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The antiviral potential of macroalgae in suppressing *Sweet potato leaf curl virus* (SPLCV) infection in sweet potatoes

LISTIHANI LISTIHANI[1,♥], I GUSTI AYU DIAH YUNITI[1], PUTU LASMI YULIANTHI SAPANCA[1], NI PUTU PANDAWANI[1], DEWA GEDE WIRYANGGA SELANGGA[2]

[1]Faculty of Agriculture and Business, Universitas Mahasaraswati Denpasar. Jl. Kamboja No.11A, Dangin Puri Kangin, Kec. Denpasar Utara, Denpasar 80233, Bali, Indonesia. ♥email: listihani9@unmas.ac.id

[2]Faculty of Agriculture, Universitas Udayana. Jl. Raya Kampus Unud, Jimbaran, Kec. Kuta Sel., Badung 80361, Bali, Indonesia

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Abstract. *Listihani L, Yuniti IGAD, Sapanca PLY, Pandawani NP, Selangga DGW. 2023. The antiviral potential of macroalgae in suppressing Sweet potato leaf curl virus (SPLCV) infection in sweet potatoes. Biodiversitas 24: 4079-4086. Sweet potato leaf curl virus* (SPLCV) was first found in sweet potatoes in Indonesia in 2022. Prevention of spread of virus is essential, especially by using macroalgae extract which is environmentally friendly and has antiviral activity. The aim of present research was to test the potential of sea macroalgae to suppress SPLCV infection and to analyze phytochemicals of potential macroalgae containing an antiviral substance. Macroalgae extract was sprayed on the test plants that were infected by SPLCV. The observed parameters were changes in symptoms, disease incidence and severity, virus confirmation by PCR, and phytochemical analysis. The test results up to day 21 showed that *Eucheuma spinosum* was found to be effective in suppressing SPLCV infection in sweet potatoes, up to symptomless infection in young leaves. *E. spinosum* and *E. cottonii* suppressed disease incidence by 80% and 40% and lower disease severity as much as 71% and 48%, while *E. serra* showed less ability to suppress SPLCV infection. The two macroalgae had flavonoid, saponin, and steroid content which may be the reason to suppress the viral infection. The results of PCR analysis showed that microalgal extract had the highest nucleotide and amino acid homology with Gianyar (LC586170) isolate with values of 99.7 and 100%. The macroalgae with the highest ability to suppress the virus were *E. spinosum* and *E. cottonii*. This showed that the application of macroalgae extract did not change the amino acid sequence of SPLCV isolate.

Keywords: Antiviral, Begomovirus, *Eucheuma cottonii*, *Eucheuma serra*, *Eucheuma spinosum*, sweet potato

