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Enhancement of bioactive compounds in *Eruca sativa* by seaweed extract

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Abstract: Aqueous extracts from three seaweeds namely *Caulerpa prolifera, Turbinaria ornata,* and *Garcilaria dura* were prepared. The three seaweeds were collected and the collection site of the seaweeds was along Hurghada shores, the Red Sea coast of Egypt during autumn 2022. Priming *Eruca sativa* L. seeds in the aqueous extracts from the three seaweeds resulted in enhancing the germination percentage, particularly with *G. dura.* Also, priming the seeds in each of the three extracts led to the increase in total phenol, total flavonoids and reduced glutathione (GSH) at different rates. Since the three compounds represent the main non-enzymatic antioxidants in *E. sativa,* therefore the present results suggest priming seeds of plants in aqueous seaweed extracts before culturing and the method is safe for human health.

keywords: Bioactive compounds, *Eruca sativa*, phenolics, flavonoids, reduced glutathione.

Introduction

The oldest type of medicine is derived from medicinal plants, which have been utilized for thousands of years in traditional medicine throughout numerous nations. Human cultures have passed down empirical knowledge regarding their beneficial impacts [1].

Biostimulants are biologically derived substances sprayed on plants, either foliar or as roots, to stimulate the plant's natural processes that lead to effective nutrient uptake, growth, and increased resistance to abiotic and biotic stresses [2].

Three categories apply to reactive oxygen species (ROS): (1) creation of ROS as a byproduct of metabolism; (2) generation of ROS in response to pathogen attack (biotic stress response); and (3) formation of ROS during the abiotic stress response. One of the reactive oxygen species (ROS), H_2O_2, damages cells and serves as a signaling chemical [3].

Seaweeds are macroalgae that play a crucial role in the rich biodiversity of marine and coastal ecosystems as well as the biosphere as a whole. Based on their color, seaweeds are classified into three classes: *Rhodophyta*, *Phaeophyta*, and *Chlorophyta* [4, 5]. Seaweed extracts have the potential to be a source of antioxidants and biostimulants [6,7]. Seaweeds include three major types of antioxidants: pigments, polysaccharides, and phenolics [8, 9].

Phenolic chemicals are a significant group of secondary metabolites found in plants that are essential to their physiological processes at every stage of development. Under both ideal and unfavorable circumstances, plants create phenolics, which are essential to several developmental processes, including cell division, hormone control, photosynthetic activity, nutrient mineralization, and reproduction. Under abiotic stress circumstances, plants manufacture polyphenols like flavonoids and phenolic acids, which help them deal with environmental limitations. The phenylpropanoid pathway is used in the biosynthesis of these polyphenols [10].

Flavonoids can promote health via a variety of cellular signaling pathways linked to the survival and proliferation of cells [11]. Flavonoids possess the potential to scavenge reactive oxygen species (ROS) and shield plants against biotic and abiotic stressors such as UV radiation, cold stress, pathogen infection, and insect feeding. Flavonoids are classified as phytoalexins or antioxidants [12].

The mitochondria and cytoplasm of chloroplasts are the sites of reduced glutathione

(GSH) production, an antioxidant substance. Cysteine, glycine, and glutamine are the three amino acids that makeup glutathione. Redundant glutathione (GSH) is mostly synthesized in chloroplasts. Both glutathione synthase and glutamyl cysteine synthetase are enzymes that catalyze the synthesis of GSH [13].

This study sought to assess how various antioxidant molecules were affected by the aqueous extracts of *Caulerpa prolifera, Turbinaria ornata,* and *Gracilaria dura*.

2. Materials and methods

Collection of seaweeds

The collection site of the seaweeds was along Hurghada shores, Red Sea coast of Egypt during autumn 2022. The seaweeds that were employed in this study were *Caulerpa prolifera, Turbinaria ornata,* and *Gracilaria dura.*

To eliminate extra salt and grit, the three seaweeds were rinsed with distilled water. Additionally, the samples were cut into the right dimensions and then preserved for one day at 40°C in the oven. After that, the samples were finely powdered. For additional examination, the granular seaweeds were obtained and then preserved at -20°C in a container that was airtight.

