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Influence of seaweed extracts on growth, phytochemical contents and antioxidant capacity of cowpea (*Vigna unguiculata* L. Walp)

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ABSTRACT

In this study, seaweed extracts were extracted from two seaweeds viz. *Sargassum swartzii* (brown seaweed) (SSE) and *Kappaphycus alvarezii* (red seaweed) (KAE). The seaweed extracts SSE and KAE were comparatively analyzed to assess the growth, yield, phytochemical content and antioxidant capacity of *Vigna unguiculata*. It was observed that the 3% SSE spray significantly improved the shoot length (33 cm), number of leaves (28), yield (40 g/pot), total phenolic content (36.64 μg GAE/g FW), protein (0.42 mg/g) and flavonoids (7.36 μg QCE/g FW) compared to the control. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity was also high in 3% SSE spray. The major antioxidant compound was identified using methanolic extracts of *Vigna unguiculata* through GC-MS analysis. The results suggested that application of brown seaweed extract (SSE) with the concentration of 3% can significantly improve the phytochemical content and antioxidant activity in *Vigna unguiculata* thus enhancing its nutritional quality.

1. Introduction

Modern agricultural practices largely depends on chemical fertilizers and pesticides to increase crop production. The sustained use of these chemical fertilizers adversely affects the soil efficiency and also have serious impact on human health (Rengasamy et al., 2015). In recent years, there is growing interest in the use of natural fertilizers than the chemical fertilizers. Seaweed extracts obtained from macroalgae has several economic importance in agriculture as soil fertilizer, growth stimulants (Khan et al., 2009), enhanced seed germination and plant growth, root development, increased yield and quality of vegetables like cucumber, tomato, broccoli, spinach and bean and also increases post-harvest shelf life (Beckett et al., 1994; Khan et al., 2009; Sarhan et al., 2011; Mattner et al., 2013; Hernández-Herrera et al., 2014). Macroalgae extracts enhance crop's tolerance towards environment stress (Zhang and Schmidt, 2000) particularly enhance drought stress, increase nutrient uptake from soil and antioxidant properties of the plants (Turan and Köse, 2004; Verkleij, 1992). All these have been attributed to the presence of minerals, nutrients, amino acids, vitamins, pigments, and complex polysaccharides that contribute to plant growth (Calvo et al., 2014). These extracts can be applied as foliar spray, granules and seaweed paste as soil manure (Thirumaran et al., 2009). Seaweed fertilizers are gaining importance over commercial synthetic fertilizer (Khan et al., 2009; Zodape, 2001) due to their properties like biodegradability, non-toxic, non-polluting and non-hazardous to humans, animals and environment (Craigie, 2011).

Cowpea (*Vignaunguiculata* L. Walp.), a staple food vegetable consumed by people all around the world especially in the developing nations, is rich in proteins, vitamins, and essential minerals. Cowpea seeds have been reported to contain about 0.18–0.59% tannins (Reddy et al., 1985), phenolic acids, such as p-hydroxybenzoicacid, protocatechuic acid, 2,4-dimethoxybenzoic acid, and cinnamic acid derivatives, which includes p-coumaric acid, caffeicacid, cinnamic acid and ferulic acid (Cai et al., 2003; Sosulski and Dabrowski, 1984).

The aim of this study was to compare seaweed extract SSE and KAE on the growth, yield, phytochemical contents and antioxidant capacity of *Vigna unguiculata* for the development and use of environmental friendly biofertilizer in sustainable organic farming.

2. Materials and methods

2.1. Chemicals

Gallic acid (Cat no.13142), 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

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(Cat no.29128), ascorbic acid (Cat no.0149100) and Folin- Ciocalteu reagent (Cat no. 39520) were purchased from Sisco Research Laboratories, India and Sigma-Aldrich, USA. All other chemicals and solvents used in the study were of analytical grade.

2.2. Preparation of seaweed extracts

Kappaphycus alvarezii (red seaweed) were collected from Munnaikadu (9°17'16.73"N; 79° 8′1.43"E) located in South East Coast of Tamil Nadu, India. The seaweed was identified using the standard systematic key reference followed by (Rao, 1987). The algae were brought to the laboratory and washed thoroughly in tap water to remove all epiphytes, sand and associated fauna. The *Kappaphycus alvarezii* extract (KAE) was prepared by grinding the seaweed (*Kappaphycus alvarezii)* using seaweed sap extractor (Indigenous model, SS Bio pulverizer 5HP capacity), filtered and stored at 4 °C (Rathore et al., 2009). The filtrate was considered as 100% KAE extract and different concentration of KAE (3%, 4% and 5%) (v/v) were prepared by diluting with distilled water.

