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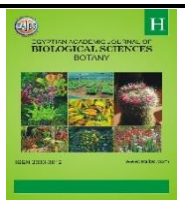

Influence of Seaweed Extracts on The Antioxidant System and Activity in *Spinacia oleracea as* Edible leafy Vegetable Plant

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ABSTRACT

The current study sought to determine the effects of *Colpomenia sinuosa and Sargassum linifolium* aqueous extracts on non-enzymatic and enzymatic antioxidants in *Spinacia oleracea* L. leaf extract. Glutathione synthase (GSS, EC: 6.3.2.3) and -glutamyl cysteine synthetase (-GCS, EC: 6.3.2.2) are involved in the glutathione biosynthesis process. Treatment of *S. oleracea* with seaweed extracts increased the level of reduced glutathione (GSH), oxidized glutathione (GSSG), and total glutathione at lower concentrations. Total phenols and total flavonoids in *S. oleracea* leaves accumulated more rapidly after treatment with seaweed extracts. The activity of the enzyme's phenylalanine ammonia lyase (PAL, EC: 4.3.1.25), chalcone synthase (CHS, EC: 2.3. 1.74), and chalcone isomerase (CHI, EC: 5.5.1.6) involved in the production of phenylpropanoid and flavonoid in *S. oleracea* leaves rose in a dose-dependent manner. Glutathione reductase (GR, EC 1.6.4.2), glutathione peroxidase (GPX, EC: 1.11.1.9), and glutathione-S-transferase (GST, EC: 2.5.1.18) are examples of antioxidant enzymes whose activity were also elevated by the treatment. In comparison to untreated plants, the treated plants' *S. oleracea* leaf extract significantly reduced superoxide anion, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and hydroxyl radicals. In light of this, the current findings imply that the use of seaweed extracts was found to boost the activity of the antioxidant system in *Spinacea oleracea*

INTRODUCTION

The scientific name for spinach is *Spinacea oleracea* Linn, a leafy green vegetable produced throughout much of the world, belonging to the family of Chenopodiaceae. Spinach is grown all over the world (Montenegro-Landvar *et al.*, 2022). It is useful as a meal and a medicine, in addition to its content of minerals including magnesium, manganese, iron, calcium, and folic acid. Spinach is a good source of vitamins A, C, and E (Sarkar *et al.*, 2022). An essential dietary crop and a typical raw material in the food processing sector, Additionally, spinach is a great source of chlorophyll, which is believed to help with digestion. Lutein and beta-carotene are abundant in spinach. It is regarded as a good source of several flavonoids that have antioxidant properties (Ko *et al.*,2014 and Estrada *et al.*, 2022).

Many synthetic antioxidants have undesirable side effects after continuous usage (some have even been pulled off the market as potential carcinogens). Antioxidants derived

from natural sources are thus advantageous for human health because they can scavenge the free radicals that cause the majority of chronic diseases. Green vegetables are one source of antioxidants since they contain different antioxidant chemicals and are considered beneficial for lowering the risk of cancer and other degenerative diseases (Kaurinovic and Vastag, 2019).

GSH, polyphenols, flavonoids, as well as additional compounds, are examples of non-enzymatic antioxidants (Patsayev *et al.*, 2017; Changxing *et al.*, 2018). The glutathione peroxidase (GPX), glutathione transferase (GST), and glutathione reductase (GR) enzymes are merely a few of the numerous enzymes that collectively make up the enzymatic antioxidant machinery (Kaur *et al.*, 2016). Cysteine, glutamate and cysteine are the three amino acids that make up the tripeptide GSH. According to Koffler *et al.* (2013), GSH is the most prevalent low molecular weight thiol in plant tissues and typically accumulates to millimolar concentrations. The most responsive GSH group for this tripeptide's action is the cysteine's sulfhydryl (SH) group. Due to its ability to scavenge reactive oxygen species (ROS), GSH is a crucial antioxidant. GSH is a hydrophilic compound with a low molecular weight that contains two COOH groups, one SH group, and one NH2 group (Gasperl *et al.*, 2022).

Glutathione S-transferase (GST) is implicated in xenobiotics, pollutants, and herbicides (Cummins *et al.*, 2011). According to Sabetta *et al.*, (2017), GSH is crucial for the growth of flower primordia and pollen germination. By using ROS-dependent pathways, GSH can modify the redox state (Foyer and Noctor, 2016). Since nitroso-glutathione serves as a NO reservoir and GSH combines with NO to create it, GSH can protect proteins from oxidation (Noctor *et al.*, 2016). According to Hasanuzzaman *et al.*, (2017), GSH aids in detoxification. According to El-Shora and Abd El-Gawad (2015), GSH can serve as a substrate for dehydroascorbate and directly interacts with free radicals, such as the hydroxyl radical, to reduce the inactivation of enzymes caused by oxidation of the crucial thiol group. Glutathione production is aided by two enzymes. Glutamylcysteine synthetase (-GCS, 6.3.2.2) and GSH synthetase (GS, 6.3.2.3) are the first and second enzymes in the production, respectively.