Preparing seaweed extract

A sample (1 g) of dry powder was extracted for 72 h with distilled water on an orbital shaker. The mixture was then centrifuged at 5000 rpm for 20 min and the supernatant was taken and kept for use.

Priming of seeds in aqueous seaweed extract

The Egyptian Ministry of Agriculture in Egypt provided the *Eruca sativa* seeds, which were then chosen for homogenous by selecting seeds with an identical size and color. The seeds were primed for 72 hours in the prepared seaweed extracts.

Effect of seaweed extracts on germination of E. sativa leaves

The method described in [14] was used to germinate the prepared seeds of *E. sativa* for 7 days in seaweed extracts. After 10 minutes of surface sterilization with 0.1% $HgCl_2$, the seeds were twice cleaned with sterilizing distilled water. Filter sheets were used to conduct the germination test in clean Petri dishes measuring 12 cm. Different concentrations (0, 25, 75, 125, 175, and 225 ml/L) of seaweed extracts were introduced to the petri dishes to effectively moisturize the seeds. Distilled water was applied to the control seeds. Three duplicates of each Petri dish were used, and the different treatments were maintained at room temperature (30-35°C). After allowing the seeds to germinate for seven days, the percentage of germination was computed.

Growth conditions of E. sativa leaves

The seedlings were placed in a growth chamber with the following parameters for 21 days: 22 ± 1°C, 16 hours of light and 8 hours of darkness, day/night temperature, 350 µmol m^2 s^{-1} photon flux density, and roughly 60% relative humidity. They were also placed in Hoagland's nutritional solutions [15].

Extraction of bioactive compounds from the plant

On the orbital shaker, samples of 1 g were extracted using distilled water for 2 hours at room temperature. After centrifuging the mixture for 20 minutes at 4000 rpm, the supernatant was poured into a 15-ml vial. The different components were determined using the supernatant that served as a plant extract.

Determination of total phenol content in E. sativa leaves

Using the Folin-Ciocalteau reagent, the total phenol content of the plant leaves under examination was measured [16]. Leaf extract (1 ml) was mixed with 7% sodium carbonate and letting the Folin-Ciocalteau reagent sit for 5 minutes, it was darkly incubated for 30 minutes at room temperature. At λ 750 nm, the absorbance was measured using spectrophotometer. The total amount of phenol was computed as mg/g D.W, or the equivalent of gallic acid.

Determination of total flavonoids content in E. sativa leaves

To determine the total flavonoids, the $AlCl_3$ technique was employed, as stated in [17]. A 0.5 ml amount of plant leaf extract was combined with 2.8 ml of distilled water, 0.1 ml of potassium acetate, and 0.1 ml of 10% $AlCl_3$. After the combination was allowed to sit at

room temperature for 30 min, the absorbance at 415 nm was calculated. The results were represented as mg catechin equivalent (CE)/g D.W. of extract from it. The total amount of flavonoids was determined using a catechin standard curve.

Determination of reduced glutathione (GSH) in E. sativa leaves

GSH estimation. The GSH in her was determined by the method of [18] One ml of PMS fraction mixed with 1 ml of sulphosalcylic acid (4%) and the samples were incubated at 4°C for at least 1 hour and then subjected to centrifugation at 1200 × g for 15 minutes at 4°C. The assay mixture contained aliquot of the extract, phosphate buffer 0.1 M, pH 7.4, and 4 mg ml^{-1} DTNB in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm and was calculated as mg GSH g^{-1} D.W tissue.

3. Results and Discussion

An investigation was conducted on the impact of seaweed extract at varying concentrations (0, 25, 75, 125, 175, and 225 ml/L) on the germination percentage of *E. sativa*. In comparison to the control sample, the results in Fig. 1 demonstrate that the germination percentage increased as the concentration of seaweed extract increased.