INTRODUCTION

Sweet potato is one of the staple foods of the people of Papua, Maluku, East Nusa Tenggara and Bali. Sweet potatoes were planted in the field by plant cutting. This method of cultivation allows the virus to be present in the field during planting into the next generations. More than 20 viruses infect sweet potatoes from the genus Begomovirus, Carlavirus, Cavemovirus, Crinivirus, Cucumovirus, Enamovirus, Ipomovirus, Nepovirus, Potyvirus, Solendovirus, and Tospovirus (Cuellar et al. 2015; Maina et al. 2018). Several important viruses in sweet potato have been reported, such as sweet potato virus C (SPVC), sweet potato feathery mottle virus (SPFMV), sweet potato feathery mottle virus strain internal cork (SPFMV-IC), sweet potato feathery mottle virus strain russet crack (SPFMV-RC), sweet potato mild mottle virus (SPMMV), sweet potato chlorotic stunt virus (SPCSV), sweet potato virus G (SPVG), and sweet potato leaf curl virus (SPLCV) (Choi et al. 2012; Clark et al. 2012; Kim et al. 2015; Maina et al. 2018; Zhang et al. 2020; Listihani and Selangga 2021; Listihani et al. 2022a). SPLCV (Geminiviridae; Begomovirus) was first infect sweet potatoes in Kenya, U.S., China, Japan, Spain, Uganda, Brazil, Korea, and Tanzania (Albuquerque et al. 2012; Choi et al. 2012; Cho et al. 2020; Wanjala et al. 2020; Andreason et al. 2021; Bachwenkizi et al. 2022). The sweet potato leaf curl virus (SPLCV) has been detected in sweet potato plants in Badung and Gianyar, Bali (Listihani et al. 2022a). Of a total of 111 plant species in 30 families tested, SPLCV infection was limited to plants of the Convolvulaceae family, genus Ipomoea (Ling et al. 2011). Apart from sweet potato (*I. batata*), 37 other *Ipomoea* species proved to be hosts for SPLCV (Ling et al. 2011). The natural spread of SPLCV is through vector insects (*Bemisia tabaci*) (Andreason et al. 2021). SPLCV causes an 80% disease incidence in sweet potato crops in Bella Vista, Corrientes province (NEA) (Pardina et al. 2012). Large insect vector populations are often found in the field, allowing the virus to spread more rapidly from one location to another and making control efforts more difficult. The plant virus maintenance performed until now involved the use of pesticides to tackle pests and virus insect vectors in plants, with the side effect being pathogen resistance against pesticides and over-accumulation of pesticide residue on the ground. Environmentally friendly SPLCV control is essential so that there is no resistance among pathogens and insect vectors. Natural products like macroalgae are often considered safe for the environment for being highly biodegradable and having a low biocidal activity (Wan et al. 2018). Macroalgae is thought to be a suitable resource of bioactive components with biological

activity, such as antibacterial, antifungal, and antiviral (Hamed et al. 2018).

Sea macroalgae can potentially be used for plant protection and development. The bioactive components, such as oil, protein (amino acid), bioflavonoid, polysaccharide, carotenoid, polyphenol, and carbohydrates are regarded to have bactericidal, antiviral, antinematode, and fungicide effects on plant pathogens (Hamed et al. 2018). The macroalgae extract is also used in agriculture as soil conditioner to improve plant productivity (González et al. 2013; Lola-Luz et al. 2014; Garcia-Gonzalez and Sommerfeld 2016; Seif et al. 2016; Thanaa et al. 2016; Barone et al. 2018; Murata et al. 2021; Ammar et al. 2022). The polysaccharide content in macroalgae has been reported to enhanced plant growth, with the ability to suppress fungi, bacteria and viruses, as well as to improve productivity in many plants (Sharma et al. 2014). Phenolic acid and flavonoid bioactive components in the methanol extract of *Sargassum vulgare* can act as an antifungal against *Pythium aphanidermatum* by inhibiting the pathogenic mycelium growth by around 51% and lowering disease severity by 82% (Nawaim et al. 2017; Nawaim et al. 2018). Brown algae *Sargassum swartzii* controls the rice sheath blight caused by *Rhizoctonia solani*. This defense mechanism is thought to be correlated with high phenolate and the early accumulation of phytoalexin compound in rice plants (Raj et al. 2016). Kappa/beta carrageenan extracted from *Tichocarpus crinitus* suppressed the TMV infection in tobacco leaves (Shukla et al. 2016; Asimakis et al. 2022).

Control of viral diseases on cultivated plants technically using plant barriers and biologically with chitosan, plant extract, and nonpathogenic bacteria has been studied previously (Selangga et al. 2018; Triwidodo and Listihani 2020; Pandawani et al. 2022). However, the potential and use of macroalgae in plant protection to control plant virus have not been studied. Thus, the aim of present research was to test the potential of sea macroalgae in suppressing SPLCV infection and to analyze the phytochemicals of potential macroalgae containing an antiviral substance.

