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BIOCHEMISTRY & PHYSIOLOGY - ORIGINAL ARTICLE

Andrographolide production and enhanced antioxidant activity in *Andrographis paniculata* (Burm f.) Nees. promoted by seaweed liquid extracts

Veeranan Uthirapandi[1] · Sivasangari Ramya Subramanian[2] · Boomibalagan Ponnerulan[3] · Eswaran Saminathan[4] · Vijayanand Narayanan[5] · Kathiresan Durairaj[6]

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Abstract
Andrographolide is important specialized metabolites of *Andrographis paniculata* (Acanthaceae) which is used to treat wide array of disorders such as cancer, diabetes, rheumatoid arthritis. An attempt was made to examine the potential of seaweed (*Sargassum wightii* Greville) on growth, andrographolide content and antioxidant activity in *A. paniculata.* Different concentrations (5%, 10%, 15% and 20%) of seaweed liquid extract (SLE) were applied through foliar application to *A. paniculata* (Burm. f.) Nees seedlings. Among the various concentrations, 10% of SLE exhibited a maximum increase in growth parameters such as plant height (113%), leaf surface area (129%), number of leaves and branch by 134% and 118%, fresh weight (178%) and dry weight (220%). Similarly, biochemical constituents such as starch (132%), glucose (122%), protein (177%) and total chlorophyll (213%) were also found to be enhanced in leaves of those plants which received 10% of SLE. A twofold (34.92 mg g^{-1}) increased synthesis of andrographolide was noticed in 10% methanolic SLE treated plants. Similarly, antioxidant activity was increased in 10% methanolic SLE which accounted for 53.94 µg/ml DPPH activity and 44.94% inhibition of nitric oxide activity. The present study suggested that SLE of *S. wightii* could be a promising biostimulant which can be attributed to the presence of growth hormones like cytokinin (3.9 mg L^{-1}), auxin (3.0 mg L^{-1}) and gibberellin (2.2 mg L^{-1}) and nutrients such as Na (45.47 mg L^{-1}), Mg (75.12 mg L^{-1}), P (4.03 mg L^{-1}), K (58.4 mg L^{-1}), Ca (0.432 mg L^{-1}), Fe (0.85 mg L^{-1}), Cu (0.346 mg L^{-1}), Mb (0.0004 mg L^{-1}) and N (2.56%) and also pave the way for sustainable environment approach in herbal cultivation.

Keywords Biochemical parameters · Biostimulation · FTIR · HPLC · *Sargassum wightii* · Specialized metabolites

1 Introduction

In the present health scenario, usage of medicinal plants to prevent or to cure diseases has gained importance due to its wide range of active pool of specialized metabolites. Emergence of new diseases and inefficacy of modern drugs have now made us to search for new alternative drug from the plant source. One such important medicinal plant with wide array of active pool of specialized metabolites is *Andrographis paniculata* (Burm.f.) Wall.

✉ Kathiresan Durairaj
kathiresansnc@gmail.com

[1] Department of Botany, Theni College of Arts and Science, Affiliated to Madurai Kamaraj University, Madurai, Veerapandi, Tamil Nadu, India

[2] Department of Biochemistry, Sri Sarada Niketen College for Women, Affiliated to Alagappa University, Karaikudi, Amaravathipudur, Tamil Nadu, India

[3] Department of Botany, Raja Doraisingam Government Arts College, Affiliated to Alagappa University, Karaikudi, Sivagangai, Tamil Nadu, India

[4] PG and Research Department of Botany, Pasumpon Thiru Muthuramalingam Thevar Memorial College, Affiliated to Alagappa University, Karaikudi, Kamuthi, Tamil Nadu, India

[5] Department of Botany, Arumugam Pillai Seethai Ammal College, Affiliated to Alagappa University, Karaikudi, Thiruppathur, Tamil Nadu, India

[6] PG and Research Department in Botany, Saraswathi Narayanan College, Affiliated to Madurai Kamaraj University, Madurai, Tamil Nadu, India

ex Nees which is an herb belonging to Acanthaceae Family. This plant is recognized for its therapeutic properties such as anti-inflammatory activity (Abu-Ghefreh et al. 2009), antipyretic (Pokala et al. 2019), hepatoprotective activity (Pan et al. 2017), immunostimulant (Pongtuluran et al. 2015). The leaves of *A. paniculata* contain many bioactive compounds including diterpene lactones (deoxyandrographolide, andrographolide, neoandrographolide and 14-deoxy-11, 12-didehydroandrographolide), diterpeneglucoside (deoxyandrographolide19-d-glucoside) and flavonoids (5,7,20,30-tetramethoxyflavanone and 5-hydroxy-7,20,30-trimethoxyflavone) (Akbar et al. 2011). Due to its wide spectrum of pharmacological applications and use in commercial formulations, this herb is being extensively collected from wild natural sources. This has led to a considerable reduction in the natural availability and thus the supply of raw materials to the industry (Raina et al. 2013).

Intensive usage of inorganic fertilizers in agriculture has led to a decrease in soil microbial population, depletion in nutrient content in the soil ecosystem and making the environment as unsuitable for agriculture (Sivasangari Ramya et al. 2015). Biostimulants are preparations made from natural raw materials which can be used in the form of soil preparations (powders, granules or solutions added to the soil) or as liquid foliar application products (Kocira et al. 2018). They have the potential to minimize or reduce the negative environmental impacts associated with applications of agrochemical pesticides and fertilizers (Calvo et al. 2014). Various categories of plant biostimulants include microbial inoculants, beneficial bacteria and fungi, plant and animal-based protein hydrolysates, and other nitrogen containing compounds, such as humic and fulvic acids, biopolymers, seaweed and botanical extracts (Jardin et al. 2015). In preparation of commercial biostimulants, seaweed extract collected from green algae such as *Ulva lactuca, Caulerpa sertularioides* and brown algae such as *Padina gymnospora* and *Sargassum liebmannii* is the most commonly used particularly brown algae dominates the ingredients (Mazhar et al. 2020). Brown alga which contains more hormones, nutrients, vitamins, etc., could be effective in enhancing the growth and physiology of certain plants than other seaweeds (Sivasangari Ramya et al. 2015). Many species of seaweeds, mainly brown algae, are widely used in agriculture as plant biostimulants, plant growth regulators, biofertilizers or metabolic enhancers (Hong et al. 2007). The objective of the current work is to explore the potential of seaweed liquid extract on andrographolide production and enhanced antioxidant activity in *A. paniculata.*

2 Material and methods

Preparation of seaweed liquid extracts (SLE) – *Sargassum wightii* Greville a brown seaweed, was collected from Mandapam (Lat 9°45′N; Long79°15′E) located in South East Coast of Tamil Nadu. The alga was brought to the laboratory, washed thoroughly in running tap water for several times to remove all sand particles, epiphytes and other debris. Then, seaweed was shade-dried and made into a fine coarse powder. Different concentrations of boiled extracts were prepared by mixing appropriate level of liquid extracts with distilled water (Rama Rao et al. 1990). The SLE concentrations used in this experiment were 5%, 10%, 15% and 20%.