In plants, particularly vegetables, polyphenols are one of the main families of naturally occurring chemicals with at least one phenol group in their structure. Secondary metabolites of the plant kingdom called polyhydroxy phytochemicals are called polyphenols. The biosynthesis of these secondary chemicals occurs via the phenylpropanoid and shikimic acid pathways. According to Kaurinovic and Vastag (2019), flavonoids are widely distributed polyphenolic chemicals that make up a large class of natural goods. Phenylalanine is converted by PAL into trans-cinnamic acid, CHS, a crucial enzyme in the biosynthesis of flavonoids and isoflavonoids, and CHI catalyzes the stereospecific cyclization of chalcones to (2S)-flavanones, which are discovered to be the only substrates for reactions to other flavonoid classes.

Xenobiotics and heavy metals are detoxified by glutathione-S-transferase (GST, EC 2.5.1.18), one of the recognized antioxidant enzymes (Kumar and Trivedi, 2018). Additionally, glutathione reductase (GR, E.C. 1.6.4.2) is an oxidoreductase that converts oxidized glutathione (GSSG) to reduced glutathione (GSH) in mitochondria, chloroplasts, and cytosols (Wu *et al.*, 2013). Glutathione peroxidase (GPX, 1.11.19) scavenges H_2O_2 (Bela *et al.*, 2015).

According to Parthiban *et al.*, (2013), seaweeds are describes as macroscopic algae that grow in the intertidal and subtidal zones of the ocean and are used as fertilizer, raw materials for industry, fodder and food. The seaweeds are divided into three main classes based on their pigments: brown seaweeds (Phaeophyta), red seaweeds (Rhodophyta), and green seaweeds (Chlorophyta) (Makkar *et al.*, 2016; Corino *et al.*, 2019; Alboofetileh *et al.*, 2021).

While seaweeds are a great source of nutrients, they are also a significant source of different bioactive compounds. Due to their ability to promote growth, extracts from seaweeds have been extensively employed as bio-stimulants in crop management (Mukherjee and Patel, 2020; Chen *et al.*, 2021). In several other crops, including spinach, extracts from seaweeds have been demonstrated to be useful in enhancing stress resistance (Xu and Leskovar, 2015). Seaweed extracts are known to cause many beneficial effects on plants, as they contain many growth-promoting hormones such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), cytokinins, trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins, and amino acids (Latique *et al.*, 2020).

The objective of the current study was to investigate the possibility of enhancing antioxidant system and antioxidant activity in *S. oleracea* leaves by seaweed extracts.

MATERIALS AND METHODS

Sampling of Seaweeds Collection and Treatment*:*

Colpomenia sinuosa and *Sargassum linifolium* from Abu Qir Bay submerged rocks on the coast of Abu Qir Bay. The samples were brought to the laboratory in plastic bags containing seawater to protect the seaweeds from drying out. The seaweeds were identified according to Aleem (1993). To remove the sand and salt content, the gathered seaweed underwent a thorough cleaning with sterilized seawater. Additionally, the sample was gently cleaned with a smooth brush to eliminate any attached epiphytes or depressed marine microorganisms. They were then shade-dried, and the dried seaweeds were ground in a commercial grinder before being stored at 4^{o}C and used for further analysis.

Preparation of Seaweeds Extracts*:*

Seaweed powder (5g) was extracted at 35 °C with 100 ml of distilled water using agitating water bath. The samples were centrifuged for 15 min at 6000 rpm after being cooled to room temperature for 72 hours. The supernatant was utilized to make a seaweed extract (Kokilam *et al*. 2013).

Plant Growth:

Spinacia oleracea L. was the plant employed in the investigation and the Egyptian Ministry of Agriculture provided the seeds. Plant growth was accomplished, according to El-Shora (2001). *S. oleracea* seeds were sterilized for 10 min. in 2% (v/v) sodium hypochlorite, followed by many rinses in distilled water. The seeds were put in 9-cm Petri dishes with Whatman paper that were sterile. The seeds were incubated at 25°C in the dark for 15 days before sprouting. Two groups of 20 seedlings, each was created from 15-day seedlings. The following treatment was given to the two groups: Group 1: untreated (control). Group 2: treatment with various seaweed extract concentrations (0, 50, 100, 150, 200, 250, and 300 ml/L). Hoagland's solution was added for 10 days to each group and then moved to plastic pots (Hoagland and Arnon, 1950).

Preparation of *S. oleracea* Leaf Extract*:*

The El-Shora *et al*. (2022) method was used to prepare the aqueous leaf extract of *S. oleracea*. The plant leaves were twice cleaned in tap water before being air dried. To obtain the plant extract, the dried leaves were pulverized, and a sample (20 g) of the powder was soaked in distilled water and agitated for 48 hours at a temperature between 23 and 28 °C.