MATERIALS AND METHODS

Inoculum multiplication

SPLCV virus source plant was obtained from previous collections of SPLCV-Tegallalang isolates from Gianyar Regency (Listihani et al. 2022a). The inoculum source was multiplied on sweet potato plants in different pots to get as much virus inoculum stock. The test plants used were sweet potato plants which had been tested by PCR and showed negative and positive results for SPLCV.

Test plant planting

Sweet potato cutting plants for SPLCV were planted by using 30 cm x 30 cm siz polybags with 6 kg planting medium. The plant medium used was manure and dirt with 1:1 ratio. The cutting plants were cut every three joints and planted on the polybag in the morning, with the requirements of one joint inside the planting medium and one leaf left attached. The planted stick was then watered to maintain the medium's humidity. Watering was performed once a day.

Sampling and macroalgae extract production

Macroalgae samples were collected from Serangan Island. Macroalgae samples were collected by pulling off the thallus and then washed with water and stored in strapped plastic containing sterile seawater. The extract of macroalgae was made from 2 g wet weight of each sample being grind in liquid nitrogen by using mortar and pestle. The grounded macroalgae were then diluted three times with 30 mL methanol and filtered by using Whatman No.41 filter paper (Santosa 2017). The extract was placed in a rotary evaporator to separate the methanol and microalgae extract.

Macroalgae extract application to test plant

The crude extract of macroalgae was diluted in 5% 2-methoxyethanol to obtain liquid extract with a concentration of 10 mg.ml-¹ (Santosa 2017). The macroalgae extract was then sprayed three times on the leaves of test plant using a mini sprayer. Treated plants were maintained and observed until light symptoms or symptomless conditions appeared from severe symptoms. The present research consisted of one factor and ten repetitions in the same test environment. The factor was comprised of three macroalgae extract treatments (*Eucheuma spinosum, Eucheuma cottonii,* and *Eucheuma serra*) applied on the test plants. Positive control (plants positive for SPLCV without treatment) and negative control (plants negative for SPLCV without treatment) were also used for the research. The variables observed were symptoms, disease incidence, and severity according to the score in Table 1.

SPLCV confirmation by PCR and sequencing analysis

The virus detection method by PCR consisted of several stages: total DNA extraction, DNA amplification, and amplification product visualization. Total DNA extraction was done manually through the CTAB method (Doyle and Doyle 1990). The virus DNA amplification was performed by using universal primer pair for *Begomovirus* (SPG2-_5'-ATCCVAAYWTYCAGGGAGCTAA-3'/SPG1_5'-CCCKGTGCGWRAATCCAT-3'). The nucleotide and amino acid homology analysis for DNA virus was performed by BLAST software in NCBi site (www.ncbi.nlm.nih.gov). The nucleotide sequences of all chosen isolates were modified by Bioedit V7.0.5 software before being used for phylogenetic analysis. The phylogenetic tree was made by ClustalW software (Bioedit V7.0.5).

Table 1. Disease score based on visual symptoms in plant

Scores	Symptoms
0	Symptomless
1	Mild vein clearing
2	Mild vein clearing and malformation leaves
3	Severe vein clearing
4	Severe vein clearing and malformation leaves

Phytochemical analysis

The macroalgae samples showing the effects of SPLCV infection were used for phytochemical analysis. Microalgae samples tested for alkaloid, flavonoid, triterpenoid, steroid, tannin, saponin, carotenoid, and coumarin (Santosa 2017).