Chemical and hormonal analysis of *S. wightii* – The physical characteristics such as colour and pH were observed using normal standard method.

Elemental analysis of S.wightii. The composition of elements such as B, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Ar, Se, Cd, Mb and Pb was estimated as per the standard method (B'Hymer et al. 2000). One gram of sample was taken in 100 ml of conical flask in which 10 ml of triacid mixture was added. This mixture was heated at 60–80 °C until all liquid evaporated from the flask. Then, the flask was double-distilled water and made upto 100 ml. Then, 10 ml was filtered using Whatman Filter paper No. 1 and the analysis was done through Inductively Coupled Plasma-Mass Spectrometer (Model:ICP-MS PerkinElmer NexION 300x (2011). Estimation of nitrogen was done by the Kjeldahl method as suggested by Bremner (1960). One gram of SLE was taken in a digestion flask in which sodium sulphate and copper sulphate were added as a catalyst in the ratio 5:1. To this, 20 ml of sulphuric acid was added and kept for 3 hrs. After the appearance of bluish-green colour, the tubes were loaded in a distillation unit and 40% NaOH was added. Distillation was observed after 3–6 min in the conical flask containing 4% boric acid with bromocresol green and methyl red indicator. The pink colour was observed when titrated against 0.1 N H_2SO_4. Burette reading was noted and calculated as per the formula

$$\text{Percentage of Nitrogen} = \frac{(14.01 \times (\text{sample titrant ml} - \text{blank titrant ml}) \times N \text{ of } H_2SO_4 \times 100}{\text{Sample volume} \times 100}$$

Estimation of auxin. Auxin was estimated in the SLE sample as per the method of Gorden and Paleg (1957). The SLE sample containing auxins was first separated by means of thin-layer chromatography. The separated spots containing IAA were eluted in 1 or 2 ml of methanol or ethanol (elution time, 30 min). To 1 ml of extract containing IAA, 2 ml of Salper reagent was added. The reagent was added dropwise but rapidly with continuous agitation. The samples were incubated in the dark (60 min). Pink colour was observed and it was measured at 565 nm against a solvent reagent blank. If the intensity was deep, the reaction mixture was diluted with the solvent. The quantity of IAA in the extract was calculated using a standard curve drawn from known concentrations of IAA.

Estimation of gibberellin. Amount of gibberellins in the extract was estimated by the method of Graham and Henderson (1961). One millilitre of gibberellin extract was pipetted out into 25 ml of volumetric flask. To this, 15 ml of phosphomolybdic acid reagent was added. The contents were mixed thoroughly in the flask, and it was placed in boiling water bath. After 1 h, flasks were removed and immersed in an ice water bath. After cooling to room temperature, volume was adjusted to 25 ml with distilled water. Absorbance was measured at 780 nm in a UV–Vis spectrophotometer using distilled water as blank. The gibberellin content was estimated with a calibration graph prepared using known concentrations of gibberellin.

Estimation of Cytokinins. Cytokinins were estimated as per the method of Syono and Torrey (1976). Dried powdered samples of seaweed were collected and thoroughly crushed in a mortar in cold 80% ethanol. The samples were filtered and the residue was extracted twice with 80% ethanol. Then ethanol (80%) extracts were combined and concentrated under reduced pressure below 40 ºC. After removal of ethanol, the pH was adjusted to 3 with 2 N HCl and extracted with methylene chloride 3 times. Then the pH was adjusted to 8 with 2 N NaOH and extracted with 1-butanol three times. Then the samples were subjected to TLC. The cytokinin content was detected by UV light absorption and by comparing with cytokinin standards.

Production of *A. paniculata* seedlings – Healthy seeds of *A. paniculata* free from visible infection, with uniform size, were purchased from Agriculture College and Research Institute, Madurai. They were surface sterilized with 0.1% mercuric chloride and then with distilled water twice. The seeds were sown in earthenware pots (12 cm diameter).

Foliar application of SLE – Foliar application of different concentrations of liquid extracts (5%, 10%, 15% and 20%) was given to potted plants after 30 days. Fifty millilitres of different concentrations of extracts was given an interval of 3 days. Four replicates for each treatment were maintained under natural uniform conditions. Growth parameters, viz., plant height, leaf area, fresh and dry weight, and the number of branch and leaves were determined. Biochemical profiles such as photosynthetic pigments (Arnon 1949), starch (Rose et al. 1991), glucose (Dubois et al. 1951) and protein (Lowry et al. 1951) were assessed in the leaves of treated plants. Both growth and biochemical parameters were observed in 6-week-old treated and control plants. Control treatment was maintained by giving water alone.

Phytochemical analysis *Preparation of different solvent extracts*. For phytochemical analysis, those treatments which exhibited better response in terms of growth and biochemical parameters alone were taken. Healthy stem and leaf of *A. paniculata* were collected and cleaned with tap water for three to four times to remove any adhering fine particles and was shade-dried. Sixty grams of powdered plant material (leaf + stem) was extracted in Erlenmeyer flasks with different solvents, viz., 90% petroleum ether, ethanol and methanol at room temperature. The obtained extract was filtered with Whatman No. 1 filter paper and kept in the Soxhlet extractor. The crude extracts were concentrated by rotary vacuum evaporator and then air-dried.

Qualitative analysis of phytochemicals. Detection of carbohydrates by Molish test (Ramakrishnan et al. 1994), protein by Biuret test (Gahan 1984), amino acids by Ninhydrin test (Yasuma and Ichikawa 1953), Alkaloids by Hager's test (Wagner et al. 1996), saponins by frothing test (Kokate 1999), phenolic compounds by lead acetate test (Harborne 1998), tannins by potassium hydroxide test (Williamson et al. 1996), flavonoids by alkaline reagent test (Raaman 2006), flavanol glycosides by magnesium and hydroxide acid reduction test (Harborne 1998), cardiac glycosidases by Keller–Killani test (Sofowara et al. 1993), phytosterols by Liebermann–Burchard test (Finar 1986), fixed oils and fats by saponification test (Kokate 1999) was estimated in crude solvent extracts.

UV-FTIR analysis – Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the plant powder sample. Five milligrams of *A. paniculata* plant (treated with SLE) powder sample was blended in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Bruker, Germany Vertex 70 infrared spectrometer. The sample was scanned from 4000 to 400 cm^{-1} (Pramila et al. 2012). The peak values of the UV–VIS and FTIR were recorded.

