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Solvent-dependent Antibacterial Activity of Brown Algal Extracts Against *Agrobacterium tumefaciens* and Suppression of Crown Gall Disease

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Abstract: Crown gall disease, caused by *Agrobacterium tumefaciens*, is a destructive bacterial disease affecting numerous dicotyledonous crops and remains difficult to manage using conventional control strategies. Marine brown algae are rich in bioactive metabolites with antimicrobial properties and represent as promising source of environmentally sustainable disease management agents. This study evaluated the antibacterial activity of ethanol, water, and benzene extracts of *Turbinaria ornata* and *Padina pavonica* brown algae against *A. tumefaciens* 27AS_Pp4 (OK559740) under *in vitro* and *in vivo* conditions and quantified major phytochemical constituents. To our knowledge, limited information is available regarding the activity of *T. ornata* and *P. pavonica* extracts against *A. tumefaciens*. All extracts inhibited the growth of *A. tumefaciens*, with efficacy depending on both algal species and extraction solvent. The ethanolic extract of *T. ornata* showed the greatest antibacterial activity, producing the largest inhibition zone (25.7 ± 0.9 mm) at concentration 10 mg /ml, followed by the ethanolic extract of *P. pavonica* (22.3 ± 0.3 mm). Both ethanolic extracts showed the lowest minimum effective concentration (MEC) (0.0391 mg/ml). In tomato pot experiments, the ethanolic extract of *T. ornata* completely inhibited gall formation (100% disease reduction), whereas ethanolic extract of *P. pavonica* achieved 93.75% disease reduction, and *T. ornata* ethanol extract markedly improved tomato plant growth. Ethanolic extracts showed the highest contents of phenolics, flavonoids, tannins, saponins, and alkaloids. These findings indicate that solvent selection markedly influences the antibacterial activity of brown algal extracts. The ethanolic extract of *T. ornata* showing the greatest potential for sustainable crown gall management.

Key words: crown gall; *Turbinaria ornata*; *Padina pavonica;* bioactive metabolites; sustainable disease management

1. Introduction

Crown gall disease, caused by the soil-borne bacterium *Agrobacterium tumefaciens*, is one of the most important bacterial diseases affecting a wide range of dicotyledonous plants. The disease is characterized by the formation of tumor-like swellings, resulting from the pathogen's ability to induce neoplastic growth in infected tissues. It is considered a wound-associated disease and represents a major constraint in the production of several economically important crops, particularly stone fruits. Peach and cherry rootstocks are among the most susceptible hosts, where crown gall infection can result in considerable economic losses **(Escobar and Dandekar, 2003; Sharma *et al*., 2004; Pulawska, 2010)**. Several approaches have been used to manage crown gall disease, including chemical treatments, antibiotics, and biological control strategies. However, chemical compounds and antibiotics often provide only partial suppression of disease and may cause phytotoxic effects **(Canfield and Moore, 1992)**. Therefore, biological control has received considerable attention as an environmentally compatible approach for managing bacterial plant diseases **(Amusa, 2005)**. Several biological agents have been investigated for the suppression of plant pathogenic bacteria, including *Agrobacterium radiobacter* K84, *Pantoea agglomerans*, and *Bacillus subtilis*, which have demonstrated effectiveness in reducing crown gall development **(Velázquez *et al*., 2010; Abd-El-Aziz, 2011; Tolba and Soliman, 2013)**. In addition to microbial antagonists, plants-derived natural products have attracted attention as potential alternatives for managing bacterial diseases. Essential oils from medicinal plants have demonstrated antibacterial activity against plant pathogenic bacteria and have shown considerable potential for disease suppression. For example, essential oils extracted from caraway (*Carum carvi* L.) and thyme (*Thymus vulgaris* L.) were reported to inhibit the development of *A. tumefaciens* under laboratory and greenhouse conditions, thereby reducing its pathogenic effects **(Abd-El-Aziz *et al*., 2021)**.