RESULTS AND DISCUSSION

SPLCV infection influences the symptoms in sweet potato leaves (Figure 1). In general, viral infection symptoms on the leaves are yellowing and curling, while fruits showed abnormalities in size, color, texture, ripeness, number of seedlings, and total productivity, and several viruses cause fruit death (Listihani et al. 2019, 2020, 2022b; Selangga and Listihani 2022; Selangga et al. 2022). As stated by Listihani et al. (2022a), SPLCV infection symptoms in young leaves are changes in leaf color into vein clearing. Based on the test result up to day 21, it was observed that *Eucheuma spinosum* showed the best performance in suppressing SPLCV infection in sweet potato plants into symptomless disease in young leaves (Table 2, Figure 2). *E. spinosum* reduced disease incidence by 80%, and disease severity by 71%. Reduction in the content of chlorophyll a, chlorophyll b, carotenoid, carbohydrate, protein, and amino acids in infected plant is caused by viral activity, causing symptoms to appear on the plants (Soni et al. 2022). The difference in the content level decrease in plants causes different disease severity scores (Ghannam et al. 2013; Ahmadi et al. 2015). In the present study, several macroalgae extract treatments, such as *Eucheuma cottonii* and *Eucheuma serra* were able to suppress SPLCV disease incidence by up to 40% with mild vein-clearing symptoms and by 10% with severe vein-clearing symptoms. In comparison, positive control plants showed disease incidence of 100% with severe vein-clearing symptoms. Disease severity in positive control plants continued to increase with growing time, ranging from 85% from 78%. In contrast, plants given macroalgae extract showed a trend of decreasing disease incidence and severity along with growing time. Viral infection in sweet potato leaves was suppressed after treatment with extracts, causing previously severe symptoms to be mild or asymptomatic. All three macroalgae types were able to suppress viral infection.

A recovery period occurs when plants infected with the virus show very severe symptoms, but over a period of time the symptoms disappear or change into mild symptoms, especially on freshly grown plant parts (Listihani et al. 2021; Selangga et al. 2021; Selangga et al. 2023). The inhibition level of SPLCV infection ranged between 5-71%, which was shown by macroalgae extract treatment (Table 2). The level of inhibition between three treatments may be influenced by certain inhibitory mechanisms activated by the application of the extract used. A similar result was also reported by Santosa (2017) who stated that macroalgae extract treatment can lower the disease incidence and severity when compared to CMV infected control plants without treatment.

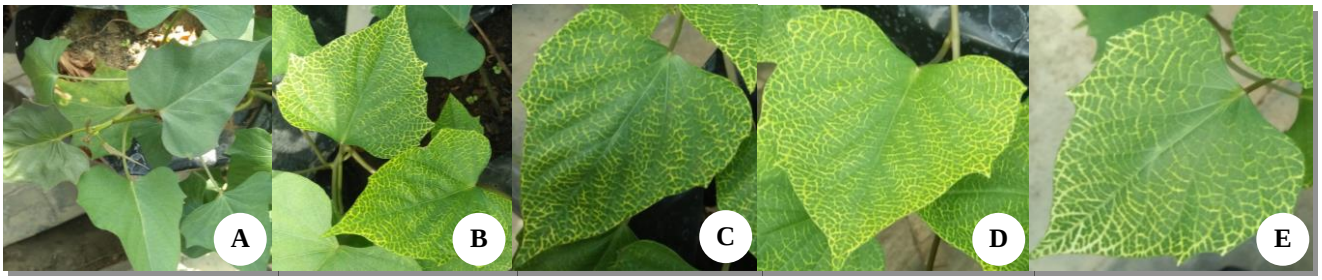

Figure 1. Vein clearing symptoms on sweetpotato leaves before macroalgae treatment: A. negative control; B. positive control; C. *E. spinosum* treatment; D. *E. cottonii* treatment; and E. *E. serra* treatment