High-performance liquid chromatography analyses – Quantification of andrographolide was carried out using Waters 2998 liquid chromatography (Waters, Milford, MA) equipped with the photodiode array detector (PDA). The data were processed with Empower 2 software. Twenty microlitres of filter-sterilized extract of the whole plant that was treated with 10% of SLE (1 mg ml^{-1}) and standard (1 mg ml^{-1} Andrographolide) was injected into the Symmetry® C18 column (4.6 mm × 250 mm, 5 µm) and eluted isocratically with HPLC grade methanol: water (with 0.2% orthophosphoric acid) in the ratio of 65:35 v v^{-1} at a flow rate of 1.0 ml min^{-1}. The andrographolide was detected with a PDA detector at 223 nm. The amount of the andrographolide present in SLE treated plant (*A. paniculata)* was calculated by comparing the standard area with the sample area. The standard and sample solutions were injected in triplicate. The spots and/or peaks were detected, and their Rf values and peak areas were calculated.

$$\text{Andrographolide (mg/g)} = \frac{\text{Peak area of the sample}}{\text{Peak area of the standard}} \times 1000$$

Analysis of antioxidant activity *2-diphenyl-2-picrylhydrazyl free radical scavenging activity (DPPH)*. The antioxidant activity of 10% SLE treated plant extracts (*A. paniculata*) was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Blois1958a, b). Sample extracts at various concentrations were taken and the volume adjusted to 100 µl with methanol. About 3 ml of 0.004% methanolic solution of DPPH was added to the aliquots of samples and standards (BHA, BHT and Rutin) and shaken vigorously. Negative control was prepared by adding 100 µl of methanol in 3 ml of methanolic DPPH solution. The tubes were allowed to stand for 30 min at 27 °C. The absorbance of the samples (*A. paniculata*) and control was measured at 517 nm against the methanol blank. Radical scavenging activity of the samples was expressed as IC50 which is the concentration of the sample required to inhibit 50% of DPPH concentration.

Nitric oxide free radical scavenging activity. The procedure is based on the method of Sreejayan and Rao (1997) where sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, 2 ml of sodium nitroprusside (10 mm) in phosphate-buffered saline (0.2 M, pH 7.4) was mixed with 100 µl sample (*A. paniculata*) solution of various extracts and standards (BHT and Rutin) and incubated at room temperature for 150 min. After the incubation period, 2 ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% N– (1-naphthyl) ethylene diaminedihydrochloride) was added to all the test tubes. The same reaction mixture without the sample was used as the negative control. The absorbance of the chromophore formed was read at 546 nm against the blank (phosphate buffer) and was calculated by using the standard formula.

Statistical analysis – All the physiological and biochemical experiments were done with triplicates, and the results were expressed as mean ± standard deviation. The data were statistically analysed using a one-way ANOVA followed by Duncan's test for phytochemical and antioxidant studies. Mean values were calculated statistically significant at $P < 0.05$.

3 Result

Physicochemical and hormonal analysis of SLE (*S. wightii)* – The extract was brown in colour, and the pH was recorded as 6.3 at room temperature. Differential quantity of micro- and macronutrients was recorded in *S. wightii* extract such as Na (45.47 g L^{-1}), Mg (75.12 g L^{-1}), P (4.03 g L^{-1}), K (58.4 g L^{-1}), Ca (0.432 g L^{-1}), Fe (0.85 g L^{-1}), Cu (0.346 g L^{-1}), Mb (0.0004 g L^{-1}) and N (2.56%). Among the micro- and macronutrients, Na, P, K, N and Mg were found to be present more when compared to other nutrients such as B, Cr, Mn, Ni, Zn, Ar, Se, Cd and Pb (Fig. 1a). Similarly, significant level of hormones such as auxin, cytokinin and gibberellin was detected in the seaweed samples. Extract recorded highest amount of cytokinin at 3.9 mg L^{-1} followed by auxin at 3.0 mg L^{-1} and gibberellin at 2.2 mg L^{-1} (Fig. 1b).

Influence of SLE on growth and biochemical constituents of *A. paniculata* – Foliar application of different concentrations of *S. wightii* expressed positive results in terms of growth and biochemical parameters. In case of total plant height, there was an appreciable increase in all the concentrations when compared to control. In case of total plant height, 10% SLE increased the plant height by 113% over control. Other treatments such as 15% and 20% also enhanced the plant height by 62 and 47%, respectively, but found to be lesser than the 10% concentration. Similarly, 10% extract increased the number of leaves and branch by 134% and 118%, respectively, which was found to be better when compared to control and other concentrations. SLE (10%) enhanced the leaf surface area by 129% followed by 28% increase in 5% concentration, 92% increase in 15% concentration and 55% increase in 20% concentration when compared to control. Eventually, there was a profound increase in fresh weight (178%) and dry weight (220%) when 10% extract of *S. wightii* was administered in the form of foliar spray. Other treatments also increased the