Sargassum swartzii extract (SSE) was prepared using *Sargassum swartzii* collected from Mandapam (9°16'54.03"N; 79°11'20.50"E) located in Gulf of Mannar coast, Tamil Nadu, India. The seaweeds were washed, dried and pulverized into fine powder. The SSE was prepared by dissolving the *Sargassum swartzii* powder in distilled water with continuous stirring for 15 min, filtered and stored at 4 °C. The filtrate was assumed as 100% SSE extract and different concentration of SSE (3%, 4% and 5%) (v/v) were prepared by diluting with distilled water.

The total organic and inorganic composition of SSE and KAE were analyzed according to the procedure described in (Mondal et al., 2015).

2.3. Plant culture and treatment

Cowpea (*Vigna unguiculata* L.Walp) seeds obtained from horticulture society of Tamilnadu, Chennai were chosen for the experiments. Seeds were sown at 1.5 cm deep in polybags (60 cm diameter; 3 seeds per pot) containing sand, coir pith and soil as pot mixture (1:1:2 w/w ratio). Plants were sprayed with SSE and KAE extracts (3%, 4% and 5%) at the rate of 50 mL per plant at an interval of 15 days for a period 60 days. Growth and yield parameters were observed after 60 days. All experiments were carried out in triplicates.

2.4. Preparations of cowpea extract

The harvested cowpea (*Vigna unguiculata* L.Walp) pods were collected and stored in a refrigerator (4 °C) within 1 h of harvest. Cowpea pods were chopped into small pieces and extracted twice with 70% aqueous methanol (v/v) at 40 °C for 2 h, centrifuged, filtered, combined and stored at 4 °C.

2.5. Measurement of total phenolic content

The total phenolic content was analyzed using the Folin–Ciocalteu method as described previously (Singh et al., 2002). Briefly, 0.2 mL of sample was pipetted into test tubes and 0.2 mL Folin–Ciocalteu reagent was added to each tube. After 8 min incubation at room temperature, 0.3 mL 15% (w/v) of sodium carbonate solution was added to stop the reaction. The mixture was left at room temperature under dark condition for 2 h and the absorbance was read at 760 nm using a Bio-spectrometer (Eppendorf kinetic, Germany). Measurements were carried out in triplicate and calculations were made based on a calculation curve obtained with Gallic acid. The total phenolics were expressed as micrograms of Gallic acid equivalents per milligram of dried sample.

2.6. Measurement of total protein content

The total protein content was determined using the Lowry's method as described previously (Lowry et al., 1951). Briefly, 0.2 mL of sample was taken in different test tubes and 2 mL of alkaline copper sulphate reagent was added to each tube. After 10 min incubation, 0.2 mL of Folin Ciocalteau reagent was added. The mixture was incubated at room temperature for 30 min and the absorbance was read at 660 nm using Bio-spectrometer (Eppendorf kinetic, Germany). The total protein content was calculated based on calibration curve obtained with Bovine Serum Albumin (BSA).

2.7. Measurement of total flavonoid content

The Total Flavonoid Content (TFC) of each *Vigna unguiculata* extract was determined using the aluminum chloride colorimetric method (Meda et al., 2005). In brief, the diluted extract or quercetin (0.5 mL) was mixed with 0.1 mL of 10% (w/v) aluminum chloride solution and 0.1 mL of 0.1 mM potassium acetate solution. The mixture was kept at room temperature for 30 min. The calibration curve was prepared by diluting quercetin in methanol (0–100 μg/mL). Then the maximum absorbance of the mixture was measured at 415 nm using a Bio-spectrometer (Eppendorf kinetic, Germany). Total flavonoid content was expressed as milligram quercetin equivalent per gram cowpea extract (mg QCN/g).