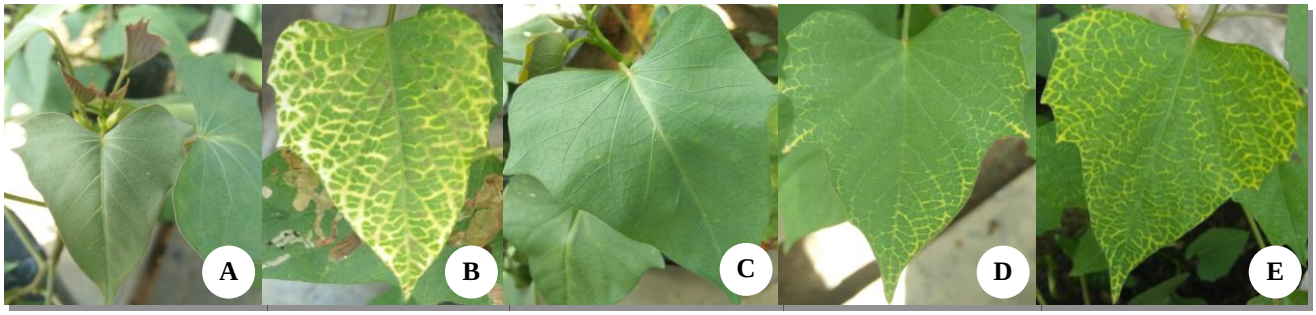

Figure 2. Vein clearing symptoms on sweetpotato leaves after macroalgae treatment: A. negative control; B. positive control; C. *E. spinosum* treatment; D. *E. cottonii* treatment; and E. *E. serra* treatment

Macroalgae extract treatment on SPLCV showed different results for each type, macroalgae extract treatment showed significantly different symptoms changes compared to leaves without any treatment (positive control) (Figures 1 and 2). According to Pandawani et al. (2022), extract treatment that has been inoculated by virus did not show symptoms because biological control can suppress infection and virus multiplication as well as inhibit replication and spread inside the plant. It has been observed that macroalgae extract could suppress virus from multiplying inside the plants so that no symptom appeared on the leaf surface. It was recorded that plants that were given the extract treatment showed varied symptoms from day 14 to day 21, while control leaf symptoms showed increasingly severe symptoms on young leaves. Plants in negative control (without extract treatment) appeared without symptom, and the PCR result showed a negative result for Begomovirus. Although symptom changes were found on plants given the extract treatments, the PCR result showed them to be positive for Begomovirus. This was because virus particles were present on the leaves in small numbers or inactive, causing no symptoms but could be detected by PCR. SPLCV isolates that were given macroalgae extract treatment were analyzed for molecular content by PCR. Results revealed that nucleotide and amino acid homology was found to be highest with SPLCV isolates from Gianyar (LC586170) with the value of 99.7 and 100% (Table 3). The phylogenetic tree analysis also showed that SPLCV that was given extract treatment showed the same group with Gianyar isolates and was different from other isolate groups available in the GenBank (Figure 3). This showed that macroalgae extract application causes several nucleotide base sequence changes but did not change the amino acid sequence of SPLCV isolate.

Table 2. The effect of macroalgae application on disease symptoms and severity of vein clearing in sweetpotato plants

Treatments	Before treatment			Four weeks after treatment			Begomovirus detection by PCR
	Disease symptoms	Disease incidence (%)	Disease severity (%)	Disease symptoms	Disease incidence (%)	Disease severity (%)	
C-	Symptomless	0 (0/10)	0	Symptomless	0 (0/10)	0	-
C+	Severe vein clearing	100 (10/10)	78	Severe vein clearing and malformation leaves	100 (10/10)	85	+
Eucheuma spinosum	Severe vein clearing	100 (10/10)	78	Symptomless and Mild vein clearing	20 (2/10)	7	+
Eucheuma cottonii	Severe vein clearing	100 (10/10)	79	Mild vein clearing	60 (6/10)	31	+
Eucheuma serra	Severe vein clearing	100 (10/10)	77	Severe vein clearing	90 (9/10)	72	+

Note: negative control, C- (SPLCV negative test plants without treatment); positive control, C+ (SPLCV positive test plants without treatment)

Table 3. Homology of nucleotide and amino acid of SPLCV isolates after treatment with SPLCV isolates from Gianyar