Estimation of Reduced and Oxidized Glutathione in The Aqueous Extract in *S. oleracea* Leaves*:*

GSH and GSSG contents were determined according to Anderson (1985). The leaf extract was neutralized in 0.5 ml of 150 mM potassium phosphate buffer (pH 7.5). The reaction mixture of 3 ml contained 0.2 ml of 6 mM 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), 1 ml of glutathione reductase (GR), 0.1 ml of 2 mM NADPH and 0.5 ml of 0.1 M of Na-phosphate buffer (pH 7.5) with EDTA. The absorbance was taken at 412 nm. 2

vinylpyridine was added to the leaf extract to determine GSSG content. GSH content was calculated by deducting the GSSG content from the total glutathione content. The values are expressed as µmoles of GSH/g fresh wt.

Determination of Total Phenolic Content in *S. oleracea* Leaf Extract:

The Folin-Ciocalteu reagent was used to calculate the total phenolic content (Dai *et al*. 1995). 1.9 ml of distilled water, 200 ml of sodium carbonate (20% w/v), and a sample of plant extract (25 ml) were combined. For 30 minutes, the mixture was heated to 60 °C. At 750 nm, the absorbance was spectrophotometric ally quantified. A standard curve was created utilizing various gallic acid concentrations.

Determination of Total Flavonoid Content in *S. oleracea* Leaf Extract*:*

The method of Lamaison and Carant (1996) was used to calculate the total flavonoid content. Using the $AlCl_3$ reagent and based on the presence of a complex of yellow color between $AlCl_3$, the hydroxyl and carbonyl groups from flavonoids. Liquid nitrogen was used to pulverize the leaf samples (0.5 g), which was then done in 80% methanol. The reaction mixture was vigorously agitated and contained a 10ml aliquot of the extract, 2.9 ml of methanol solution, 1 ml of 10% sodium potassium tartrate, 1 ml of 10% $AlCl_3$, and 1 ml of distilled water. At 415 nm, the absorbance was measured by spectrophotometric ally. Quercetin created the standard curve for all flavonoids. In terms of the dry weight of plant leaves, the results were reported as mg quercetin equivalent g^{-1}.

Preparations of Enzymes Extract from *S. oleracea* Leaf Extract:

The preparation of the enzyme extract was done at 4 °C according to El-Shora and Abo-Kassem (2001). The leaf tissue (0.5g) was homogenized with polyvinyl pyrrolidone, and the resultant homogenate was centrifuged for 10 min at 4 °C and 8,000 g to get the supernatant for enzyme analysis.

Assays of Enzymes in *S. oleracea* Leaf Extract

The activities of the following enzymes were measured in *S. oleracea* leaf extract: glutamyl-cysteine synthetase (GCS, EC: 6.3.2.2) according to Nagalakshmi and Prasad (2001), glutathione synthetase (GSS, EC: 6.3.2.3) according to Volohonsky *et al*. (2002), phenylalanine ammonia lyase (PAL, EC: 4.3.1.24) according to Wang *et al*. (2006), chalcone synthase (CHS, EC: 2.3.1.74) according to Obinata *et al*. (2003), chalcone isomerase (CHI, EC: 5.5.1.6) by the method of Dixon *et al*. (1978), glutathione peroxidase (GPX, EC: 1.11.1.9) according to Nagalakshmi and Prasad (2001), glutathione S-transferase (GST, EC: 2.5.1.18) by the method of Lee (1991), glutathione reductase (GR, EC 1.6.4.2) according to Goldberg and Spooner (1983).One unit (U) of each enzyme activity is defined as the mount of the enzyme needed to convert 1 µmol of its substrate to product in one min. Protein Content was determined according to Bradford (1976).

Determination of the Antioxidant Activity of *S. oleracea* Leaf Extract:

i- Superoxide Anion Scavenging Activity:

.The experiment was predicated, with minor changes, on the sample's ability to prevent the photochemical reduction of NBT in the nicotinamide adenine dinucleotide-nitroblue tetrazolium-phenazine methosulfate (NADH-NBT-PMS) system. NBT (78 mol/l in 20 mmol/l potassium phosphate buffer, pH 7.4), NADH (468 mol/l in 20 mmol/l potassium phosphate buffer, pH 7.4), and a properly diluted sample totaled 1 ml of the reaction mixture. 0.4 ml of PMS (60 mol/l in 20 mmol/l potassium phosphate buffer, pH 7.4) was added to the mixture to start the reaction. A spectrophotometer (TU-1800) was used to measure the absorbance at 560 nm after the tubes were incubated at room temperature for 5 min. The reaction mixture's decreased absorbance was a sign of improved superoxide anion scavenging activity. Using the following formula, the % suppression of superoxide anion formation was determined: Where A0 is the absorbance without a sample and As is the absorbance with a sample, % inhibition is calculated as 1 As A0 100.

ii-DPPH Scavenging Activity:

The radical-scavenging activity of the sample against DPPH free radical was measured using the method of Yu et al. (2007) with some modifications. A 1.5 ml of ethanolic solution of DPPH (2 × 10−4 mol/l) was mixed with an equivalent aliquot of different concentrations of sample in a tube. Absorbance at 517 nm was measured at 2-minute intervals by the use of a spectrophotometer. After standing in the dark for 30 min when the absorbance reached a plateau, it was measured against ethanol. Controls containing ethanol instead of the antioxidant solution and blanks containing ethanol instead of DPPH solution were also made. DPPH scavenging activity was calculated with the equation: (As of control –As of sample)/ (As of control) × 100%. Ascorbic acid was used as a reference compound.