2.8. DPPH radical scavenging activity

DPPH radical scavenging activity was measured based on (Brand-Williams et al., 1995). Then 0.1 mL of sample was added to 0.9 mL of 0.1 mM DPPH radical solution, prepared in methanol. The solution was rapidly mixed and allowed to stand in dark at 37 °C for 30 min. The blank was prepared in a similar way without sample. The decrease in absorbance of each solution was measured at 517 nm using Bio-spectrometer (Eppendorf kinetic, Germany). Ascorbic acid was used as a positive control. The percentage of radical scavenging activity of tested extracts was calculated by using the following formula:

$$\text{DPPH Scavenging activity}(\%) = [(A_0 - A_1 / A_0)] \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.9. Phytochemical analysis using GC-MS

This analysis was performed in this study to identify the bioactive compounds in 3% SSE treatment group which showed significant improvement in the crop quality. Gas Chromatography–Mass Spectrometry (GC–MS) (QP2010 Ultra, Shimadzu, Japan) equipped with Rtx-5SilMS column (30 m × 0.25 mm × 0.25 μm) was used to analyze bioactive compounds in the extract. For GC-MS detection, an electron ionization energy system with ionization energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1 mL/min and an injection volume of 1 μL was employed (split ratio: 10); Injector temperature 200 °C; Ion-source temperature 200 °C. The oven temperature was programmed from 80 °C (isothermal for 2 min), with an increase of 5 °C/min up to 300 °C for 2 min. Mass spectra were taken at 70 eV; at scan interval of 0.5 s with scan range of 45–1000 *m/z*. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. Software adopted to handle mass spectra and chromatograms was a GC-MS solution ver.2.53 with NIST 11 and WILEY 8 library.

2.10. Statistical analysis

Statistical analysis was performed using graph pad prism 7.0 software package. The results of all the experiment were represented as mean ± SD of triplicates employed. Significant differences among the means of samples were analyzed by Tukey's test and analysis of variance was performed by one-way ANOVA.

Table 1
Chemical constituents of SSE and KAE.

Constituents	Concentration in SSE	Concentration in KAE
Macro nutrients		
Nitrogen	1.32%	0.16%
Phosphorus	0.29%	0.043%
Potassium	0.8%	1.82%
Secondary nutrients		
Calcium	1.43%	0.10%
Magnesium	0.1%	0.01%
Sulphur	0.23%	0.045%
Micro nutrients		
Molybdenum	3 ppm	2 ppm
Boron	0.16 ppm	0.13 ppm
Copper	111.86 ppm	24.62 ppm
Manganese	23.54 ppm	2.93 ppm
Zinc	28.42 ppm	5.68 ppm
Iron	783.07 ppm	49.58 ppm

Table 2
Effect of seaweed extracts on Plant height, Number of leaves and yield of *Vigna unguiculata.*

Treatment (%)	Plant height (cm)	No. of leaves	Vegetable Yield (g FW/ plant)
Control	31 ± 1.73	18 ± 2	12
3% KAE	33.67 ± 1.53	19.33 ± 3.51	32
4% KAE	34.33 ± 3.51	20.33 ± 3.06	36
5% KAE	33 ± 1	22 ± 1.53*	38
3% SSE	36.69 ± 3.79*	28 ± 1***	40
4% SSE	33.33 ± 3.51	23.33 ± 3.06**	36
5% SSE	34.43 ± 3.61	26.67 ± 0.58***	36

All values are expressed as mean ± S.D.
* p < 0.05.
** p < 0.005.
*** p < 0.005.

3. Results

3.1. Chemical composition of SSE and KAE extracts

The composition analysis of SSE and KAE showed the presence of macro and micronutrients (Table 1). While nitrogen concentration was higher in SSE, potassium concentration was higher in KAE. The concentration of iron was higher in SSE followed by zinc and other micronutrients. The calcium concentration was higher in SSE than the other nutrients. The composition of secondary nutrients in KAE was comparatively equal for the nutrients such as calcium, magnesium and sulphur.

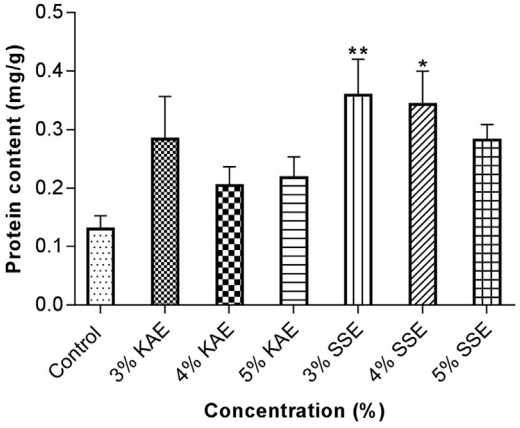

Fig. 2. Effects of KAE and SSE on *Vigna unguiculata* protein content. All the values are mean ± SD of triplicates. Significant differences are indicated by *p < 0.05, **p < 0.005, ***p < 0.005 Vs Control.