Isolates	Geographical origin	Hosts	Symptoms	Homology (%) SPLCV after treatment		Accession numbers
				nt	aa	
U Ubud-Ubud-1	Gianyar, Indonesia	*Ipomoea batatas*	Vein clearing	99.7	100	LC586170
hu194 Hu-194	Hunan, China	*Ipomoea batatas*	Vein clearing	97.7	98.8	MK052985
ZJ	Zhejiang, China	*Ipomoea setosa*	Leaf curling	96.5	97.4	JF736657
202	South Korea	*Ipomoea batatas*	Leaf curling	96.1	97.1	KT992065
169	South Korea	*Ipomoea batatas*	Leaf curling	96.4	97.6	KT992062
GE-21	Muan, South Korea	*Ipomoea batatas*	Unknown	96.0	97.1	JX961673
7	South Korea	*Ipomoea batatas*	Leaf curling	95.7	96.9	KT992048
Sp3-2	Spain	Unknown	Unknown	89.0	90.9	KT099145
P213-11	Southern Portugal	*Ipomoea indica*	Vein clearing	88.6	90.2	MG254543
P213-8	Southern Portugal	*Ipomoea indica*	Vein clearing	88.3	90.0	MG254542
409	Khartoum, Sudan	*Ipomoea batatas*	Lef curling	88.8	90.4	KY270782
Uk-2008	Kampala, Uganda	*Ipomoea setosa*	Leaf curling	88.8	90.4	FR751068
648B-9	South Carolina, USA	*Ipomoea batatas*	Leaf curling	88.2	90.0	HQ333144
BR-Uti-08	Bahia, Brazil	*Ipomoea batatas*	Leaf curling	88.2	90.0	HQ393447
WS1-4	South Carolina, USA	*Ipomoea setosa*	Leaf curling	88.5	90.2	HQ333141
MP3-09	Pernambuco, Brazil	*Ipomoea batatas*	Leaf curling	87.8	89.7	HQ393470
*TYLCV	Masan, South Korea	*Lycopersicon esculentum*	Leaf curling	66.5	69.6	HM130912

Note: *TYLCV: *Tomato yellows leaf curl virus* as *out group*; nt (nucleotide) and aa (amino acid)