iii-Hydroxyl Radical-Scavenging Activity:

Malondialdehyde is produced when the Fenton reaction oxidizes 2-deoxyribose (Kim and Minamikawa 1997.) In a screw-capped test tube, 0.2 ml of $FeSO_4.7H_2O$ and ethylenediamine tetraacetic acid (EDTA) mixed solution were made, along with 10 mmol of each. The sample solution, 10 mM of 2-deoxyribose solution, and 0.1 M of phosphate buffer (pH 7.4) were then added to create a total volume of 1.8 ml. This reaction mixture was then given 200 l of a 10 mmol H_2O_2 solution, and everything was allowed to sit at 37 °C for 4 hours. After this period of incubation, 1 ml of each of the solutions of trichloroacetic acid (2.8%) and thiobarbituric acid (1.0%) were added to the reaction mixture. The entire mixture was then heated for 10 minutes, and cooled on ice, and its absorbance was measured at 520 nm. The scavenging activity was estimated using the formula: 2-deoxyribose Scavenging activity (%) = 1 As Ab0 Ac A0 100, where A0 is the absorbance at 520 nm with no treatment; Ac is the absorbance of the treated control at 520 nm; and As is the absorbance of the treated sample at 520 nm.

RESULTS AND DISCUSSION

The GSH, GSSG, and total glutathione content of *S. oleracea* leaves, as well as the activities of the enzymes involved in glutathione biosynthetic biosynthesis, have increased significantly as a result of the biostimulatory actions of seaweed extracts (Fig. 1a, 1b, and 1c). These findings concur with those made public by El-Shora *et al.* (2016).

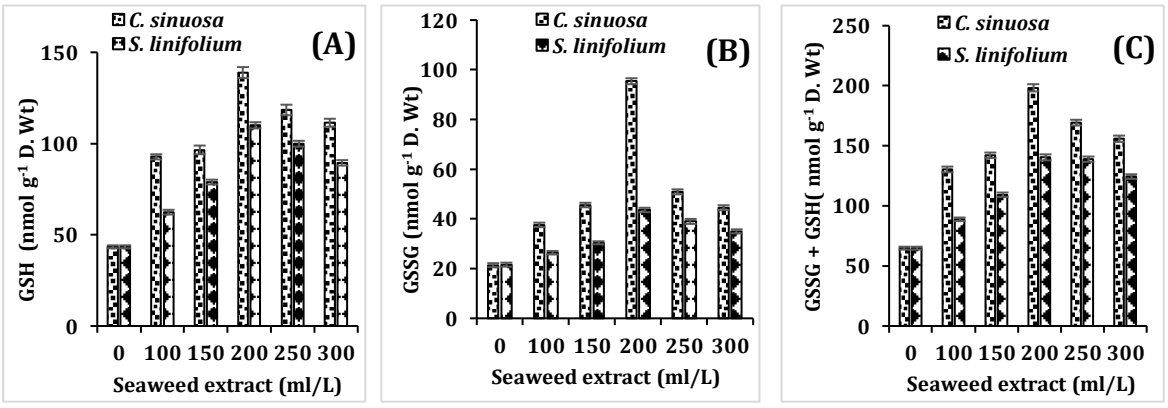

Fig. 1: Effect of aqueous seaweeds extracts on the content of: (A) reduced glutathione (GSH), (B) oxidized glutathione (GSSG) and (C) total glutathione in *S. oleracea* leaves.

The growth regulators found in seaweed extract, which are involved in glutathione production, including glutamyl-cysteine synthetase and glutathione synthetase, may have enhanced the activities of the biosynthetic enzymes that produce glutathione (Fig. 2a and

2b). The transport and synthesis of amino acids into proteins and DNA, as well as other redox events, all include the GSH in plants (Frendo *et al.,* 2013).

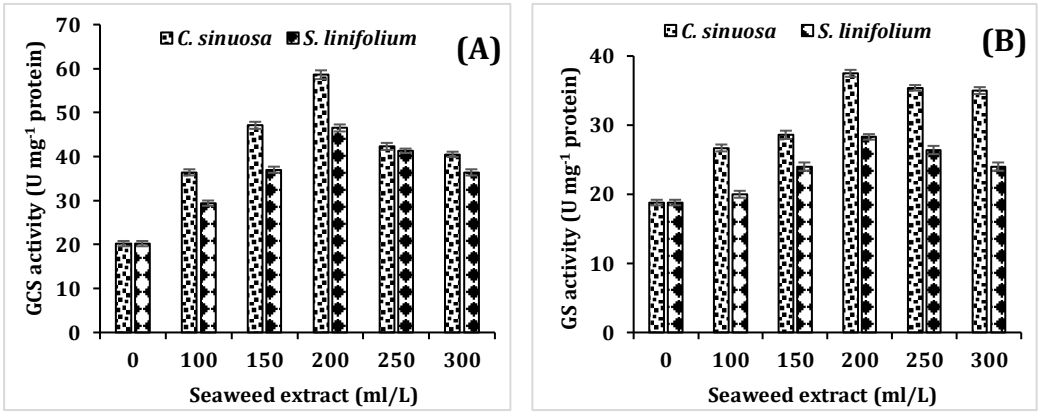

Fig. 2: Effect of aqueous seaweeds extracts on the activity of: (A) glutamyl-cysteine synthetase (GCS) and (B) glutathione synthetase (GS) in *S. oleracea* leaves.

