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Impact of Algal Extract and Algal-Mediated Nanoparticles on Modulation of Secondary Metabolites Profile of *Coriandrum sativum* L.

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ABSTRACT
This research delves into the exploration of the impact of algal extract and algal-mediated nanoparticles, on the alteration of secondary metabolites composition of Coriandrum sativum L. Methodologically, Oscillatoria algae was collected and made as an extract, one exclusively algal extract and one with the synthesis of zinc nanoparticles. Coriandrum sativum seeds were sowed for germination and subsequently treated with algal extract, and algal extract-mediated nanoparticles. After sufficient growth, the entire plant was harvested for extraction and evaluation of secondary phytoconstituents, which included total phenols, terpenoids, and alkaloids. The research was also focused on assessing the antioxidant activity of treated plant sets. The findings revealed a significant difference in the plant metabolite concentrations. This research sheds light on both the algae and nanoparticles' ability to enhance metabolite concentrations in the plant and contributes to the existing body of knowledge in agriculture and agro-based plant growth mechanisms as well as the exploitation of high content of phytoconstituents to be employed in pharmaceuticals. Additionally, this study also acknowledges the limitations it faces in terms of its lack of diversity in applying to a variety of plant species. Nevertheless, this research advances our understanding of variations possible in the concentrations of plant metabolites under different treatments and sets the stage for future investigations in alternatives of harnessing crop yield in the agricultural domain and harvesting phytoconstituents at an amassing rate.
Keywords: *Algal extract, Oscillatoria, zinc nanoparticles, secondary metabolites, Coriandum sativum L.*

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INTRODUCTION

Coriandrum sativum, commonly known as coriander or cilantro, stands as a botanically marvelous plant with multifaceted attributes encompassing culinary, medicinal, and aromatic dimensions. Its cultural and historical significance spans centuries, and its ubiquitous presence in various global cuisines attests to its culinary importance. Beyond its flavoursome leaves and seeds, *Coriandrum sativum* harbors a rich repository of secondary metabolites and complex compounds that contribute to its diverse properties and responses to environmental stimuli [1]. This research embarks on a comprehensive exploration of the variations in secondary metabolites within *Coriandrum sativum*, particularly when subjected to growth conditions facilitated by algal extracts [2-3]. It also aims to understand the phytochemical modulations of the secondary metabolites in the plant promoted by algal nanoparticle extracts. Certain algal extracts used in agriculture systems have the potential to act as biostimulants and biofertilizers [4-5]. Nanotechnology shows considerable promise in the fields of biomedical, chemical, electronics, energy, and drug delivery applications. Nanoparticles are advantageous for plant growth, development, and protection. Nanoparticles bestow specificity in pesticide delivery, enhance nutrient supply, and manage pathogenicity, increasing the photosynthetic capacity and germination rate [6-7]. Until now, chemical and physical methods used to fabricate nanoparticles of different sizes and shapes are regarded as toxic and expensive, respectively. The biological systems that have shown potential for the fabrication of different nanoparticles include algae, plants, fungi, bacteria, etc. The shape and size of the nanoparticle produced remained the same regardless of the biological system used for its production. Algal extracts are well-suited to produce