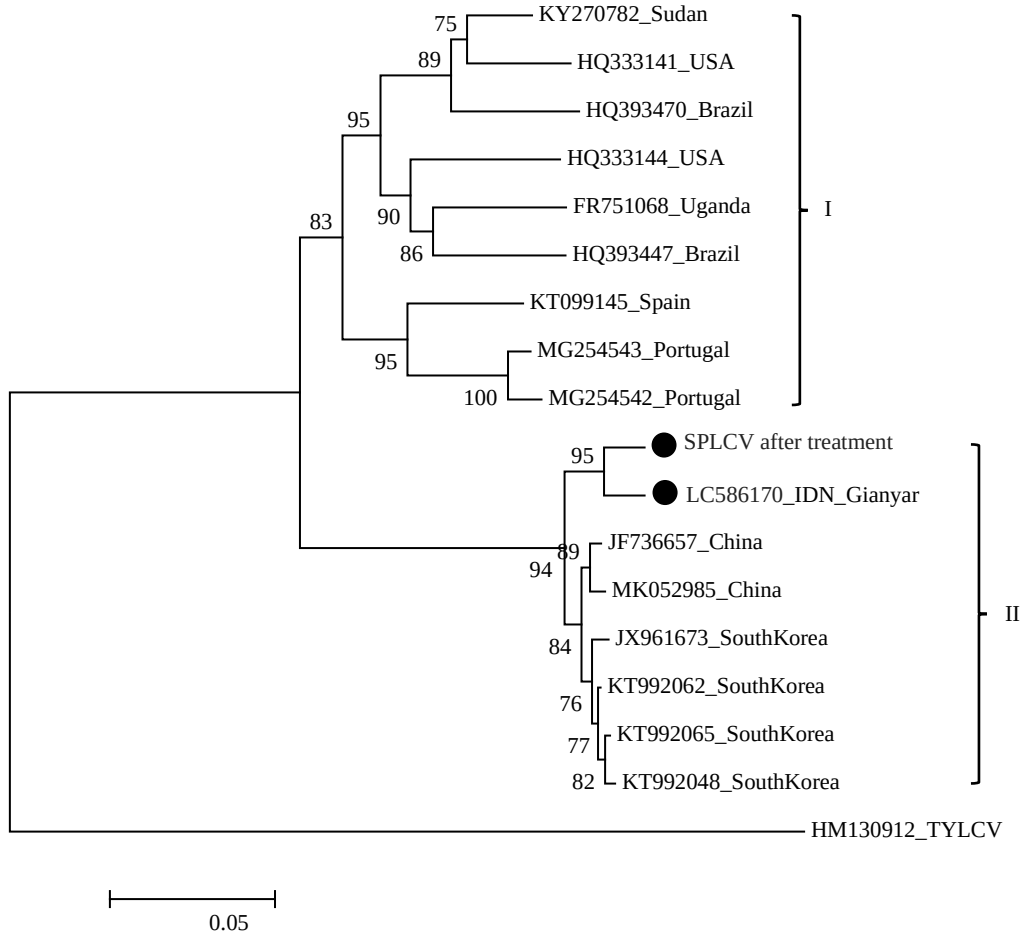

Figure 3. Phylogenetic tree of AC1 and AC2 SPLCV genes for Bali isolates based on the SPLCV nucleotide sequence using *Tomato yellows leaf curl virus* (TYLCV) as the out group. IDN-Indonesia

Table 4. Phytochemical analysis of selected macroalgae species

Species	Alkaloid	Flavonoid	Saponin	Steroid
Eucheuma spinosum	-	+	++	+++
Eucheuma cottonii	-	++	++	++

Note: -: Absent, +: Little content, ++: Medium content, +++: High content

The phytochemical analysis was only tested on two of three macroalgae species, which were *E. spinosum* and *E. cottonii*. *E. spinosum* and *E. cottonii*. The species were chosen to show their ability to suppress SPLCV moderately (*E. cottonii*) and highly (*E. spinosum*). The phytochemical analysis showed that *E. spinosum* and *E. cottonii*. did not contain alkaloids, but both contained steroids in different amounts (Table 4). *E. spinosum* species contained high steroid content, but *E. cottonii* species had low steroid content. Both species showed negative results for tannin, quinone or even triterpenoid contents, but saponin content was found in *E. spinosum* and *E. cottonii*. Flavonoid content was found in both species, but highest content was found in *E. cottonii.*

Extract application in suppressing viral infection enhances several enzyme activities, such as peroxidase, polyphenol oxidase, and phenol (Abdelkhalek et al. 2021). Healthy plants contain a lot of highly soluble proteins that are the components of virus coat protein synthesis (Martins et al. 2022). The induction of macroalgae extract in the plants can lower the level of soluble protein, making the environment less advantageous for virus replication.

Macroalgae and plants have the ability to produce bioactive substances that can suppress pathogens. Flavonoid has a role in plant protection against biotic factor (herbivores, pathogen) and abiotic pressure (UV radiation, heat) (Mierziak et al. 2014). Flavonoid also tightens plant and tissue structures by controlling the auxin activity (IAA) which prevents pathogen infection and is related to pathogenic enzyme inhibition, especially those that damage plant cell wall (Mierziak et al. 2014).