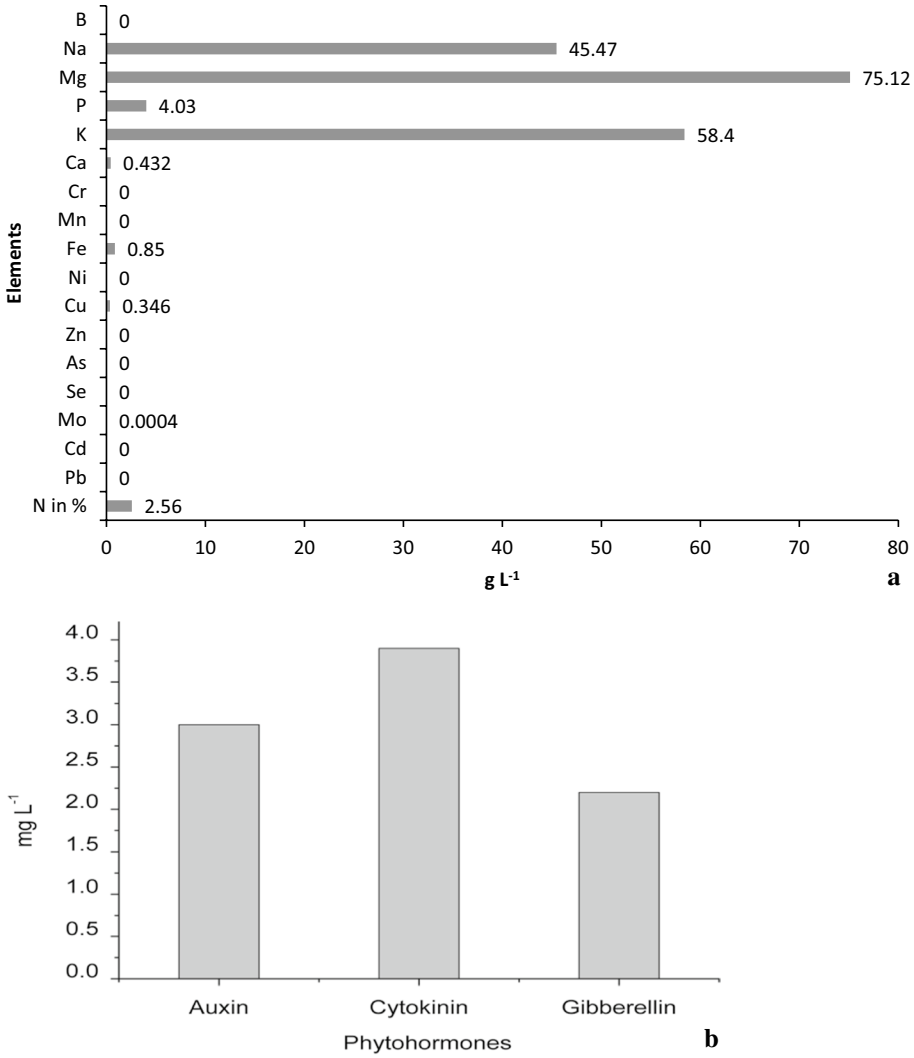

Fig. 1 Chemical (**a**) and hormonal analysis (**b**) of *S.wightii* extracts. N (nitrogen), Pb (lead), Cd (cadmium), Mo (molybdenum), Se (selenium), As (arsenic), Zn (zinc), Cu (copper), Ni (nickel), Fe (iron), Mn (manganese), Cr (chromium), Ca (calcium), K (potassium), P (phosphorus), Mg (magnesium), Na (sodium), B (boron)

fresh weight and dry weight but found to be lesser than 10% extract (Fig. 2a–d).

In case of biochemical constituents, differential responses in biochemical constituents were recorded in *A. paniculata* plant when different concentrations of SLE were given as foliar spray. Increase in starch (132%), glucose (122%), protein (177%) and total chlorophyll (213%) was noticed in those plants which received 10% extract of *S. wightii.* Other treatments such as 5%, 15% and 20% also enhanced the biochemical constituents but were found to be lesser when compared to 10% concentration. In general, all the concentrations of SLE exhibited positive response in terms of growth and biochemical constituents of *A. paniculata* when compared to control. Among the different concentrations, 10% extract was found to be the optimum concentration (Fig. 3a, b).

Qualitative phytochemical analysis of untreated and SLE treated *A. paniculata*. In this study, it was noticed that methanolic extracts of whole plant of untreated *A. paniculata* revealed the presence of 6 phytochemical compounds (proteins, amino acids, alkaloids, phenolic compounds, flavonoids and glycosides) followed by ethanol extracts which exhibited 4 compounds (proteins, amino acids, phenolic compounds and flavonoids) and petroleum ether extract which revealed only one compound (protein). Carbohydrates, saponins, tannins, flavonolglycosides, cardiac glycosides and fixed oils and fats were absent in all the three solvents extracts of untreated *A. paniculata* (Table 1).

Methanolic solvent extract of 10% SLE treated *A. paniculata* increased the synthesis of 4 new phytochemical compounds (tannin, flavanol glycosides, cardiac glycosides and Phytosterols), and ethanolic extract exhibited three new compounds (alkaloids, flavanol glycosides and cardiac glycosides) when compared to untreated plants. But, petroleum ether extract exhibited only two new compounds

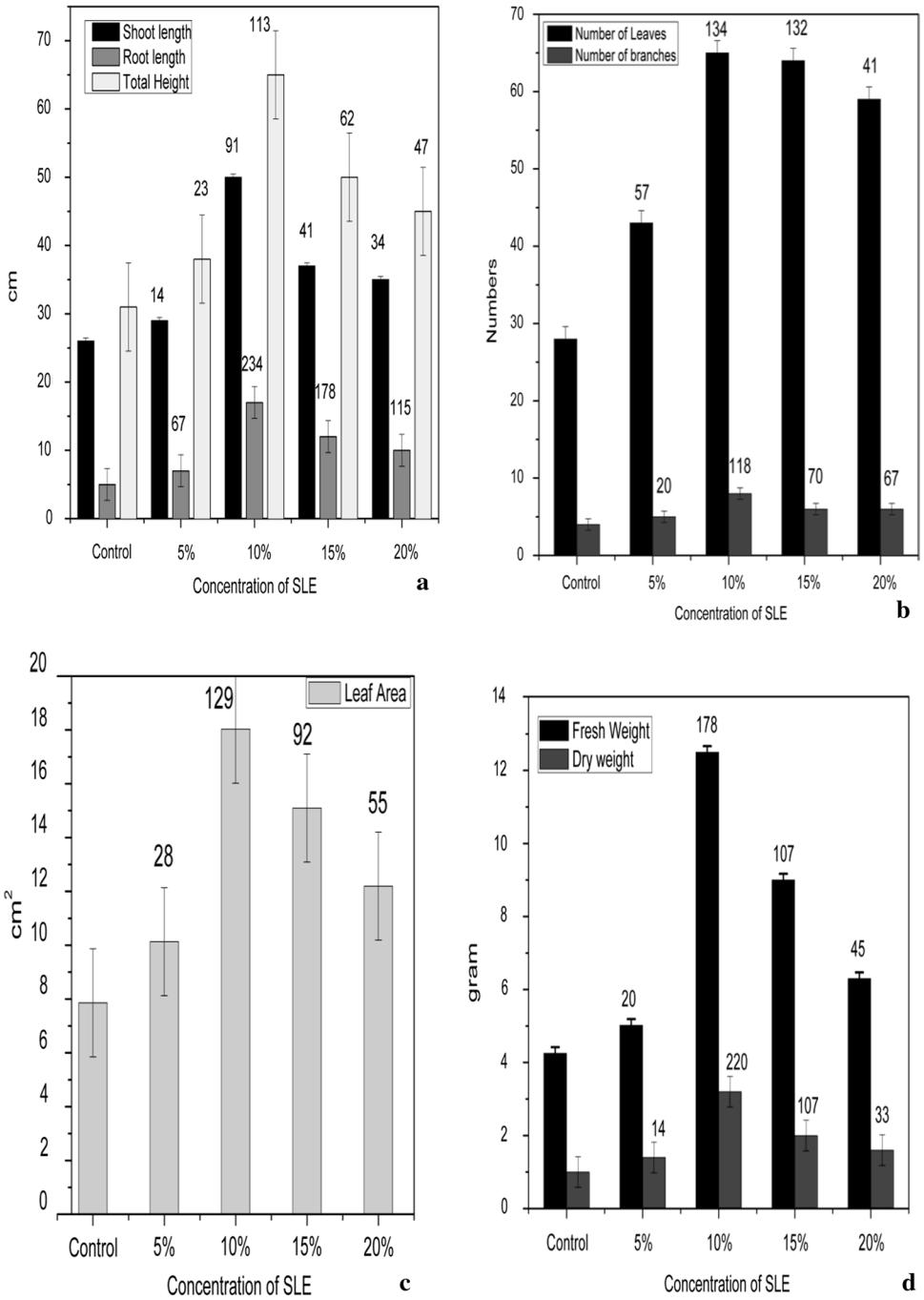

Fig. 2 Influence of different concentrations of *S. wightii* on growth parameters of *A. paniculata*. **a.** Shoot length, root length and total plant height. **b**. Number of leaves and branches. **c**. Leaf area, and **d**. fresh and dry weight. Values given above each bar are percent increase over control

(amino acid and flavonol glycosides) when compared to compounds present in untreated plant (Table 2).