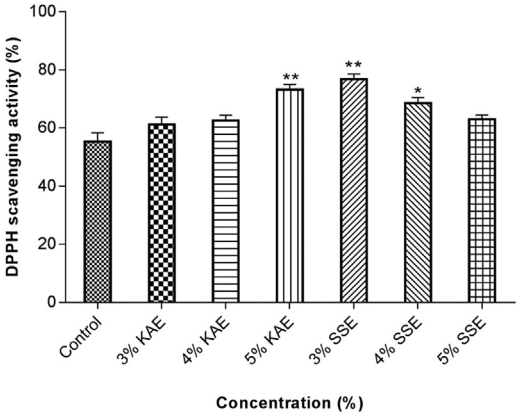

Fig. 3. Effects of KAE and SSE on *Vigna unguiculata* antioxidant activity. All the values are mean ± SD of triplicates. Significant differences are indicated by *p < 0.05, **p < 0.005, ***p < 0.005 Vs Control.

3.2. Effect of SSE and KAE extracts on growth of Vigna unguiculata

Both KAE and SSE treatments showed significant improvement on the growth of *Vigna unguiculata*, especially shoot length, number of

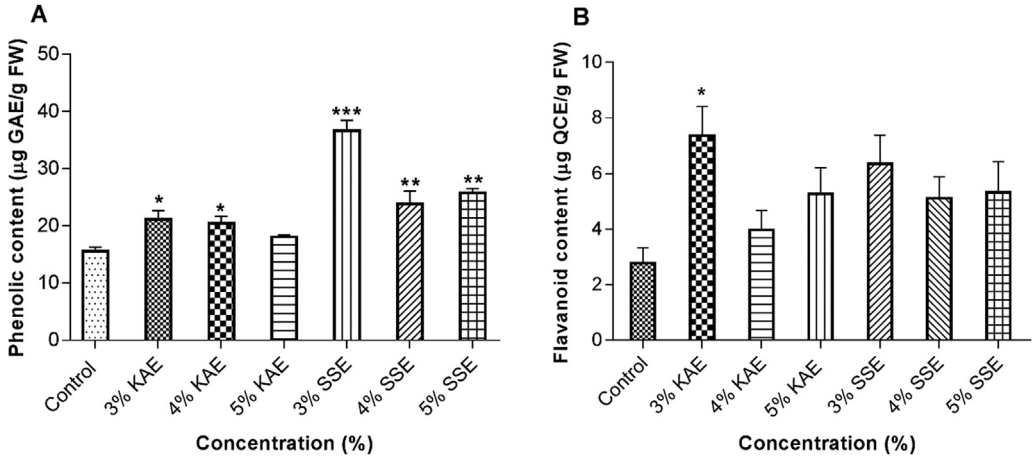

Fig. 1. Effects of KAE and SSE on *Vigna unguiculata*. A. Phenolic content, B. Flavonoid content. All the values are mean ± SD of triplicates. Significant differences are indicated by *p < 0.05, **p < 0.005, ***p < 0.005 Vs Control.