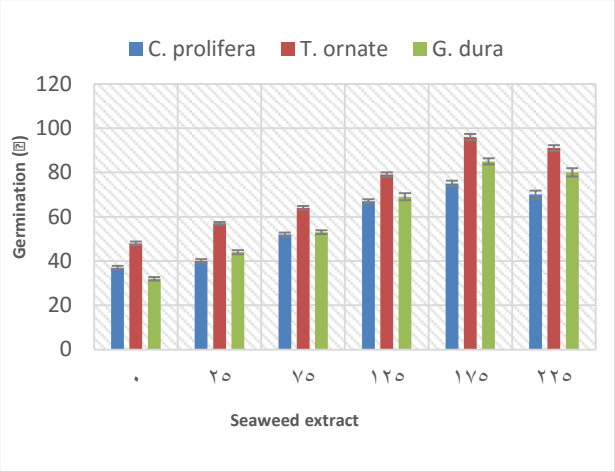

Fig. 1: Effect of seaweed extracts on seed on the percentage of germination of *E. sativa*

The impact of seaweed extract on total phenol as an antioxidant are shown in Fig. 2. The findings show that total phenol increased continuously as seaweed extract concentration increased to 175 ml/L before declining following treatment with 225 ml/L. Compared to the effect of *C. prolifera*, *T. ornata*, and *G. dura* it was found that *T. ornata* extract exhibited the highest phenol content.

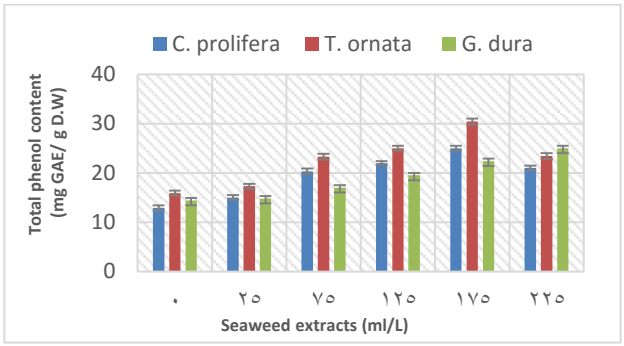

Fig. 2: Effect of seaweed extracts on total phenol content in *E. sativa* leaves

Three different seaweed extracts have been studied for their influence on the total flavonoids content of *E. sativa*. The concentrations of seaweed that were examined were 25, 75, 125, 175, and 225 ml/L. Following the treatment of seeds with the seaweed extracts, the total flavonoids concentration showed a steady increase, as illustrated in Fig. 3. In general, the intervention with seaweed extracts was efficient in enhancing total flavonoids, and this was observable compared to the control. The three stimulating seaweed extracts showed apparent advantages depending on the quantity.

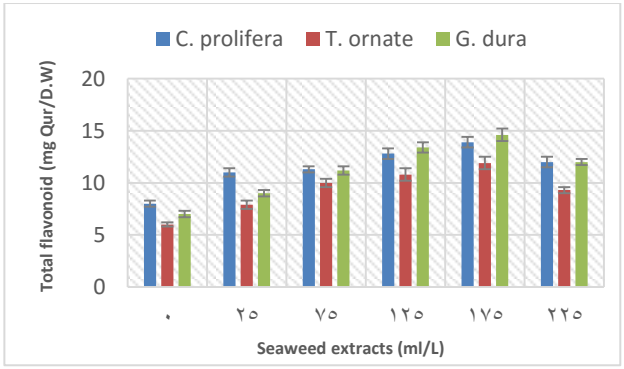

Fig. 3: Effect of seaweed extracts on total flavonoids content in *E. sativa* leaves

The amount of reduced glutathione (GSH) in *E. sativa* leaves after priming seeds treated with extracts *C. prolifera*, *T. ornata*, and *G. dura* was measured. According to Fig. 4 the plant extract consistently raised glutathione levels to a maximum of 19.4 at 175 ml of *T. ornata* before they started to decrease. At higher concentrations the GSH content was reduced with the three seaweed extracts. The samples that were treated with seaweed extracts generally demonstrated a higher concentration of GSH.