nanoparticles due to their abundance. There are sufficient reports of the algal-mediated biosynthesis of nanoparticles [8-10) and their impact on plant growth and in shifting the plant metabolites profile [11-12]. Nanoparticles play a key role in enhancing drought stress tolerance in plants. Nanoparticles maintain membrane stability, induce the expression of stress-related proteins, improve nutrient and water uptake, increase photosynthesis, and increase grain yield and harvest index [13-15]. The algal extract utilized in this study was meticulously crafted through a systematic process involving collection, drying, powdering, and extraction using methanol. This method ensures the concentration of bioactive compounds derived from algae, which are known for their diverse secondary metabolite content [16]. Taking innovation a step further, the algal nanoparticle extract was utilized for zinc nanoparticle synthesis. This addition introduces a novel dimension, exploring the potential synergistic effects of algal extracts and nanoparticle-mediated responses in plants [17-18]. In contrast, the controlled growth sample undergoes cultivation under standard conditions, devoid of exposure to algal extracts or nanoparticles. This control group establishes a foundation for comparative analysis, enabling a nuanced understanding of how the introduction of algal and algal nanoparticle extracts influences the secondary metabolite repertoire of *Coriandrum sativum*. The culmination of the growth phase sees the harvested plants transformed into plant extracts and subsequently subjected to both quantitative and qualitative analyses to elucidate the intricate biochemical composition of *Coriandrum sativum*. Quantitative assessments encompass total phenolic, flavonoid, and terpenoid content, along with an evaluation of anti-oxidative properties using spectrophotometry. The amalgamation of advanced analytical techniques with traditional qualitative methods forms the bedrock of this research. The ensuing data is meticulously interpreted and visualized through scatter plot graphs, offering an accessible representation of the dynamic variations in secondary metabolites among the distinct treatment groups of *Coriandrum sativum* plants. Beyond the realms of fundamental plant science, the outcomes of this study may have profound implications for agriculture, pharmacology, and other interdisciplinary domains. Through unraveling the intricate relationships between these extracts and plant metabolites, this research aspires to open novel avenues for optimizing plant growth and harnessing the phytoconstituents for the therapeutic potential of *Coriandrum sativum* in diverse applications.

MATERIAL AND METHODS

Algal extract and algal-mediated nanoparticle preparation

Biomass of algae *Oscillatoria* was collected, rinsed, and washed to remove excess dirt and unwanted debris, and subsequently identified under a microscope for confirmation. They were then kept in the incubator at 38°C for three days for drying. The dried alga was powdered using a mechanical blender and sieved using a sieve; the bigger particles were further ground and made into a powder. 1 gm of algal powder was mixed with 100 ml of methanol and was kept in constant agitation in a mechanical shaker for 3 days. It was further kept in the dark for 24-48 hours. The mixture was filtered out using a Whatman no: 1 filter paper Typically, 10 ml of filtrate was used as an algal extract after raising the volume to 1000 ml with distilled water. To another 10 ml of filtrate, 900 ml of 1mM Zinc sulfate solution was added and kept in the dark for 24 hours to promote the synthesis of Zinc nanoparticles. The solution was continuously tracked for colour change and confirmation of nanoparticle synthesis absorbance was measured at 280-800 nm using a UV-Vis spectrometer for absorbance peak between 320-360 nm, specific for ZnO NPs (Zinc oxide nanoparticles) [19-20].

Treatment of seedlings with algal extract and algal-mediated zinc nanoparticles

Three different green bags suitable for plant growth, marked “Control”, “Algal Extract”, and “Algal Nanoparticle Extract” were taken and filled with an equal amount of soil with a uniform concentration of garden soil, vermicompost soil, granules, and cocopeat. Seeds of coriander were taken in equal amounts in three different containers and soaked overnight for better germination. The soaked seeds in the morning were pat dried and crushed gently into two halves and spread over the soil of the three different bags. They were gently pressed and were further layered by another coat of soil. 40 ml of algal extract to one pot and 40 mL of algal nanoparticle extract to another pot was added every day along with water. For standard usage and comparison, the third pot with the seeds named “Control” was grown with water only. After optimum growth was reached after 25 days, they were appropriately harvested and transferred into different plastic bags (Figures 1 and 2).

Preparation of plant extract

The entire *Coriandrum sativum* from all three treatments (Control (A1), Algal extract treated (A2), and Algal mediated nanoparticles treated (A3)) were placed in the incubator for drying. After three days at 38^{o}C, the dry weight was noted. The dry plant samples were added into a mechanical blender and powdered. The powdered plant samples were then sieved and the bigger residues were ground again into a fine powder. 1 g of dry plant powder of the treatment group was dissolved in 100 ml of methanol (Figure 3). The plant

samples were kept in a shaker under warm conditions for three days for constant agitation. After three days, all three samples were filtered in different conical flasks using Whatman's no: 1 filter paper. The methanol was evaporated to make a dry residue which was dissolved in 100 ml of methanol to make a final concentration of 1mg/ml.