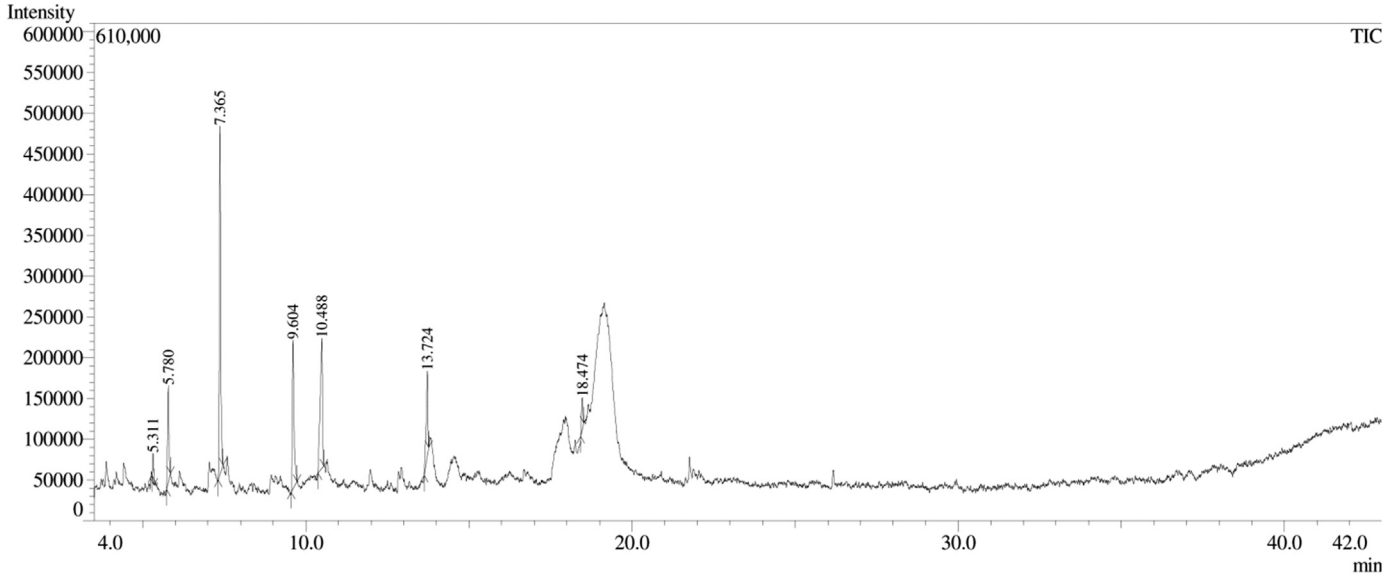

Fig. 4. GC-MS chromatogram of *Vigna unguiculata* treated with 3% SSE.

Table 3
Phytocomponents identified in the methanolic extract of *Vigna unguiculata* by GC-MS.

Peak	Retention time	Name of the compound	Area	Area%
1	5.311	Benzeneacetaldehyde	62,612	1.81
2	5.780	N-(3-butenyl)-N-(methylcyclohex)	317,533	9.16
3	7.365	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	1,205,051	34.78
4	9.604	3-Acetoxy-3-hydroxypropionic acid, methyl	627,282	18.10
5	10.488	beta.-Alanine, N-acryloyl-, isobutyl ester	769,362	22.21
6	13.724	trans-2-Decenoic acid	373,309	10.77
7	18.474	2-Tridecenoic acid, (*E*)-	109,650	3.16
			3,464,799	100.00

leaves and yield (Table 2) compared to control ($p < 0.05$). The treatment with 3% SSE registered highest value of shoot length (36 cm), number of leaves (28) and yield (40 g). (Table 2). The treatment with 3% SSE was found optimal for the growth of cowpea. However, treatment with higher concentrations displays no significant effect on its growth.

3.3. Effect of SSE and KAE extracts on phytochemical content of Vigna unguiculata

The treatment with 2–5% of KAE & SSE improved the quality of *Vigna unguiculata* plants. The phenolic content (36.64 µg GAE/g FW, Fig. 1. A) and protein content (0.42 mg/g, Fig. 2) in vegetables was high in the 3% SSE treatment when compared to the control. However, the flavonoid content was high in 3% KAE treatment (7.36 µg QCE/g FW, Fig. 1. B). There was no significant difference observed among SSE treatment group in flavonoid content. In addition, the antioxidant activity was significantly higher in 3% SSE treated *Vigna unguiculata*. L (Fig. 3).

3.4. Phytochemical analysis by GC-MS

Seven compounds were identified in methanolic extract of *Vigna unguiculata* using the Gas Chromatography-Mass Spectrometry (GC-MS) analysis (Fig. 4). The major constituents identified were Benzene acetaldehyde (peak area, 1.81%), N-(3-butenyl)-N-methylcyclohex) (peak area, 9.16%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy (peak area, 34.78%), 3-Acetoxy-3-hydroxypropionic acid, methyl (peak area, 18.10%), beta.-Alanine, N-acryloyl-, isobutyl ester (peak area, 22.21%),trans-2-Decenoic acid (peak area, 10.77%), 2-Tridecenoic acid, (E) (peak area, 3.16%) (Table 3).