Marine macroalgae have recently gained considerable attention as promising natural sources of biologically active compounds for plant disease management. These plant-like organisms are widely distributed in coastal ecosystems and produce a diverse array of secondary metabolites contains antibacterial, antifungal, antioxidant, and other biological activities products, making them attractive candidates for controlling plant pathogenic microorganisms **(Bhadury and Wright, 2004; Saleh and Al-Mariri, 2017; Hamed *et al*., 2018; Ishmayana *et al*., 2025)**. Different solvent extracts of marine algae have exhibited inhibitory effects against a wide range of phytopathogenic bacteria. For example, extracts of *Sargassum wightii*, *Padina boergesenii*, and *Turbinaria conoides* showed antibacterial activity against *Pseudomonas syringae*, while *Sargassum polyceratium* exhibited activity against several bacterial species, including *Staphylococcus aureus*, *Pectobacterium carotovorum* and *Escherichia coli* **(Kumar *et al*., 2008)**. Additionally, *Sargassum swartzii* extracts inhibited the growth of *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial blight of rice **(Arunkumar *et al*., 2005)**. Furthermore, brown seaweed extracts of *Cystoseira myriophylloides*, *Laminaria digitata*, and *Fucus spiralis* were reported to reduce tomato crown gall disease, which was associated with their antioxidant properties **(Esserti *et al*., 2017)**. Among marine algae, brown algae have received particular attention because of their abundance of bioactive natural substances and their potential applications in the control of plant pathogenic bacteria **(Ara *et al*., 2002; Abd-El-Aziz, 2020)**. The biological activities of brown algae are largely attributed to their rich chemical composition, including fucoidan, laminarin, alginates, phlorotannins, phenolic acids, flavonoids, fucoxanthin, fatty acids, fucosterol, vitamins, and minerals, which have been associated with antioxidant, anticancer, and antimicrobial properties **(Barahona *et al*., 2025)**. Recent studies have highlighted the agricultural applications of seaweed extracts, not only as antimicrobial agents but also as materials used for improving soil conditions, enhancing nutrient uptake, and increasing crop productivity **(Chiheb *et al*., 2009; Abdel-Raouf *et al*., 2012; Farag *et al*., 2017)**. It's well known that the biological activity of algal extracts is strongly influenced by both the extraction method and the solvent used, as different solvents vary in their ability to extract specific classes of bioactive compounds. Previous studies have demonstrated that alcoholic solvents can provide higher extraction yields of secondary metabolites with high biological activities compared with other solvents **(Generalić Mekinić *et al*., 2021)**. Similarly, variations in antibacterial activity among algal extracts have been attributed to differences

in solvent polarity as well as the type and quantity of extracted compounds **(Kumar *et al*., 2008; Ibrahium *et al*., 2020)**.

In spite of several studies have investigated the antimicrobial properties of marine algae against different plant pathogens, but limited information is available regarding the activity of *T. ornata* and *P. pavonica* extracts against *A. tumefaciens*. Therefore, the present study aimed to evaluate the antibacterial activity of different solvent extracts of *T. ornata* and *P. pavonica* against *A. tumefaciens* under *in vitro* and *in vivo* conditions and to investigate the relationship between their antibacterial activity and the phytochemical constituents of the produced algal extracts.

2. Materials and Methods

Source of algae

Two marine brown algal species, *T. ornata* and *P. pavonica,* were used in this study. Both species belong to the class Phaeophyceae: *T. ornata* to the family Sargassaceae, and *P. pavonica* to the family Dictyotaceae. The algal samples (approximately 3kg) were kindly provided by the National Institute of Oceanography and Fisheries (NIOF), Hurghada, Red Sea Governorate, Egypt. The algal samples were washed thoroughly with seawater followed by distilled water to remove adhering salts, sand, and epiphytic organisms. The samples were then shade-dried at room temperature until reaching a constant weight. By the aid of an electric blender the dried algal materials were ground into a fine powder and stored in airtight containers until further extraction.

Extraction of algae

Three solvents, namely benzene, ethanol, and distilled water, were used for extraction bioactive compounds from the algal materials. The extraction process was performed according to the methods described by **Hellio *et al*. (2001)** and **Hamed and Messiha (2018)**. Five hundred grams of each dried algal powder were soaked separately in 3 L of tested solvent at room temperature for 72 h. Obtained extract solutions were filtered through Whatman No. 1 filter paper. The obtained filtrates were concentrated under reduced pressure using a rotary evaporator (Senco, China), and the concentrated extracts were further air-dried at room temperature until constant weight. The dried crude extracts were stored at 4 °C in sealed containers until further use.

Source of *A. tumefaciens* isolate

The virulent isolate of *A. tumefaciens* 27AS_Pp4 (OK559740) used in the study was previously isolated from infected peach and identified by **Abd-El-Aziz *et al.* (2021)**.