Qualitative Analysis for Phytochemicals

Qualitative phytochemical screening was performed by following standard methods [21].

Tannins

To a few drops of the plant extract, a few drops of alcoholic 0.1% ferric chloride were added to observe brownish-green or blue-black coloration indicative of the presence of tannins.

Flavonoids

Approximately 3 ml of a diluted ammonia solution was introduced into 2 mL of plant extract for each sample. Subsequently, 1 ml of concentrated sulphuric acid (H_2SO_4) was added. The appearance of a yellow hue in each extract indicated the presence of flavonoids.

Terpenoids

To test the presence of terpenoids, the Salkowski test was employed. 3 ml of plant extract was added to 1 ml of chloroform and 1 ml of H_2SO_4. An intense red-brown coloration formed was indicative of the presence of terpenoids.

Phenols

A ferric chloride test is done for the test of phenols. To a few drops of the sample, a few drops of 5% Ferric chloride were added. A dark blue coloration obtained confirms the presence of phenolic compounds.

Alkaloids

Dragendorff's test was conducted to detect the presence of alkaloids. 1 ml of Dragendorff''s reagent was added to 2 ml of extract, and an orange-red precipitate formation indicates the presence of alkaloids.

Saponins

The I ml of plant extract was diluted to 6 ml by adding distilled water and shaken vigorously. The extract was observed for the formation of persistent foam indicating the presence of saponins.

Quantitative Analysis

Total phenolic content

The methanolic plant extract (1 ml) was transferred into a test tube and raised to a 3 ml volume with distilled water. To this 0.5 ml of 10% Folin-Ciocalteu reagent. The solution was mixed well and incubated at room temperature in the dark for 3 minutes followed by the addition of 2 ml of 2% Sodium carbonate and incubation at room temperature for 30 minutes. The optical absorbance was then taken at 765 nm using a UV-Vis spectrophotometer. The same procedure was used for all three samples. Gallic acid was used as a standard. The phenol content present was expressed in micrograms of gallic acid equivalent to 1 mg of extract (mg GAE/g dry extract) [22].

Total flavonoid content

To 1 ml of methanolic plant extract, 1 ml of 10% Aluminium chloride was added and mixed well. Furthermore, 1 ml of 1M of Sodium acetate was added and incubated in the dark at room temperature for 40 minutes. The solution was mixed properly and the absorbance was taken at 420 nm using a UV-Vis spectrophotometer. A similar procedure was applied to all three samples. Quercetin was used as a standard. The Total flavonoid content was shown in micrograms of quercetin equivalent to 1 g of extract (mg QE/g dry extract) [23].

Total terpenoid content

The estimation of total terpenoid content was conducted using the standard procedure described in the literature with minor modifications [24]. To 1 ml of the plant extract, 2 ml of chloroform was added and vortexed vigorously for 3 minutes and left for another 3 minutes. Furthermore, 200µl of conc. H_2SO_4 was added to the solution and kept in the dark for incubation for 1.5-2 hours. A reddish-brown precipitate was obtained when removed, after which the supernatant was carefully discarded, and 3 ml of methanol was added to the precipitate and was vortexed until the precipitate completely mixed with the methanol. The absorbance was measured at 530 nm with a UV-Vis spectrophotometer. The total terpenoid content of the plant sample was calculated as mg of linalool per gram of extract using a regression equation obtained from the linalool calibration curve.

Antioxidant activity-DPPH assay

Three different concentrations of each plant extract (Control, Algal extract treated, Algal mediated nanoparticles treated); 100 µl, 200µl, and 300µl, were taken separately and were raised to 3 ml using methanol as solvent. To this, 1 ml of 0.004% of DPPH (1,1-diphenyl-2-picrylhydrazyl) was added and kept in the dark for 30 minutes. After removing it from incubation, absorbance was measured at 530 nm. Ascorbic acid was used as standard. The DPPH along with methanol was used as the solution for control.

Pure methanol was used as a blank. The formula for calculating DPPH radical scavenging activity is given below [25].