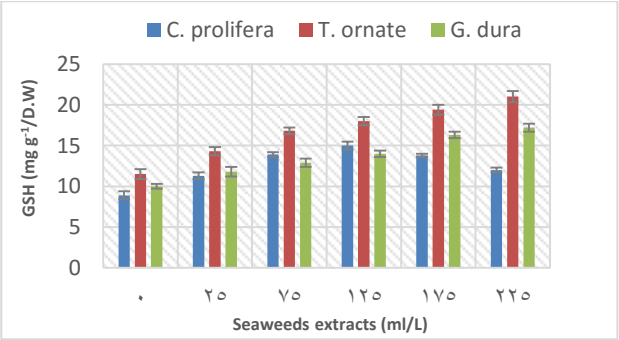

Fig. 4: Effect of seaweed extracts on reduced glutathione (GSH) content in *E. sativa* leaves

Due to the nontoxicity of seaweeds and their environmentally friendly nature, seaweed extracts are becoming increasingly popular as low-cost substitutes for traditional fertilizers [19,20,21]. Because they increase the plant defense mechanism and growth response, algae extracts are considered biological stimulants [22].

According to the current conclusions, when three different quantities of aqueous seaweed extracts were applied, the total phenol level increased significantly. In plants, both total phenol and total flavonoids have been associated with biological functions, including the ability to function as antioxidants. When seaweed extracts were applied to *E. sativa* leaves, their overall phenolic content and total amount of flavonoids were enhanced.

According to previous data, it has been shown that treating cabbage and spinach with seaweed enhanced their phenolic content [23]. Similar effects have been demonstrated for broccoli and cabbage, where the application of seaweed extracts enhanced the amount of phenolic and flavonoids components [24].

Polyphenols as chemical compounds are found in seaweed extracts [25], and applying seaweed increases the amount of total phenol and total flavonoids in plant tissues. The activation of phenolic content synthesis by seaweed growth hormones may account persistent increase in phenolic content of treated *E. sativa* with increasing seaweed concentration.

Seaweed extracts cause higher phenolic synthesis [26]. As a result, the rise in polyphenols may be connected to total phenols' function as a crucial regulator of plant growth and metabolic activities [23] Since phenolics represent hydrogen, they can be used as antioxidants [27]. Several processes have been suggested to account for the antioxidant properties of phenolic compounds, including (i) scavenging reactive oxygen species, (ROS), (ii) increasing antioxidant defense, and (iii) preventing ROS generation [28].

The total amount of flavonoids after treatment with seaweed extract followed the same general structure as the total phenol. After being treated with seaweed extracts, the flavonoid content of *E. sativa* leaves increased. Total flavonoids can absorb various oxidizing species, such as hydroxyl radicals, peroxy radicals, and superoxide anions. They may also serve as singlet oxygen quenchers [29].

According to their chemical structure, flavonoids, which are polyphenolic compounds, are divided into flavones, flavonols, flavanones, isoflavones, anthocyanidins, catechins, and chalcones [30]. It has been hypothesized by [31] that flavonoids can protect cells by enhancing the levels of glutathione, another effective antioxidant. Through a variety of processes, including the scavenging of free radicals, including superoxide, hydroxyl, and peroxyl radicals, as well as the inhibition of the enzymes that produce free radicals, flavonoids demonstrate their antioxidant capacity [32].

The main reactive component of GSH is the sulfhydryl (SH) group of cysteine in GSH, which was raised to the ideal concentration by treating it with seaweed extracts. Because of its ability to scavenge reactive oxygen species (ROS), GSH is a significant antioxidant.

Numerous groups that are hydrophilic and have a low molecular weight characterize GSH [33]. By oxidizing the necessary thiol group, GSH can directly interact with free radicals, particularly hydroxyl radicals, preventing the activity of the enzyme [34].GSH is required for several enzymes to remain stable. Additionally, GSH can function as a substrate for dehydroascorbate and directly combat free radicals, such as hydroxyl radicals, which decrease the inactive state of the enzyme by oxidizing its necessary thiol group [35].

The current findings imply that by boosting the various bioactive components in seaweed extracts, agricultural techniques in the future may be able to boost the antioxidant activity of

numerous food plants, including the leaves of *E. sativa*.