UV-FTIR analysis – The methanol extract of 10% *S. wightii* treated *A. paniculata* revealed the presence of 26 peaks with absorption band ranging between 459.06 and 3402.6 cm^{-1} (Table 3; Fig. 4). The strong absorption bands were observed between 2360.87 and 2341.58 cm^{-1}, 1637 cm^{-1}, 1629.85 cm^{-1}, 1610.56 cm^{-1} and 1037.70.

HPLC analysis of andrographolide in *A. paniculata* – HPLC analysis of methanol extract of whole plant of *A. paniculata* was scanned under UV 223 nm and compared with appropriate standard andrographolide. The densitometric scanning at 223 nm revealed that methanolic solvent of 10% SLE treated

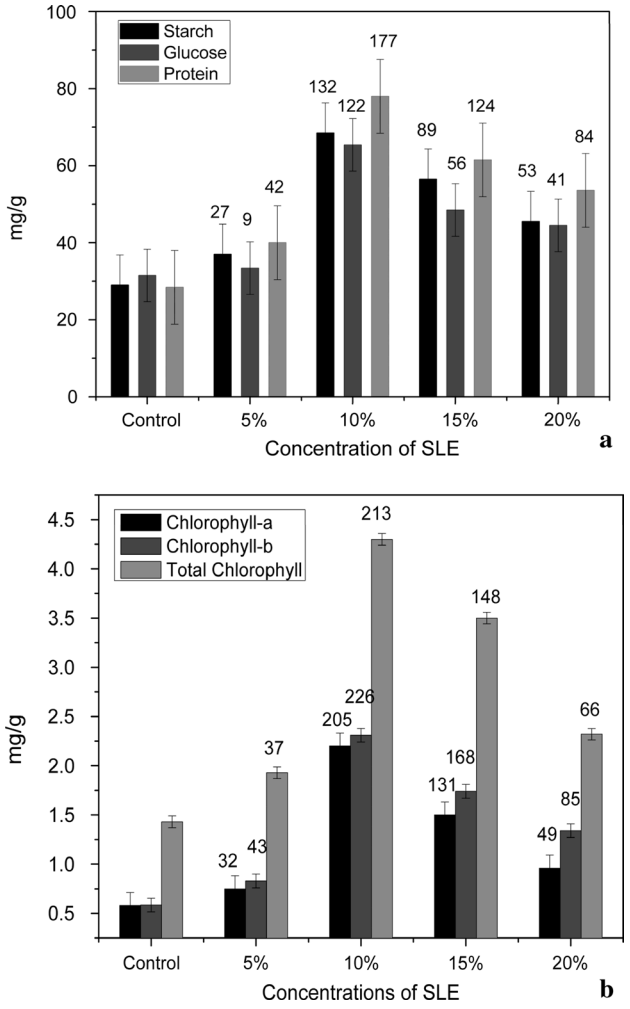

Fig. 3 Influence of different concentrations of *S. wightii* on biochemical parameters of *A. paniculata.* **a.** Starch, glucose and protein level and **b**. photosynthetic pigments. Values given above each bar are percent increase over control

plant exhibited the presence of 10 peaks in 20 µl concentration in the chromatogram with an peak area of 253,168 with retention time up to 6 min.

In this study, there was a twofold increase in andrographolide content (34.92 mg g^{-1}) in those plants which received 10% SLE when compared to untreated plants (17.41 mg g^{-1}) (Table 4; Figs. 5a–c).

Antioxidant activity of *A. paniculata*. *DPPH scavenging activity of A. paniculata.* Methanol, ethanol and petroleum ether solvent extracts of 10% treated SLE exhibited various degrees of antioxidant. Lower IC_{50} value indicates higher antioxidant activity. Our results inferred that only methanolic extracts of 10% treated SLE exhibited a relatively high antioxidant activity with a relatively low IC_{50} (53.940 µg ml^{-1}) when compared to untreated plants. Moreover, ethanolic solvent extract of 10% SLE treated *A. paniculata* expressed low antioxidant activity when compared to control (Fig. 6a).

Table 1 Phytochemical analysis of untreated *Andrographis paniculata*

S. No	Test	Solvents		
		Pet. Ether	Ethanol	Methanol
1	Detection of carbohydrates	–	–	–
2	Detection of protein	+	+	+
3	Detection of amino acids	–	+	+
4	Detection of alkaloids	–	–	+
5	Detection of saponins	–	–	–
6	Detection of phenolic compounds	–	+	+
7	Tannins	–	–	–
8	Detection of flavonoids	–	+	+
9	Detection of glycosides	–	–	+
10	Detection of flavonol glycosides	–	–	–
11	Cardiac glycosides	–	–	–
12	Detection of phytosterols	–	–	–
13	Detection of fixed oils and fats	–	–	–

(+)- Present and (–)- Absent

Table 2 Phytochemical analysis of *Andrographis paniculata* treated with 10% seaweed liquid extract *Sargassum wightii*

	Test	Solvents		
		Pet. Ether	Ethanol	Methanol
1	Detection of carbohydrates	–	–	–
2	Detection of protein	+	+	+
3	Detection of amino acids	+	+	+
4	Detection of alkaloids	–	+	+
5	Detection of saponins	–	–	–
6	Detection of phenolic compounds	–	+	+
7	Tannins	–	–	+
8	Detection of flavonoids	–	+	+
9	Detection of glycosides	–	–	+
10	Detection of flavonol glycosides	+	+	+
11	Cardiac glycosides	–	+	+
12	Detection of phytosterols	–	–	+
13	Detection of fixed oils and fats	–	–	–

(+)- Present and (–)- Absent

Table 3 FTIR analysis of *Andrographis paniculata* treated with 10% seaweed liquid extract of *Sargassum wightii*

Peak Number	Peak	Group	Compound
1	3402.43	OH	Alcohol and phenols
2	3348.42	NH_2	Primary amides
3	3304.06	OH	Oximes
4	3288.63	NH	Secondary amides
5	3267.41	CH	Acetylenes
6	3192.19	NH_2	Primary amides
		NH_3^+	Amino acids
7	3091.89	CH	Aromatic and unsaturated hydrocarbons
8	2850.79	CH_3	O or N
9	2360.87	PH	Phosphines,
		NH_3^+	Amines Hydrohalides
10	1718.58	C=O	ketones
11	1637.56	C=O	Tertiary amides,
		NH_3^+	β Ketones esters, Amino acids
12	1610.56	NH_2	Amino acids
13	1500.62	NO_2	Aromatic nitro compound,
		NH_3^+	Amino acids or hydrochlorides
14	1411.89	OH	Carboxylic acids
15	1398.39	COO-	t-Butyl group, Carboxylic acids salts
16	1388.75	SO2	Sulphonyl chloride
17	1321.24	CF_3	Benzene ring
18	1238.30	C-N	Aromatic amines
		C-N–C	Ester, lactones,
		SO_3H	sulphonic acids
19	1207.44	C–O–C,	Ethers,
		C-O-N	amines
		C–O–C	vinyl ethers
20	1035.77	P-O-C	Organophosporus compound
21	893.04	$CH=CH_2$	Vinyl compound
22	854.47	Si–C	Organosilion compound
23	582.50	SO_2	Sulphonyl chloride,
		C-I	Iodo compound
24	536.21		Naphthalenes
25	518.85	NO_2	Nitro compound
26	459.06	C-N–C	amines