DPPH radical scavenging activity (%)

$$= \frac{\text{Absorbance of control - Absorbance of treated}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

The results of our research reveal compelling insights into how chemically efficient plant biochemicals can influence plant growth. Through rigorous analysis and experimentation, we uncovered that with the appropriate extract for administration for plant growth, there is a significant change in phytochemical concentrations, shedding light on the potential production of cheaper biofertilizers. Notably, our data elucidates the importance of improvisation in plant quality, especially in a competitive and more demanding world, underscoring the significance of our study. These results provide valuable implications in the fields of Nanoscience and agro-based mycology, suggesting avenues for further exploration and potential real-world applications. Overall, the findings advance knowledge in using biofertilizers and offer valuable contributions to the scientific community.

Synthesis of algal-nanoparticles

The colour of the solution changed slowly from brown to light yellow during the reaction which turned to white on 4th day of inoculation, indicating the formation of ZnO nanoparticles. Secondary metabolites present in algal extract reduce the zinc ions present in the zinc oxide solution. The absorption peak maximum was observed at 358 nm which is specific for zinc oxide nanoparticles

Qualitative analysis

The results for the presence of various phytochemical tests are tabulated in Table 1. The results show that the plant *Coriandrum sativum* is immensely rich in flavonoids, terpenoids, alkaloids, tannins, and phenols.

Quantitative analysis

Total phenol content, flavonoid content, and terpenoid content are presented in Table 2.

The regression equation obtained from the standard graph was used to calculate the total phenolic content, flavonoid content, and terpenoid content. (Figures 4, 5, and 6). In each regression equation, Y is the absorbance and X is the concentration. The coefficient of X is the slope. The constant is the intercept.

Results show that there is a significant increase in the amount of phenol content in algal extract-grown plants (73.88±5.04 mg/g) followed by algal nanoparticle extract-grown plants (59.60±3.12 mg/g) when compared to the control set of plants. All three sets of plant samples exhibited a modest amount of flavonoid content displaying an insignificant variation. The values obtained for total terpenoid content are significantly higher in algal extract-grown plants (3.30±1.05 mg/g) while very close values, 1.71±0.28 and 1.42±0.31mg/g were recorded for algal nanoparticle extract-grown plants and a controlled set of plants respectively (Figure 7).

Anti-oxidant activity- DPPH assay

The DPPH method with an organic radical, 1,1-diphenyl-2-picrylhydrazyl was applied to determine the free radical scavenging activity and expressed in the percentage of radical scavenging activity (Table 3), which is interpreted as the amount of antioxidants present in the respective plants grown in different extract. All three samples exhibited a high percentage of radical scavenging activity at the lowest concentration (100 µg). The algal nanoparticle extract-treated plant shows more radical scavenging activity than that of the control set of plants followed by the algal extract-treated group (Figure 8). The findings of this study suggest a notable disparity in plant metabolite concentrations when subjected to extracts derived from algae and algae-zinc nanoparticles. The observed increase in metabolite concentration hints at the potential of these extracts to influence the biochemical pathways within *Coriandrum sativum*. The distinctiveness in the composition of these extracts, one being algae-derived and the other incorporating algae-zinc nanoparticles, underscores the complexity of plant-nutrient interactions [26]. This variation in metabolite concentrations could stem from a range of factors, including the unique biochemical composition of the extracts, their mode of action within the plant, and the specific metabolic pathways affected [27]. The antioxidant activity conducted using different plant extracts suggests that ZnO NPs are fairly efficient in neutralizing the adverse effect of free radicals. The exact mechanism of nanoparticles-mediated antioxidant activity is yet to be decoded but few studies have proved that ZnO NPs activate the intracellular enzymatic and non-enzymatic antioxidant system [28]. The plethora of phytoconstituents such as phenols, flavonoids, and terpenoids on the surface of nanoparticles is also associated with their high free radicals scavenging activity [29].