Based on the research result, test plants given macroalgae extract after being inoculated by SPLCV changed their symptom to mild and symptomless. They

also showed lower disease incidence and severity compared to untreated test plants. This is because compounds trigger salicylic acid inside the plant, which induces plant's defense against virus (Sharma et al. 2014; Sudirman et al. 2018). Thus, it improves the plant resistance and allows it to endure virus attach. The mechanism of macroalgae antiviral interaction is explained as the inhibition of virus adsorption into the host cell, after passing the competition with attaching virus or through a synergic combination between polysaccharides and the host. The mechanism is that polysaccharide blocks the cell host, so the virus cannot get in and infect (Hamed et al. 2018). Macroalgae mechanism in inhibiting plant virus replication has previously been reported. Zhao et al. (2017) reported that macroalgae inhibit the replication of *Potato virus* X (PVX) up to 95% in potatoes. Zhao et al. (2015) and Stadnik and De Freitas (2014) also reported that *Dictyota* spp. has antiviral activity against plant viruses for containing of polysaccharides. A study from Santosa (2017) also showed that *Dictyota cervicornis* has antiviral potential as it can supressed the number of local lesions on *C. amaranticolor* infected by CMV for up to 96.25%; the possible use of *D. cervicornis* as antiviral is also due to the high level of polysaccharides in it. Sami et al. (2021) reported that *E. spinosum* and *E. cottonii* has high polysaccharide content which makes them great for use as antiviral. *E. cottonii* contain various nutrition, such as protein, lipid, carbohydrate, and bioactive compounds (Biris-Dorhoi et al. 2020; Hentati et al. 2020; Martins et al. 2022). The bioactive compounds present in various algae are polyphenol (Hentati et al. 2020), alkaloid, terpen, pigment, sterole, fatty acid (Barbosa et al. 2014), carrageenan, fucan, and several other compounds that have been proven to potentially be used as antiviral, antibacterial, and anticancer (Kalitnik et al. 2013; Prajapati et al. 2014). *Eucheuma spinosum* and *Eucheuma cottonii* contain carrageenan which is proven to act as an antiviral. Carrageenan's mechanism as an antivirus is by inactivating viral particles so that virus cannot replicate (Sangha et al. 2015). *Eucheuma cottonii* is a type of red algae that can produce polysaccharides such as carrageenan (Sudirman et al. 2018; Lomartire et al. 2022). The polysaccharide content in *Eucheuma cottonii* is crucial for all organisms and has various biological functions, including as anti-inflammatory agent, anticoagulant, antibacterial, antioxidant activity, and inhibit virus attack (Hentati et al. 2020; Carpena et al. 2022). The faster the plant symptom turning into mild indicates that the extract application can inhibit damages due to viral activity in plants. Suppression of symptoms probably occur due to salicylic acid function as an indirect inhibitor of virus systemic movement through the host vascular tissue, resulting in symptoms delay (Ghannam et al. 2013). The given macroalgae extract showed its activity on virus-infected plant as a virus inhibitor. Viral inhibitor is a compound that can prevent viral infection which is present in the sap of certain plants (Duarte et al. 2021).

Several compounds are produced by plants as pathogen inhibitors are flavanoid, chitinase, phytoalexin, peroxidase, polyphenol oxidase, and lipoxidase (Duarte et al. 2021). Improving plant defense against pathogen infection can be done by inducing systemic defense in the plant itself (Selangga et al. 2018). Systemic defense induction by induction agent (*Systemic Acquired Resistance*/SAR) is a method that has been developed to produce plants that are more resistant to disease. The flavonoid and steroid compounds in macroalgae extract act as systemic defense inducing agent which activates the plant defense system and thus improves the plant defense mechanism against virus. Phenolic compounds, and flavonoids are known to be important as a signal molecule in several plant defense responses. The expression of SAR is very dependent on the accumulation of flavonoids or salicylic acid and associated with pathogenesis-related protein (PR protein) which has anti-pathogen activity (Zhou et al. 2021).

Conclusions

Macroalgae extract treatment showed that treated sweet potato had lower disease incidence and severe and mild symptoms or even symptomless, compared to positive control plants that were only infected by virus. *E. spinosum* and *E. cottonii* were very effective in controlling SPLCV infection in sweet potatoes. Phytochemicals like, steroid and flavonoid may be play an important role to suppress viral infection in plants.

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