Preparation of bacterial suspensions

A. tumefaciens 27AS_Pp4 (OK559740) isolate was inoculated on Petri dishes containing King's B agar medium (20 g proteose peptone; 1.5 g MgSO4; 1.5 g K_2HPO_4; 10 ml glycerol; 20 g agar; distilled water to 1000 ml; pH was adjusted to 7.2). The plates were incubated for 48 hours at 28 ± 1 °C. After that, bacterial growth was harvested, and the obtained suspension was diluted with sterile distilled water until the optical density of the tested isolate reached at 600 nm (OD600) 0.1 (corresponding approximately to 10^8 CFU/ml) **(Daly *et al*., 2017)**.

Inhibitory activity of algae extracts on *A. tumefaciens* 27AS_Pp4 *in vitro*

The antibacterial activity of *P. pavonica* and *T. ornata* extracts against *A. tumefaciens* 27AS_Pp4 was evaluated using the filter paper disc diffusion technique. Disks of 5-mm diameter (Whatman standard filter paper discs, No.3) were soaked in each extract (10 mg extract/ml of 0.5% dimethyl sulfoxide (DMSO)), and then placed on King's B agar medium previously inoculated with the bacterium (1 ml of 10^8 CFU/ml was added to 250 ml of medium before hardening). Three replicates for each treatment were used. The plates were left at room temperature for 1 h to allow diffusion of the algal extracts into the medium, and then sealed with Parafilm. After 24 h of incubation at 28 °C, the antibacterial activity was measured as the diameter of the appearing inhibition zone

around each disk and compared with that of control disks soaked in 0.5% DMSO **(Vasinauskiene *et al.*, 2006; Esserti *et al.*, 2017)**.

Minimum effective concentration (MEC)

The extract concentrations (10 mg extract/ml of 0.5% DMSO) were diluted serially to reach 5, 2.5, 1.25, 0.625, 0.3125, 0.1563, 0.0781, 0.0391, and 0.0195 mg/ml **(Alsenani *et al.*, 2020)**. In agar plates previously inoculated with *A. tumefaciens* 27AS_Pp4 cultures, three wells with a diameter of 0.5 cm were made in each plate and filled with 40 µl of each tested extract concentration. Three plates were used for each algal extract. The MEC was defined as the lowest tested concentration of the extract that produced a detectable inhibition zone against *A. tumefaciens* 27AS_Pp4 after 24 h of incubation at 28 ± 1 °C. Microbial growth inhibition was determined by measuring the diameter of the inhibition zone around each well **(Hassanein and Eldoksch, 1997; Velmurugan *et al.*, 2012; Hamed and Hussein, 2020)**.

Inhibitory activity of algae extracts on *A. tumefaciens in vivo*

Tomato plants (cv. Super Strain B) 3 weeks old were used (one plant per pot, 20 cm in diameter, containing 2 kg of soil). Each treatment was replicated five times. By the aid of scalpel all plants were wounded in the crown region before they were inoculated with the bacterium. A 0.31 mg amount of each extract was dissolved in 1 ml of 0.5% DMSO, and treatments were applied as follows: (1) Soil infested with *A. tumefaciens* 27AS_Pp4 (1×10^8 CFU/ml; 50 ml/pot), with another 50 ml/pot of 0.5% DMSO added to the pot as the positive control. (2) Soil inoculated with *A. tumefaciens* 27AS_Pp4 (1×10^8 CFU/ml) and treated with algal extracts at a total volume of 100 ml/pot (v:v; 50 ml pathogen suspension + 50 ml algal extract). (3) Soil was treated with 50 ml/pot of 0.5% DMSO and 50 ml/pot of sterile distilled water to serve as the negative control. Disease readings were recorded after 45 days of inoculation and were rated based on the number and weight of galls as well as plant growth characteristics (shoots and roots/ plant and length of plant). Disease reduction (%) was calculated based on the reduction in the average number of galls per plant relative to the positive control, using the following formula:

Disease reduction (%) = [(number of galls in the positive control − number of galls in the treatment) / number of galls in the positive control] × 100.