Furthermore, this research contributes to our understanding of agricultural practices by highlighting potential strategies for enhancing plant metabolite production. By demonstrating the efficacy of algae and algae-zinc nanoparticle extracts in augmenting metabolite concentrations, this study offers insights into

alternative methods for promoting plant growth and productivity. These findings not only expand our knowledge of plant biochemistry but also hold promise for the development of cost-effective and sustainable approaches to agricultural management [30]. In addition to this, it is plausible enough to state that though with the presence of either algal extract or algal nanoparticle extract, the plant showed a significant difference chemically when compared with the control group plant as metabolomic studies support the fact that nanoparticles modulate reactive oxygen or nitrogen species and signaling pathways hence affecting the concentration of secondary metabolites [31]. It is noteworthy to also consider the insignificant divergence of the phytochemical data of the algal nanoparticle extract treated test group from the control group of plants. This might be speculated to be a result of various reasons, the higher concentration in which the nanoparticle extract was prepared being the most important of all. However, it is essential to acknowledge the study's limitations, particularly its focus on a single plant species. The research also puts forth another hypothesis that a higher concentration of nanoparticles might inhibit the extensive growth of the plant [32], thus serving as a limitation for this study.

Figure 1: Coriander plants grown using algal extract and algal-mediated nanoparticles