4. References

1. Khan, H. (2014). Medicinal plants in light of history: recognized therapeutic modality. *Journal of Evidence-Based Complementary and Alternative Medicine*, **19(3),** 216-219.
2. Yakhin, O. I., Lubyanov, A. A., Yakhin, I. A., & Brown, P. H. (2017). Biostimulants in plant science: a global perspective. Frontiers in Plant Science, **7**, Article ID 2049.
3. Smirnoff, N., and Arnaud, D. (2019). Hydrogen peroxide metabolism and functions in plants. New Phytologist, 221(3), 1197-1214.
4. Corino C, Modina SC, Di Giancamillo A, Chiapparini S & Rossi R. (2019). Seaweeds in Pig Nutrition. Animals. **9(12)**, Article ID1126.
5. Alboofetileh, M., Hamzeh, A. & Abdollahi, M. (2021). Seaweed proteins as a source of bioactive peptides. Current Pharmaceutical Design. **27(11),**1342–1352.
6. Farvin KHS and Jacobsen C. (2013). Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. Food Chemistry, **138(2–3)**,1670–1681.
7. Manlusoc JKT, Hsieh CL, Hsieh CY, Salac ESN, Lee YT &Tsai PW. (2019). Pharmacologic application potentials of sulfated polysaccharide from marine algae. Polymers (Basel). **11(7)**, Article ID 1163.
8. Øverland M, Mydland LT & Skrede A. (2019). Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. Journal of the Science of Food and Agriculure, **99(1)**,13–24.
9. Matos GS, Pereira SG, Genisheva ZA, Gomes AM, Teixeira JA & Rocha CMR. (2021). Advances in extraction methods to recover added-value compounds from seaweeds: sustainability and functionality. Foods,**10(3)**,516.
10. Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., & Zheng, B. (2019). Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. Molecules, **24(13)**, Article ID 2452.
11. Farzaei, M. H., Bahramsoltani, R., Abbasabadi, Z., Braidy, N., & Nabavi, S. M. (2019). Role of green tea catechins in prevention of age- related cognitive decline: pharmacological targets and clinical perspective. *Journal of Cllular Physiology*, **234(3)**, 2447-2459.
12. Zhang, P., Du, H., Wang, J., Pu, Y., Yang, C., Yan, R., & Yu, D. (2020). Multiplex CRISPR/Cas9- mediated metabolic engineering increases soya bean isoflavone content and resistance to soya bean mosaic virus. *Plant Biotechnology Journal*, **18(6)**, 1384-1395.
13. Kalinina, E. V., Chernov, N. N., & Novichkova, M. D. (2014). Role of glutathione, glutathione transferase, and glutaredoxin in regulation of redox-dependent processes. Biochemistry (Moscow), **13(79)**, 1562-1583.
14. El-Shora, H. M., El-Farrash, A. H., Kamal, H. and Abdelrazek, A. (2015). Enhancement of antioxidant defense system by UV-Radiation in fenugreek as a medicinal plant. *International Journal of Advanced* Research, **3**, 529-535.
15. Hoagland, D. R., and Arnon, D. I. (1950). The water-culture method for growing plants without soil. Circular. California Agricultural Experiment Station, 347 (2nd edit).
16. Ganesan, K., Kumar, K. S. & Rao, P. S. (2008). Antioxidant potential of solvent extracts of Kappaphycus alvarezii (Doty) Doty–An edible seaweed. Food chemistry, **107(1)**, 289-295.
17. Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, **10(3)** https://doi.org/10.38212/2224-6614.2748.
18. Jollow, D. J., Mitchell, J. R., Zampaglione, N. A., & Gillette, J. R. (1974). Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide

as the hepatotoxic metabolite. Pharmacology, **11(3)**, 151-169.
19. Hernández-Herrera, R. M., Santacruz-Ruvalcaba, F., Ruiz-López, M. A., Norrie, J., & Hernández-Carmona, G. (2014). Effect of liquid seaweed extracts on growth of tomato seedlings (Solanum lycopersicum L.). *Journal of Applied Phycology*, **26**, 619-628.
20. Hernández-Herrera, R. M., Santacruz-Ruvalcaba, F., Briceño-Domínguez, D. R., Filippo-Herrera, D., Andrea, D., & Hernández-Carmona, G. (2018). Seaweed as potential plant growth stimulants for agriculture in Mexico. Hidrobiológica, **28(1)**, 129-140.
21. Sohn, S.I., Rathinapriya, P., Balaji, S., Jaya Balan, D., Swetha, T.K., Durgadevi, R., Alagulakshmi, S. Singaraj, P & Pandian, S. (2021). Phytosterols in seaweeds: An overview on biosynthesis to biomedical applications. *International Journal of Molecular Sciences*, **22(23),** Article ID 12691.
22. Ali, O., Ramsubhag, A., & Jayaraman, J. (2021). Biostimulant properties of seaweed extracts in plants: Implications towards sustainable crop production. Plants, **10(3),** Article ID 531
23. Lola-Luz, T., Hennequart, F., & Gaffney, M. (2013). Enhancement of phenolic and flavonoid compounds in cabbage (Brassica oleraceae) following application of commercial seaweed extracts of the brown seaweed, (Ascophyllum nodosum). Agricultural and Food Science, *22*(2), 288-295.
24. Lola-Luz, T., Hennequart, F., & Gaffney, M. (2014). Effect on yield, total phenolic, total flavonoid and total isothiocyanate content of two broccoli cultivars (Brassica oleraceae var italica) following the application of a commercial brown seaweed extract (Ascophyllum nodosum). Agricultural and Food Science, **23(1),** 28-37.
25. Jaworowska, A., and Murtaza, A. (2022). Seaweed derived lipids are a potential anti-inflammatory agent: a review. International *Journal of Environmental Research and Public Health*, **20(1),** Article ID 730.
26. Patanè C., Pellegrino, A., Cosentino, S. L. & Testa, G (2023). Allelopathic Efeects of Cannabis sativa *L.* aqueous leaf extracts on seed germination and seedling growth in Durum Wheat and Barley. Agron., **13(2),** Article ID 454.
27. Grgić, J., Šelo, G., Planinić, M., Tišma, M., & Bucić-Kojić, A. (2020). Role of the encapsulation in bioavailability of phenolic compounds. Antioxidants,**9(10),** Article ID 923.
28. Kuźniak, E., and Kopczewski, T. (2020). The chloroplast reactive oxygen species-redox system in plant immunity and disease. Frontiers in Plant Science, **11,** Article ID 572686.
29. Amić, A., & Mastiľák Cagardová, D. (2023). A DFT study on the kinetics of HOO•, CH_3OO•, and $O_2^{\bullet-}$ Scavenging by quercetin and flavonoid catecholic metabolites. Antioxidants, **12(6),** Article ID 1154.
30. Subedi, L., Timalsena, S., Duwadi, P., Thapa, R., Paudel, A., & Parajuli, K. (2014). Antioxidant activity and phenol and flavonoid contents of eight medicinal plants from Western Nepal. *Journal of Traditional Chinese Medicine*, **34(5),** 584-590.
31. O'Byrne, D. J., Devaraj, S., Grundy, S. M., & Jialal, I. (2002). Comparison of the antioxidant effects of Concord grape juice flavonoids *α*-tocopherol on markers of oxidative stress in healthy adults. The American *Journal of Clinical Nutrition*, **76(6),** 1367-1374.
32. Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: location and functional significance. Plant Science, **196**, 67-76.
33. Gasperl, A., Zellnig, G., Kocsy, G., & Müller, M. (2022). Organelle-specific localization of glutathione in plants grown under different light intensities and spectra. Histochemistry and Cell Biology, **158(3),** 213-227.
34. El-Shora, H M & Abd El-Gawad. (2015). Physiological and biochemical responses of Cucurbita pepo L. mediated by

Portulaca oleracea L. allelopathy. Fresenius Environmental Bulletin. **24**, 386 - 393.
35. Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complementary and Alternative Medicine, **12**, 1-12.