According to El-Omari *et al.* (2016), a high ratio of GSH/GSSG speeds up the H_2O_2 scavenging mechanism. The biological activities of total phenol and total flavonoids, which are frequently found in plants, have been documented to include antioxidant capabilities. When *S. oleracea* leaves were treated with seaweed extracts, their content of total phenols (Fig. 3a) and total flavonoids (Fig. 3b).

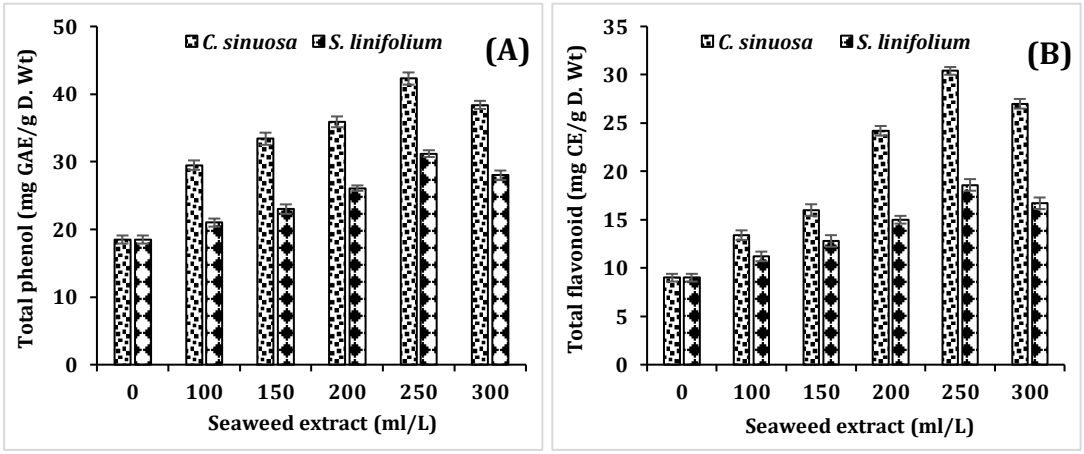

Fig. 3: Effect of aqueous seaweeds extracts on the content of: (A) total phenol and (B) total flavonoid in *S. oleracea* leaves.

In support, it has been found that seaweed treatment improved the phenolic content of cabbage and spinach (Lola-luz *et al.,* 2013). Similar outcomes have been noted for broccoli and cabbage, where treatment with seaweed extracts raised their level of phenolic and flavonoid components (Lola-Luz *et al.,* 2014). According to Holdt and Kraan (2011), seaweed treatment increases the total phenol and total flavonoid content in plant sections. Seaweed extracts also include polyphenol components. Seaweed growth hormones may be responsible for inducing the phenolic content of treated *S. oleracea* to continuously rise as seaweed concentration increases. Seaweed extracts encouraged tomato plants to produce more phenolics including flavonoids like rutin and naringenin (Deolu-Ajayi *et al.,* 2022).

It could be mentioned that simple phenols like hydroxycinnamic acids like caffeic, *p*-coumaric, ferulic, and sinapic acid, as well as hydroxybenzoic acids like gallic, vanilic, 4-hydroxybenzoic, protocatechuic, syringic, and gentisic acid, are found in seaweeds.

Polyphenols, which include flavonoids and non-flavonoids, are also present (De Quiros *et al.*,2010). These substances have the ideal structural chemistry to neutralize reactive oxygen species and scavenge free radicals (Sánchez, 2017). As a result, the rise may be connected to total phenols, which are important regulators of plant metabolism and growth (Ali *et al.*, 2021). Additionally, a rise in the expression of glutathione reductase, thylakoid-bound ascorbate peroxidase (APX), and monodehydroascorbate reductase was linked to the rise in total phenol and total flavonoid levels.

According to Fan *et al.* (2013), these genes were connected to the phenylpropanoid pathway, which are known to promote the manufacture of phenolic compounds. It could be mentioned that simple phenols like hydroxycinnamic acids like caffeic, p-coumaric, ferulic, and sinapic acid, as well as hydroxybenzoic acids like gallic, vanilic, 4-hydroxybenzoic, protocatechuic, syringic, and gentisic acid, are found in seaweeds. Polyphenols including flavonoids and non-flavonoidsare also present (De Quiros *et al.*, 2010).

