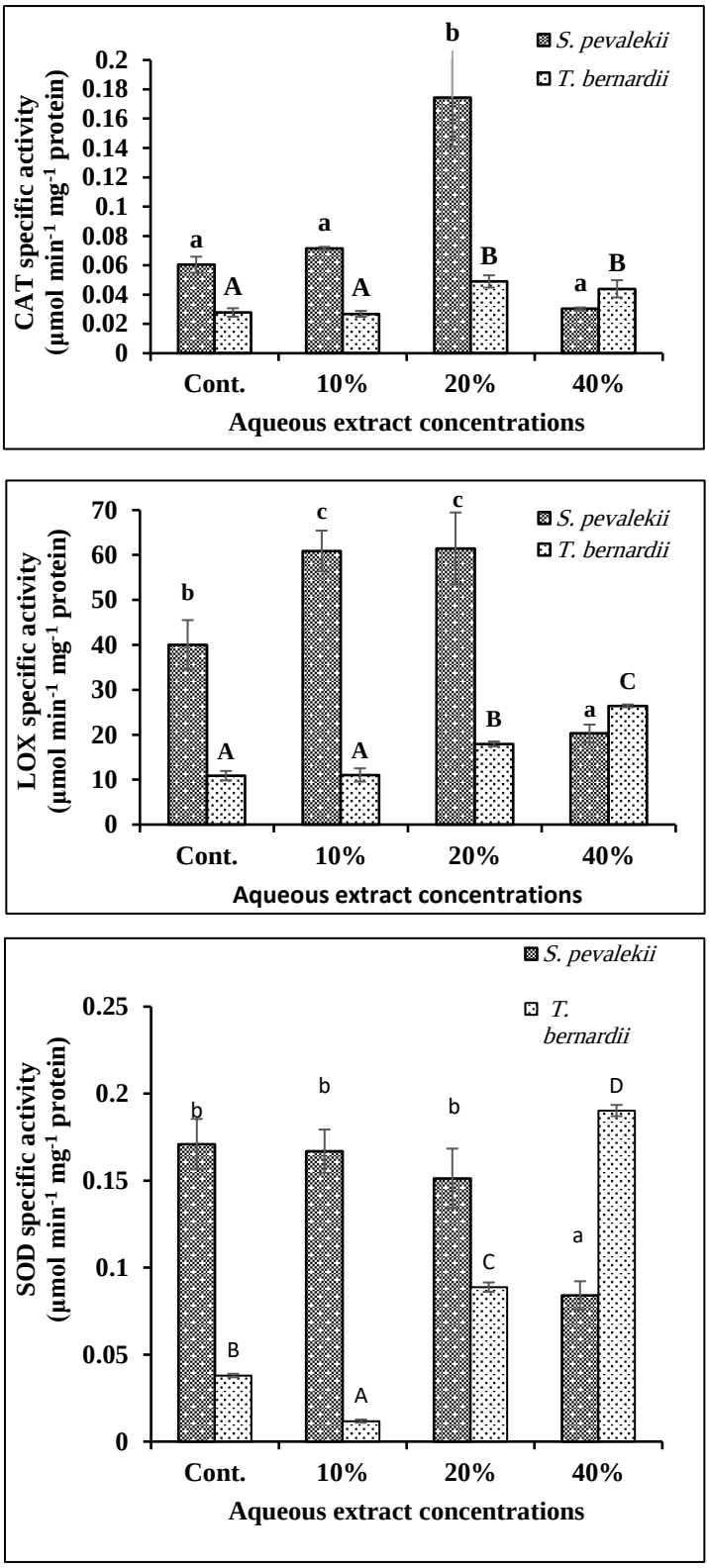

Figure 3. Effect of different concentrations of aqueous extracts of *P. salicifolia* (10, 20, and 40%) on the antioxidant enzymes activity of *S. pevalekii* and *T. bernardii*.
Cont.: control, CAT: catalase, LOX: lipoxygenase, and SOD: superoxide dismutase.

Aquatic macrophytes can control the growth of algae via inhibition or stimulation by releasing allelopathic substances [32]. The chl. *a* and dry weight of *S. pevalekii* in this investigation decreased at low conc. (40%) of *P. salicifolia* crude extract, and they were reduced significantly at all crude extract concentrations. These results are consistent with those of Chia *et al*. [33] who found that the chlorophyll *a* and dry weight of *Scenedesmus quadricauda* decreased with increasing the aqueous crude extract of *Azadirachta indica*. Fawzy *et al*. [34] also reported that the growth of *Cyanosarcina fontana* decreased with an increasing the concentration of the root extract of *Verbesina encelioides*.

The current study showed that the total carbohydrates, total proteins and proline contents were significantly decreased by increasing the concentrations of aqueous extract of *P. salicifolia* in *S. pevalekii* and *T. bernardii*. However, the amino acid content was increased with increasing the aqueous plant extract in the tested algae. The maximum total lipid contents of *S. pevalekii* and *T. bernardii* were recorded at 10% of aqueous extract. Fawzy *et al*. [34] revealed that the total carbohydrates, total proteins, and total lipids were significantly decreased by increasing the concentrations of the root extract of some medicinal plants. The ample evidence indicated that manipulation of algal growth media could also achieve improvements in lipid biosynthesis [35].

An excessive production of oxygen radicals dring algal metabolism is reported, especially when exposed to toxic or stress conditions [36]. As a result of increased reactive oxygen species (ROS), microalgae tend to up-regulate the biosynthesis and activities of ROS combating enzymes [37]. Catalase activity was stimulated in *S. pevalekii*, when treated with 20% extract of *P. Salicifolia* and significantly increased in *T. bernardii* with increasing the aqueous extract concentrations of *P. Salicifolia*. Cordeiro-Araújo *et al*. [38] reported a decline in CAT activity that may be related to its inactivation via the binding of thiol groups with the bioactive components of the extract investigated. The presence of toxic or bioactive substances in plants induces incresaed production of compounds like nitric oxide and H_2O_2, which have the potential to inhibit CAT activity [39]. The activity of the LOX in *S. pevalekii* was increased at a low concentration of the aqueous extract of *P. salicifolia* and increased with increasing the aqueous extract concentrations of *P. Salicifolia* in *T. bernardii* [40].

The SOD activity was also decreased in the case of *S.* extract of *P. salicifolia.* However, in the case of *T. bernardii*, SOD activity was increased with increasing the concentrations of the tested plant. In this respect, the allelochemicals of *Phragmites communis* reduced the activities of superoxide dismutase (SOD) *in Chlorella pyrenoidosa* and *Microcystis aeruginosa* [41-42].

Conclusions

The current investigation found that aqueous extract of *P. salicifolia* has the potential to inhibt the growth of *S. pevalekii* and *T. bernardii*. Furthermore, increasing the amount of aqueous extract of *P. salicifolia* improved the antioxidative enzyme activity of *T. bernardii*. As a result, *P. salicifolia* may be used as a biological agent to suppress nuisance algal blooms and undesired algae. Further research into the allelopathic effects of *P. salicifolia* in natural environments is also required, as the major microalgal species and environmental variables in lakes and water bodies differ from the *in vitro* conditions which may affect the outcomes.

Conflict of interest

The authors declare no conflict of interest

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