Nitric oxide scavenging activity of A. paniculata. The plants exhibited antioxidant activity through competing with oxygen to scavenge for the nitrite radical which was generated from sodium nitroprusside at physiological pH in an aqueous environment. In this study, different solvent extracts of 10% SLE expressed positive results by inhibiting the NO radical when compared to control. Among the different solvent extracts, methanolic extract of 10% SLE treated plant (44.945%) had highest capability of quenching the NO radical followed by ethanolic (38.720%) and petroleum ether extract (33.333%) (Fig. 6b).

4 Discussion

Our study inferred that different concentrations of *S. wightii* exhibited positive response on growth and physiology of *A. paniculata.* This positive effect may be attributed to the presence of phytohormones, macro- and micronutrients and other essential growth-promoting hormones compounds present in the SLE. In our study, cytokinin was found to be present more in the SLE than auxin and gibberellin. Cytokinins are supposed to play an important role in plant growth regulation (Werner et al. 2001). Kulkarni et al.

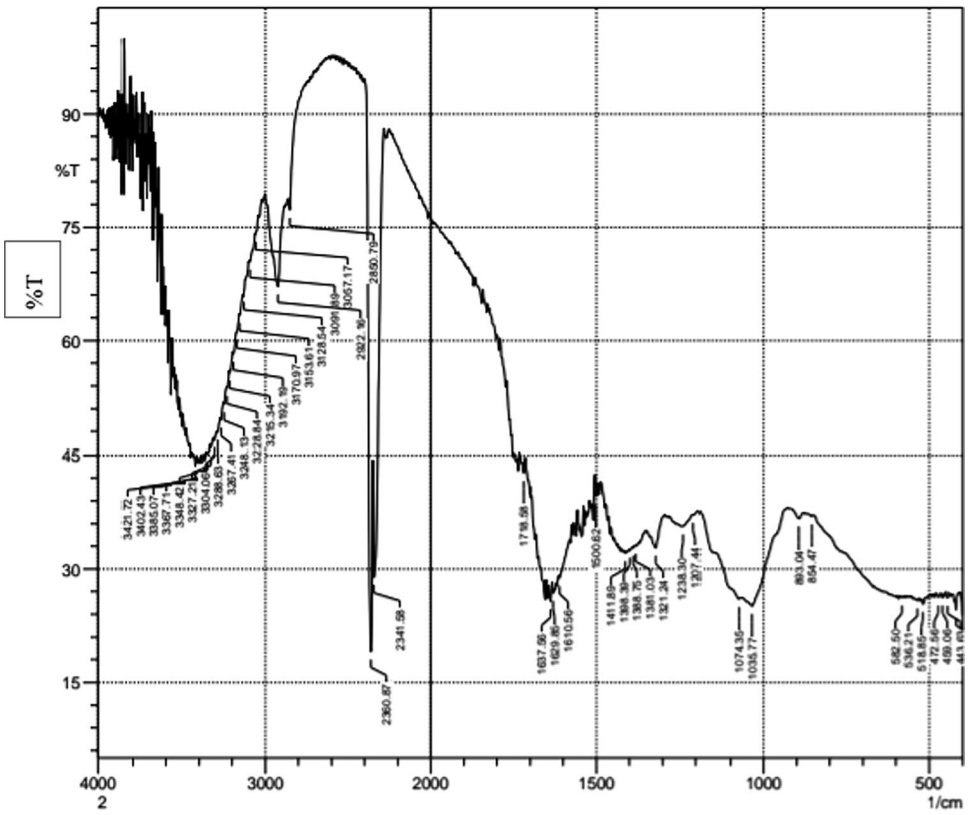

Fig. 4 FTIR analysis of *A. paniculata* treated with 10% SLE

Table 4 HPLC analysis of andrographolide in *A. paniculata* treated with bio-organic fertilizers

S. No	Treatment	RT (min)	Area	Height (h)	Absorbance (nm)	Andrographolide (mg/g)
1	Andrographolide	3.565	7,404,097	822,357	223	Std
2	Untreated	3.570	128,949	10,170	223	17.41
3	SLE (10%)	3.572	253,168	18,032	223	34.92

(2019) revealed that cytokinins, cis-zeatin, dihydrozeatin and isopentyladenine significantly were increased in plants treated with a pure eckol compound isolated from *Ecklonia maxima* (Osbeck) Papenfuss. Interestingly, brown seaweeds were reported to contain higher macronutrients (N, P and K) compared to other seaweed species (Hernandez-Herrera et al. 2014). Our results also corroborated with the earlier findings in *Codium decorticatum* (Woodward) Howe (Vijayakumar et al. 2019); *Ulva intestinalis* L. (Ghaderiardakani et al. 2019); *Cystoseira trinodis* (Forsskal) C. Agardh (Sathya et al. 2013); *Sargassum vulgare* C.Agardh (Sami et al. 2019); *Sargassum swartzii* C.Agardh and *Kappaphycus alvarezii* (Doty) L.M.Liao (Vasantharaja et al. 2019); *Ulva reticulata* Forsskal (Ganapathy Selvam et al. 2013); *Sargassum polycystum* C. Agardh (Bharath et al. 2018) *Ecklonia maxima* (Kulkarni et al. 2019). In addition, Mg is considered as one of the chief component for biosynthesis of chlorophyll molecule in plant system. Chemical and physiological analysis of SLE of *S. wightii* revealed the presence of more amount of magnesium (75.12 g l^{-1}) which could have induced the biosynthesis of chlorophyll molecule resulting in enhanced production of energy for growth and development of the plant.