The present results indicate the enhancement of the enzymes involved in phenol and flavonoids including PAL (Fig. 4a), CHS (Fig. 4b) and CHI (Fig.4c). These results are congruent with those of Panjehkeh and Abkhoo, (2016) who reported upregulation of the PAL gene in tomato plants by the marine algal extract. An extract from *Ulva* spp. applied to barrel clover led to an increase in the activity of defense enzymes such as PAL, CHS and isoflavone reductase (Cluzet *et al.*, 2004). Fan *et al.* (2011) reported an increase in the biosynthesis CHI by treatment with seaweed extracts.

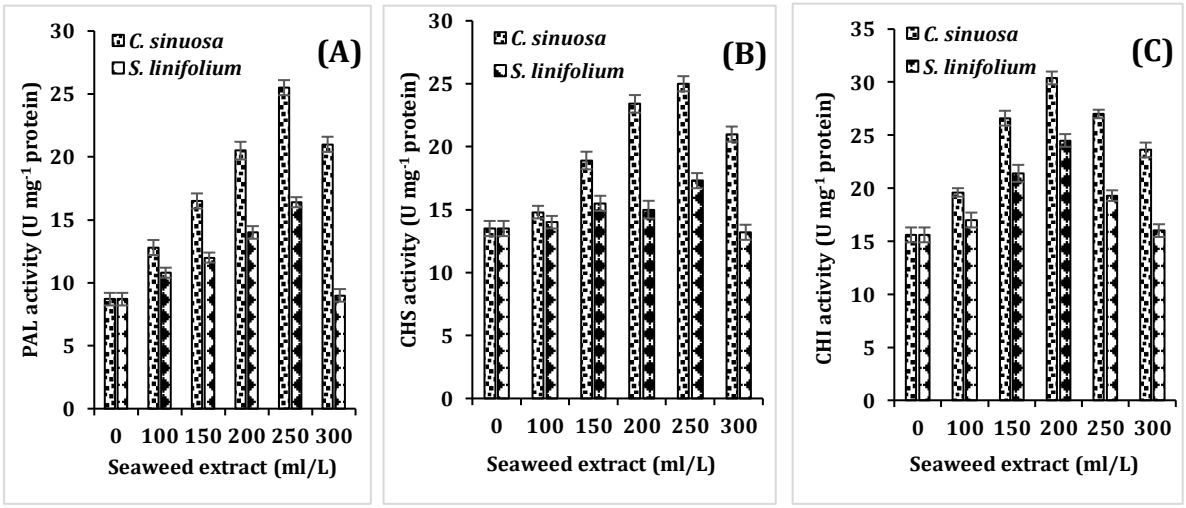

Fig. 4: Effect of aqueous seaweed extracts on the activity of: (A) phenylalanine ammonia lyase (PAL), (B) chalcone synthase (CHS) and (C) chalcone isomerase (CHI) in *S. oleracea* leaves.

The activation of antioxidant enzymes: GR (Fig. 5a), GPX (Fig. 5b) and GST (Fig. 5c) is one of the defense mechanisms that plants have evolved to keep ROS at normal levels (Roussi *et al.*, 2022). Our findings showed that three enzymes' activity increased in a concentration-dependent manner. In the presence of GSH, GST is known to aid in the reduction of a variety of organic hydroperoxides (Roussi *et al.*, 2022). According to research by Frendo *et al.* (2013), the GST enzyme can protect plants against salt stress and counteract its effects on lipid peroxidation. Another study found that GST is involved in the detoxification route to lessen the effects of high levels of cadmium in *Phragmites australis* (Srikanth *et al.*, 2013). Under conditions of lower quantities of seaweed extracts, GPx activity was increased in the leaves of *S. oleracea*. High seaweed extract concentrations, however, have a detrimental effect and might cause oxidative stress in the treated plant.

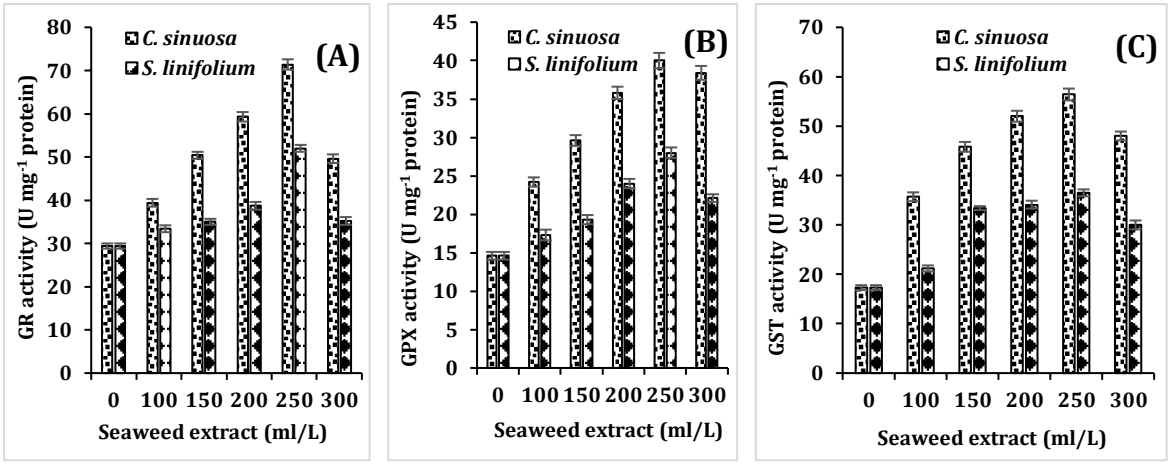

Fig. 5: Effect of aqueous seaweeds extracts on the activities of: (A) glutathione reductase (GR), (B) glutathione peroxidase (GPX) and (c) glutathione transferase (GST) in *S. oleracea* leaves.