Figure 2: Harvested coriander plants

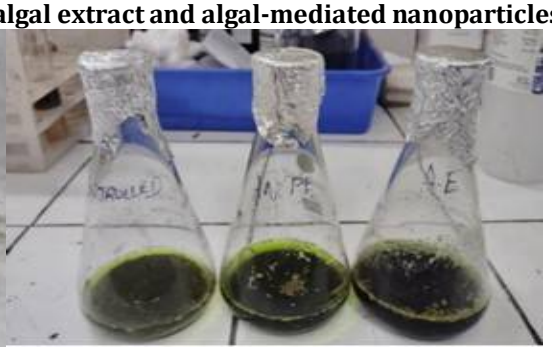

Figure 3: Dried plant sample in methanol

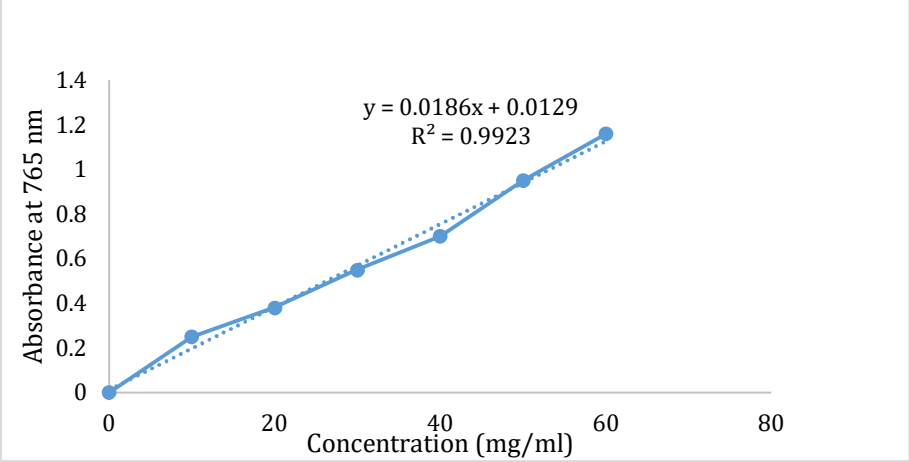

Fig 4: Standard curve of gallic acid

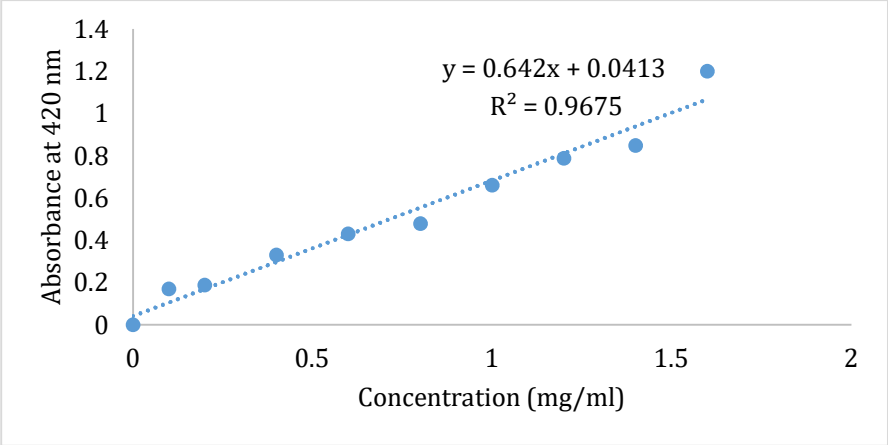

Fig 5: Standard curve of quercetin

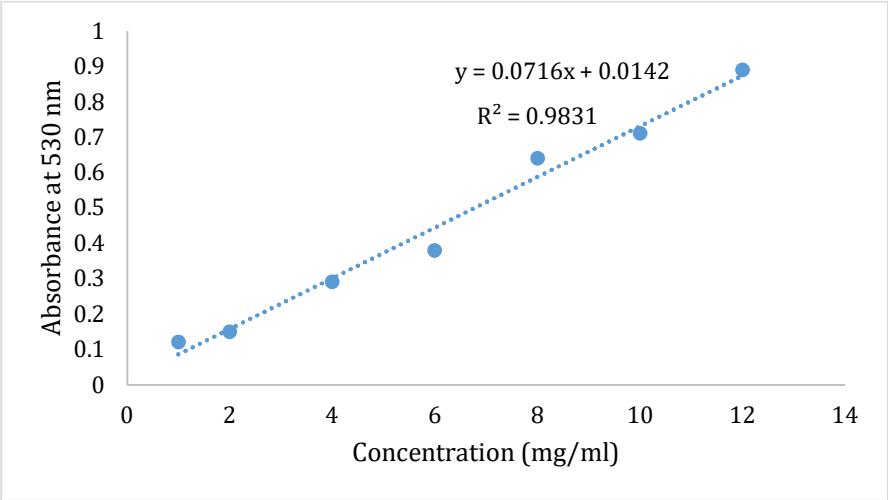

Fig 6: Standard curve of linalool

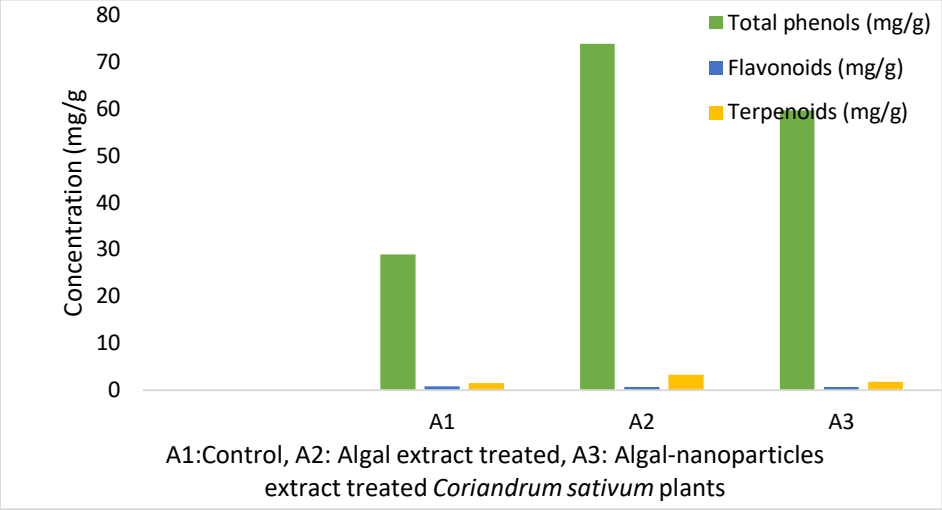

Figure 7: Total Phenols, Flavonoids, and Terpenoids content in different test groups of *Coriandrum sativum*