In our study, 10% SLE exhibited produced better results in growth parameters such as total plant height, fresh and dry weight, leaf area, number of leaves and number of branches in *A. paniculata* when compared to other concentrations (5%, 15% and 20%). Seaweed products contain growth regulators (auxins, cytokinin and gibberellins), amino acids and mineral nutrients that accordingly exhibited positive effect on plant growth and division as reported by Yokoya (2010). Khan et al. (2009) and Rengasamy et al. (2015) reported that plant growth hormones play a vital role in increase of cell size and cell division and also stressed that cytokinin is effective in shoot formation and auxin in root development by complementing

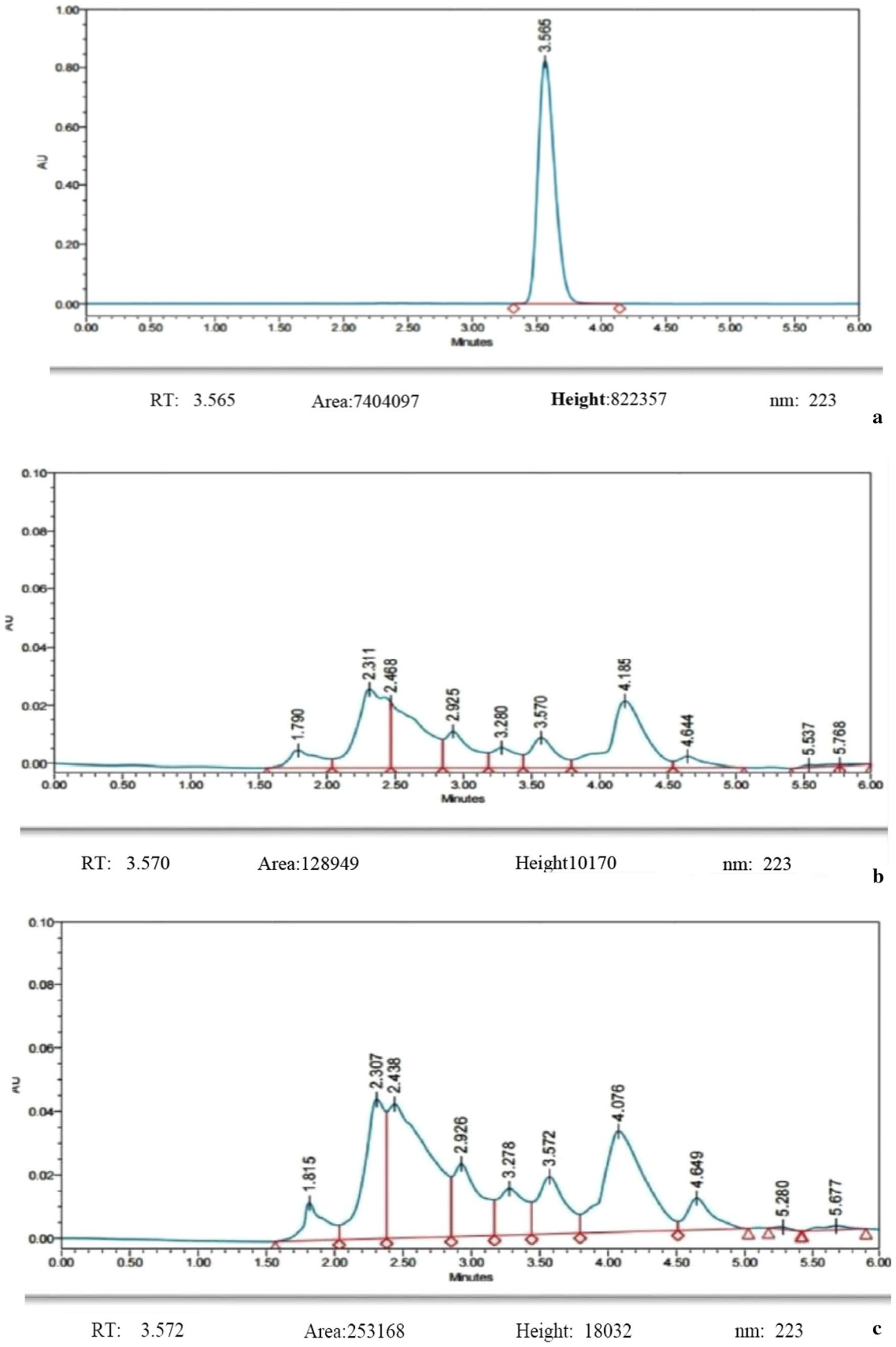

Fig. 5 HPLC analysis of andrographolide content. **a**. Standard, **b**. control, **c**. 10% SLE treated plant

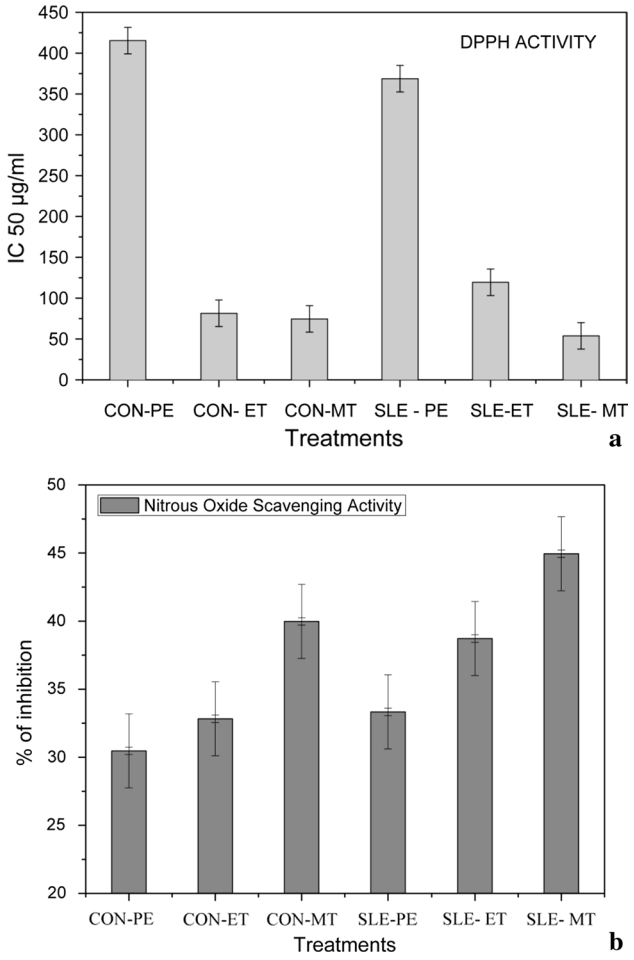

Fig. 6 Influence of *S. wightii* on antioxidant activity of *A. paniculata.* **a**. DPPH activity. **b**. Nitric oxide free radical activity

each other. Hernández-Herrera et al. (2014) also justified that enhancement in plant vegetative growth might be due to the presence of natural phytohormones and nutrients present in the SLE. This increase in growth parameters exhibited by brown algae may be due to the presence of phycocolloids and polyphenols that have the ability to chelate complex ions to usable forms present in the soil, thus promoting growth activity. Our work also found to be conformity with the earlier works of Abd El-Samad et al. (2019) on tomato, Sami et al. (2019) on red radish plants and Vasantharaja et al. (2019) on cowpea plants, Hehata et al. (2011) on celeriac plants.