According to Mrid *et al.* (2021), the rise in the GR enzyme level caused by the use of seaweed extracts is expected to boost the production of high levels of GSH, which are necessary for both the detoxification of H_2O_2 from plant cells and for other metabolic processes involved in plant growth and development. Additionally, it's possible that the presence of growth hormones in seaweed extracts is what causes them to induce GPX, GR and GST in *S. oleracea* leaves.

According to Zhang *et al*. (2003), the presence of cytokinin may be the reason why seaweed extracts promote plant development and antioxidant enzymes. According to Delaunois *et al*. (2014), phytohormones including gibberellins, cytokinins, abscisic acid and auxins can be found in seaweed extracts. Other plant enzymes, such as urease, were activated in *Cucurbita pepo* when auxins such as kinetin, zeatin, and benzylamine were applied externally (El-Shora and Ali, 2016).). However, it is possible that the presence of heavy metals, including Cd and Pb, in the investigated seaweed extracts, which were responsible for the studied enzymes' inhibition after treatment with large amounts of seaweed extract.

The results of Fig. 6a show how seaweed extracts may scavenge superoxide radicals in treated and untreated *S. oleracea* leaves in comparison to the same dosages of BHT. *S. oleracea l*eaf extract was less effective than BHT at all concentrations for scavenging superoxide anion. Both of them demonstrated the scavenging of superoxide radicals in a concentration-dependent manner. With the use of extracts from *C. sinuosa, S. linifolium*, and BHT, the IC_{50} values were, respectively, 18.3, 13.6, and 20.3 ml/L respectively. Among reactive oxygen species (ROS), the hydroxyl radical is the most reactive and has the shortest half-life when compared to other ROS.

A spare electron that is delocalizing around the entire molecule causes the DPPH, a stable nitrogen radical, to appear dark purple. A non-radical version of DPPH is created when DPPH's solution and a hydrogen atom donor (H-A) are combined. Its color is light yellow. According to El-Mekabaty and El-Shora (2018), the antioxidant's ability to scavenge free radicals is demonstrated by the purple DPPH radical's transformation into a yellow color.

The results in Fig. 6b demonstrated the ability of the extract from *Spinacia* leaves to scavenge DPPH radicals. The DPPH radical was scavenged by all samples in a dose-dependent way. *Spinacia* leaf extract demonstrated lower DPPH radical scavenging activity than BHT at all doses, with IC_{50} values of 17.9, 16.3, and 19.6 ml/L under treatment with extracts of *C. sinuosa, S. linifolium*, and BHT, respectively. Superoxide anion, a very

poisonous species, is produced in numerous biological interactions. The superoxide anion produced by the PMS/NADH coupling reaction and dissolved oxygen decreases NBT in the PMS/NADH-NBT system. Thus, the depletion of superoxide radical, by the antioxidants, in the reaction medium is illustrated by the reduction in the absorbance at 560 nm.

According to Richards *et al.* (2015), the hydroxyl radical is the most reactive oxygen radical and causes significant harm to nearby biomolecules. The effectiveness of *S. oleracea* leaf extract as a hydroxyl radical scavenger as measured by the 2-deoxyribose oxidation technique is shown in Fig.6c. The leaf extract of *S. oleracea* plants treated with the various tested seaweed concentrations exhibited good hydroxyl radical-scavenging activity, and the scavenging action was concentration-dependent. Compared to BHT, the activity of *S. oleracea* leaf extract was lower. Treatment with extracts of *C. sinuosa, S. linifolium*, and BHT, respectively, resulted in IC_{50} values of 13.9, 10.8, and 16.8 ml/L.