4. Discussion

Brown seaweeds were reported to contain higher macro nutrients (N, P, K) compared to other seaweed species (Hernández-Herrera et al., 2014). Though mineral contents were reported to vary depending on the seasons, harvest location and analytical methods, the nutrients content found in the seaweed extracts in this study was in agreement with the levels of other previous studies on seaweed species (Pramanick et al., 2017; Sivasankari et al., 2006).

Seaweed fertilizers exhibit strong growth promoting activities and act as bio stimulants in the crop production. Increase in the shoot length, leaf number and yield obtained in this study could be attributed to the presence of growth promoting macro and micronutrients in the seaweed extracts. Earlier aqueous extracts of *S. wightii* at 20% concentration was reported to improve the growth, shoot and root length of *Vigna sinensis* (Sivasankari et al., 2006). However, our study shows treatment at lower concentration of SSE (3%) improved the shoot length, leaf number and yield of *Vigna unguiculata*. (Battacharyya et al., 2015) also reported that seaweed extracts were applied to improve the quality of many plants at lower concentrations. The presence of polysaccharides as sugars in extracts were reported to enhance plant growth (Rolland et al., 2002). Furthermore, it has been reported that seaweed extracts contain nutrients, vitamins, plant growth hormones (auxin and cytokinins) which may have effects on cellular metabolism (Khan et al., 2009). Hernández-Herrera et al. (2014) and coworkers have reported that phosphorous in seaweed extracts enhance root proliferation by increasing the root/shoot ratio. Increase in vegetative growth, leaf number, area, plant dry weight, and plant height under seaweed extract

treatments was also reported earlier (Elansary et al., 2016; Khan et al., 2009).

Phenolic compounds found in plants are major secondary metabolites that have important role to act as antioxidant resource in crops. Also, they act as free radical scavengers due to their high reactivity as electron donors (Podsedek, 2007). Flavanoid compound in plants constitute an important group of polyphenolic compounds which has strong antioxidant potential and exerts many health benefits (Wang et al., 2011). An earlier study on the application of brown seaweed extract on spinach has reported an increase in the antioxidant activity along with enhanced phenolic and flavonoid contents (Fan et al., 2011). Studies suggests that seaweed extract have more influence on plant metabolism such as antioxidants, total soluble proteins, total phenolics, and flavonoids (Battacharyya et al., 2015; Fan et al., 2011; Sharma et al., 2014). In addition, carbon, nitrogen and sulphur metabolism as well as photosynthesis were also reported to significantly stimulated by the seaweed extracts (Jannin et al., 2013). Moreover, foliar application of seaweed extracts was recommended in different types of plants as the nutrients are directly absorbed by the leaves when applied onto the shoots of plant (Battacharyya et al., 2015).

Phytochemicals like terpenoids, alkaloids and fatty acid esters have been reported to be potent antioxidants and play a major role in the metabolism of plants (Grassmann et al., 2005). GC-MS analysis revealed the presence of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy (peak area 34.78%), a major antioxidant compound in the methanolic extract of *Vigna unguiculata.* Previous study also reported the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-(4 H)-pyran-4-one in antioxidant capacity of prunes (Cechovska et al., 2011).

5. Conclusion

Seaweed extracts prepared from *Sargassum swartzii* (brown seaweed) (SSE) and *Kappaphycus alvarezii* (red seaweed) (KAE) were tested as foliar spray for the growth promotion of *Vigna unguiculata.* 3% (w/v) *Sargassum swartzii* extract (SSE) application significantly improved the phenolic, flavonoid and protein contents and antioxidant capacity in *Vigna unguiculata* than *Kappaphycus alvarezii* extract (KSE) and control. The increase in leaf numbers, shoot length and yield could be attributed to increased stomatal conductance which enhances photosynthetic rate and vegetative growth. Furthermore, increased antioxidants activities obtained in terms of DPPH assay could be attributed to the increased phenolic and flavonoid content in plants treated with 3% SSE. Considering the stimulatory effects on plant growth and metabolism, *Sargassum swartzii* extract (SSE) at 3% could be considered as optimum concentration for foliar spray to obtain enhanced yield and quality produce in organic agriculture.

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Conflict of interest

Authors declare no conflict of interest.

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