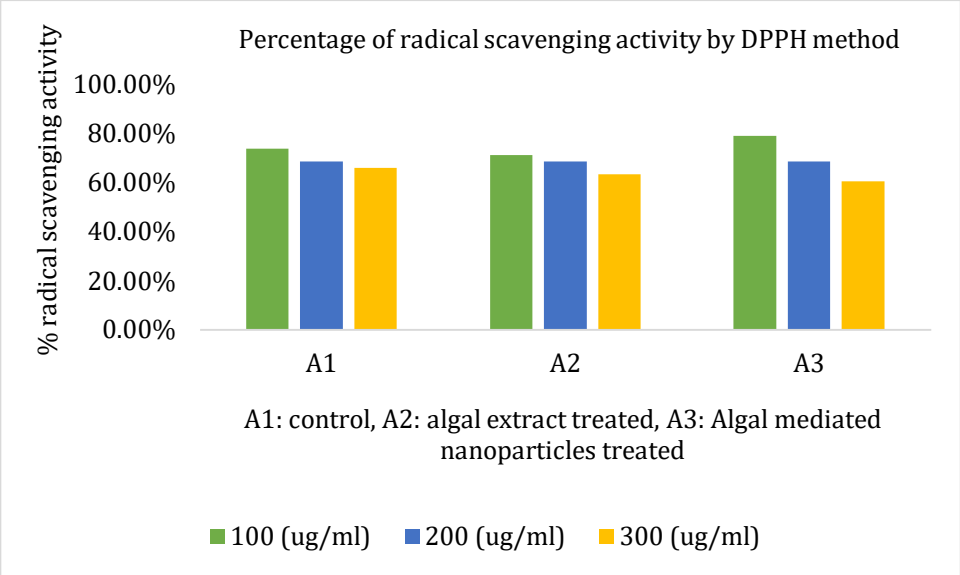

Fig 8: Percentage of radical scavenging activity by DPPH method

Table 1: Phytochemical Test

S.No.	Secondary metabolite	Inference
1	Alkaloid (Dragendorff's test)	+
2	Flavonoid	+
3	Terpenoid (Salkowski test)	+
4	Tannins (Ferric chloride test)	+
5	Phenols (Ferric chloride test)	+
6	Saponins	-

(+ = present, - = absent)

Table 2: Total Phenols, Flavonoids, and Terpenoids content

S. No.	*Coriandrum sativum* test group	Plant part	Total phenols (mg/g)	Flavonoids (mg/g)	Terpenoids (mg/g)
1	Control	Entire	28.90±3.35	0.78±0.10	1.42±0.31
2	Algal extract treated	Entire	73.88±5.04	0.71±0.12	3.30±1.05
3	Algal nanoparticle extract treated	Entire	59.60±3.12	0.71±0.09	1.71±0.28

Values represent Mean±SD (n=3)
(SD: Standard deviation, n=no. of observations for each set)

Table 3: Percentage of radical scavenging activity

S. No.	Concentration (µg/ml)	A1 (Control)	A2 (Algal extract treated)	A3 (Algal nanoparticles extract treated)
1	100	73.68±0.45	71.05±0.85	78.94±1.45
2	200	68.42±1.50	68.42±0.34	68.42±0.67
3	300	65.78±1.7	63.15±0.12	60.52±0.50

Values represent Mean±SD (n=3)
(SD: Standard deviation, n=no. of observations for each set)

CONCLUSION

The current study offers insight into the use of algal extracts and green synthesized nanoparticles to amend the phytochemical constitution of medicinal and crop plants. Future research should explore the

applicability of these findings across a broader range of plant species to establish their utility in diverse agricultural contexts.

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AUTHOR CONTRIBUTION STATEMENT
Babita Rana has guided the complete research work, interpreted data, and contributed to the drafting of the manuscript. Vedantha has executed the experimental work and compiled the whole data.

DATA AVAILABILITY STATEMENT
All the data is available in the manuscript. However, if additional or raw data is required then it will be made available upon request.

CONFLICT OF INTEREST
Authors declare no conflict of interest in terms of content and writing of the manuscript.

DECLARATION OF COMPETING INTEREST
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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