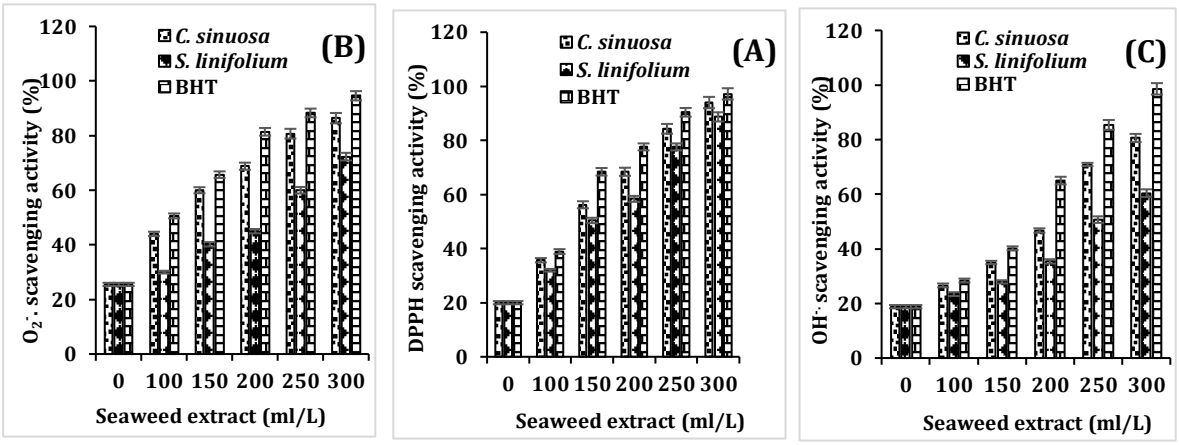

Fig. 6: Influence of seaweeds extracts on: (A) superoxide anion scavenging activity, (B) DPPH scavenging activity and (c) hydroxyl radical scavenging activity of *Spinacia* leaf extract.

The present results show a significant association between *S. oleracea* antioxidant activity and total phenol and flavonoid concentrations, which is in agreement with reports from other researchers employing seaweeds as a treatment (Farasat *et al.*, 2014). Additionally, it has been shown that seaweed extracts boost antioxidant activity (Hashmi *et al.*, 2012). According to Latique *et al.* (2021), macroalgae can boost the activities of the antioxidant enzyme system by creating more non-enzymatic antioxidants such as phenols and flavonoids.

Additionally, due to their reactivity as electron or hydrogen donors, which aid in stabilizing and delocalizing unpaired electrons, as well as their function as chelators of transition metal ions, phenolic compounds have an antioxidant impact as free radical scavengers (Santos-Sánchez *et al.*, 2019). According to evidence, marine algae are a significant source of bioactive chemicals because they generate a wide range of secondary metabolites with antioxidant characteristics, which may improve the plant's antioxidant capacity following treatment (Athukorala *et al.*, 2007).

In conclusion, the current results imply that seaweed extracts might be utilized to strengthen the antioxidant system in leafy edible vegetables like *S. oleracea.*

Ethical Approval: Ethical Approval is not applicable.

Competing interests: The authors declare no conflict of interest.

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ARABIC SUMMARY

تأثير مستخلصات الأعشاب البحرية على النظام المضاد للأكسدة في أوراق نبات السبانخ كنبات من الخضروات الورقية الصالحة للأكل

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إهتمت هذة الدراسة إلى تعيين تأثير المستخلصين المائيين لكل من *Colpomenia sinuosa* و *Sargassum linifolium* على مضادات الأكسدة غير الأنزيمية والإنزيمية في مستخلص أوراق *Spinacia oleracea* L. يشارك الجلوتاثيون سينسيز (GSS، EC: 6.3.2.3) وجلوتاميل سيستين سينثيتاز (GCS، EC: 6.3.2.2) في عملية التخليق الحيوي للجلوتاثيون. أدت معالجة نبات السبانخ بمستخلصات الأعشاب البحرية إلى زيادة مستوى الجلوتاثيون المختزل (GSH)، والجلوتاثيون المؤكسد (GSSG)، والجلوتاثيون الكلي بتركيزات أقل. تراكمت الفينولات والفلافونويدات الكلية في أوراق نبات السبانخ بسرعة أكبر بعد المعالجة بمستخلصات الأعشاب البحرية. زاد نشاط إنزيم فينيل ألانين أمونيا لياز (PAL، EC: 4.3.1.25)، وسينسيز الشالكون (CHS، EC: 2.3.1.74)، وإيزوميراز الشالكون (CHI، EC: 5.5.1.6) المشاركين في إنتاج فينيل الفينولات والفلافونويدات. في أوراق نبات السبانخ بطريقة تعتمد على التركيز. ومن أمثلة الإنزيمات المضادة للأكسدة جلوتاثيون ريدكتيز (GR، EC 1.6.4.2)، الجلوتاثيون بيروكسيداز (GPX، EC: 1.11.1.9)، والجلوتاثيون-S-ترانسفيراز (GST، EC: 2.5.1.18) و قد زاد نشاطها أيضًا بعد المعالجة بمستخلصات الأعشاب البحرية. وبالمقارنة مع النباتات غير المعالجة فإن مستخلص أوراق نباتات السبانخ المعالجة بالمستخلصات الطحلبية قلل بشكل كبير من الشق الحر سوبر أكسيد وكذلك شق 1، 1-ثنائي فينيل-2-بيكريل هيدرازيل (DPPH)، وشق الهيدروكسيل. وفي ضوء ذلك، تشير النتائج الحالية إلى أن استخدام مستخلصات الأعشاب البحرية عزز نشاط النظام المضادا للأكسدة في أوراق نبات السبانخ.