Although seaweeds could potentially benefit plant growth by providing macronutrients, including nitrogen (N), phosphorus (P), ammonium (NH_4^+) and potassium (K), studies have consistently shown that seaweed extracts' beneficial effects are not due to macronutrients, particularly at the concentrations used in the field (Khan et al. 2009). Very dilute seaweed extracts (1:1000 or below) still have biological activity, and the beneficial effects may involve several plant growth promoters working synergistically (Fornes et al. 2002). This is in accordance with the earlier reports that lower concentrations of seaweed extracts enhanced the biochemical constituents in *Citrullus lanatus* (Abdel-Mawgoud et al. 2010), *Trigonella foenum-graecum* (Pise and Sabale 2010), *Solanum melongena* (Bozorgi 2012; Sivasangari Ramya et al. 2015) and *Abelmoschus esculentus* (Sasikumar et al. 2011).

Specialized metabolites are those biologically active chemical compounds which occurs naturally in plants. They act as a natural defence system for host plants and provide colour, aroma and flavour. In our study, 10% ethanolic SLE treated *A. paniculata* plant increased the synthesis of six new phytochemical constituents when compared to untreated plants followed by petroleum ether (3 compounds) and methanol (1 compound). Vasantharaja et al. (2019) reported that seaweed extracts prepared from 3% of *Sargassum swartzii* C.Agardh (brown seaweed) improved the phenolic, flavonoid and protein contents and antioxidant capacity in *Vigna unguiculata* (L.) Walp than *Kappaphycus alvarezii* extract and control. Similarly, Elansary et al. (2016) reported that *Ascophyllum nodosum* (L.) Le Jolis SLE when administered as foliar spray and soil drench significantly enhanced the total phenol, flavonols and tannin contents of *Calibrachoa* cultivars leaf that subsequently increased the antioxidant potential of *Calibrachoa* cultivars leaf extracts. Further, the differential occurrence of phytoconstituents recorded in our study can be attributed to polarity of the compounds extracted by each solvent and their efficiency of the solvents to dissolve the phytochemical compounds. In fact, the regulation of phytochemical synthesis of specific compounds in the plant is determined by environmental conditions of growing place and time and also response to outside signals. Such compounds are included in seaweed extracts which could have triggered or activate specific biochemical pathways that accounts for synthesis of specialized metabolites in plants (Cheynier et al. 2013).

FTIR analysis indicated highest O–H absorption ranging between 3400 and 3000 cm^{-1} related main functional groups alcohol and phenols, namely primary amides, secondary amides, oximes, acetylenes and primary amine groups present on the cell walls of seaweed. Presence of phenolic compounds could have associated with increasing the antioxidant activity of *A. paniculata.* Further, the strong absorption bands observed between 2360.87 and 2850.79 cm^{-1} confirmed the presence of functional groups such as methyl and amino group which arises due to the carbonyl stretch and N– H vibrations in the amide linkage (Abdel-Rauf et al. 2013), 1637 and 1610.56 peak can be attributed to β- ketones and tertiary amides, and 1035.77 represents the functional group of P–O–C groups (El-Rafie et al. 2013).

Andrographolide is one of the active principles specialized metabolites of *A. paniculata* (Sareer et al. 2014).

Our study indicated that there was a twofold increase in the andrographolide in those plants which received 10% of SLE. This positive effect may be due to rapid penetration of plant growth-promoting substances such as auxin, cytokinin and gibberellin, micro- and macronutrients and other necessary constituents such as vitamins, amino acids, by foliar application which could have triggered the synthesis of specialized metabolites such as andrographolide etc. Similarly, several studies have reported that application of seaweed has enhanced the specialized metabolites such as stevioside in *Stevia rebudiana* Bertoni (Salama et al. 2016); β-pinene, α-phellandrene, α-terpinene, α-bisabolol in *Rosemarinus officinalis* (L.) (Tawfeeq et al. 2016); IAA in *Prunus mahaleb* (L.) (Szabó et al. 2014); linoleic acid, oleic acid, palmitoleic and stearic acid in *Olea europaea* (L.) (Chouriaras et al. 2009). One can conclude that the cumulative synergistic interactions of phytonutrients and endogenous growth hormones (auxins, gibberellins and cytokinin) present in brown seaweed extracts might be the primary reason for the increase in specialized metabolites of andrographolide in *A. paniculata.*

Macroalgal extracts are known to enhance antioxidant activities in plants (Zhang et al. 2004). Studies suggest that SLE had more influence on plant metabolism such as antioxidants, total soluble proteins, total phenolics and flavonoids (Battacharyya et al. 2015). In cucumber plants, the antioxidant capacity was found to be higher in the foliar treatment of *Macrocystis pyrifera* (L.) C. Agardh followed by *Bryothamnion triquetrum* (S.G.Gmelin) M.Howe and also justified that the highest antioxidant activity in the plants might be due to the synthesis of phenols by plants as a defence mechanism to counteract the negative effects of oxidative stress (Valencia et al. 2018). Vasantharaja et al. (2019) reported that seaweed extracts prepared from 3% of *Sargassum swartzii* (brown seaweed) improved the phenolic, flavonoid and protein contents and antioxidant capacity in *Vigna unguiculata* than *Kappaphycus alvarezii* extract and control and stated that increased antioxidants activities obtained in terms of DPPH assay could be attributed to the increased phenolic and flavonoid content in plants treated with 3% *S. swartzii* extract. Methanolic extracts of medicinal plants are viable source of antioxidant activity which corroborate with many researchers reported in *Phyllanthus freternus* G.L. Webster, *Triumfettar homboidae* Jacq and *Casuarina littorea* (L.) against nitric oxide free radicals (Parul et al. 2013), *Bougainvillea glabra* Choisy against DPPH, nitric oxide, superoxide radical, metal ion and hydrogen peroxide (Trinichia et al. 2017).

Based on our findings, it can be concluded that the presence of growth hormones, micro- and macronutrients and other biologically active specialized metabolites present in the SLE facilitated and channelized the various plant metabolisms for growth and development by increasing the photosynthetic activity, inducing the synthesis of phytochemical constituents, enhancing the andrographolide content and increasing the antioxidant activity. Presence of higher concentration of Mg which is the chief component for chlorophyll biosynthesis, might have induced the biosynthesis of chlorophyll molecule in the plant, thus maximizing the photosynthetic activity and other biosynthetic pathway. However, further studies are still needed to find out the optimum dosage or concentration on the basis of plant responses and physiological targets and other metabolic activities to establish their efficiency in sustainable management of agricultural systems. Thus, we confirm that as of now, SLE can act as cost-effective, viable and eco-friendly organic input to eradicate the mushroom growth of synthetic fertilizers in agricultural field and also for the production of primary and specialized metabolites in the medicinal plants for commercial drug preparation.

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Author's contribution UV designed, outlined and carried out FTIR and biochemical analysis. SRS collected the plant material and performed phytochemical screening and antioxidant activity. ES and BP carried out HPTLC analysis. VN collected the seaweed samples, authenticated and carried out hormonal analysis. KD supervised the whole experiment, interpreted the data and drafted the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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