Estimation of phenolic compounds from algae

The tested two algae were extracted with water, ethanol, and benzene. The dried residue was dissolved in 50% isopropanol and kept in the freezer until analysis. The extracts were used for subsequent analysis of phenolic compounds. To determine the content of total and free phenolic compounds, the Folin–Ciocalteu method was used according to (**Simons and Ross, 1971)**. For free phenolic compounds, diluted Folin–Ciocalteu reagent, 20% sodium carbonate, and water were added to an aliquot of the extract. For total phenolic compounds, the extract was first hydrolyzed with concentrated hydrochloric acid for 15 minutes at boiling temperature. It was then cooled, and 20% sodium carbonate, water, and diluted Folin–Ciocalteu reagent were added. Total and free phenolic compound contents as mg/g were determined spectrophotometrically at 520 nm, using catechol as a standard.

Estimation of flavonoid compounds from algae

Total flavonoid content (TFC) was determined as described by **Zhishen *et al.* (1999)**. The reaction was measured at 510 nm using a spectrophotometer (Spectronic 601). The results were expressed as mg quercetin equivalents (QE)/g of extract.

Estimation of tannins, saponins, and alkaloids from algae

Total tannin content (TTC)

Total tannin content was determined according to **Peri and Pompei (1971)** and **Wizi *et al.* (2022)**. One ml of extract was combined with one ml of distilled water. Then, 0.5 ml of Folin's phenol reagent (1:2) was added, followed by 5 ml of 35% sodium carbonate solution, and the mixture was

allowed to stand for 5 min. The color intensity was measured at 640 nm. The tannin content was expressed as mg of tannic acid equivalents/g of extract.

Total saponin content

Total saponin content was assessed using a modified version of the vanillin-sulphuric acid colorimetric reaction according to **(Le *et al.*, 2018)**. The results were quantified as diosgenin equivalents (mg DE/g extract) using a standard curve.

Alkaloid content

The alkaloid content was estimated in algal extracts according to **Martinez Nadal *et al*. (1963)** and **Prabakaran *et al.* (2018)**. One ml of each extract (1 mg/ml) was dissolved in water, followed by the addition of 0.1 ml of FeCl3 (2.5 mM FeCl3 in 0.5 M HCl) and 0.1 ml of 1,10-phenanthroline. The mixture was incubated for 30 min at 70 °C, and the absorbance was measured at 500 nm. Total alkaloid content was expressed as mg of gallic acid equivalents (GAE).

Statistical analysis

The normality of data distribution was assessed using the Shapiro–Wilk test. For datasets meeting the assumption of normality (plant characteristic parameters), one-way analysis of variance (ANOVA) was employed to determine significant differences between treatments, according to **Montgomery (2017).** In cases where the ANOVA yielded significant results, Tukey's honestly significant difference (HSD) post hoc test **(Tukey, 1949)** was applied for pairwise multiple comparisons to identify specific treatment differences while controlling for Type I error.

For datasets that violated the normality assumption (pathological parameters), the non-parametric Kruskal–Wallis H test was utilized. Significant findings were further analyzed using Dunn's post hoc test **(Dunn, 1964)** with Bonferroni correction to adjust for multiple comparisons. A P-value < 0.05 was considered statistically significant for all tests.

3. Results

Inhibitory activity of algae extracts on *A. tumefaciens in vitro*

The antibacterial activity of the algal extracts against *A. tumefaciens in vitro*, as shown in Table 1 and Fig. 1, was demonstrated by the inhibition zones formed around the disks soaked in each tested extract. The largest inhibition zone was recorded for the *T. ornata* ethanol extract (25.7 ± 0.9 mm), followed by *P. pavonica* ethanol and *T. ornata* water (both 22.3 ± 0.3 mm), and *P. pavonica* water (18.7 ± 0.7 mm). The smallest inhibition zones were produced by the benzene extracts of *T. ornata* and *P. pavonica* (13.0 ± 0.6 and 15.0 ± 0.6 mm, respectively).

Table (1). Diameter of the inhibition zones (mm) for different extracts of *Padina pavonica* and *Turbinaria ornata* algae against *Agrobacterium tumefaciens* 27AS_Pp4 *in vitro*

Algae	Extracts	Inhibition zone (mm)
P. pavonica	**Benzene**	15.0 ± 0.6
	Ethanol	22.3 ± 0.3
	Water	18.7 ± 0.7
T. ornate	**Benzene**	13.0 ± 0.6
	Ethanol	25.7 ± 0.9
	Water	22.3 ± 0.3
Dimethyl sulfoxide (DMSO) 0.5% (control)		0.0 ± 0.0

Values are presented as mean ± standard deviation (SD) (n = 3).

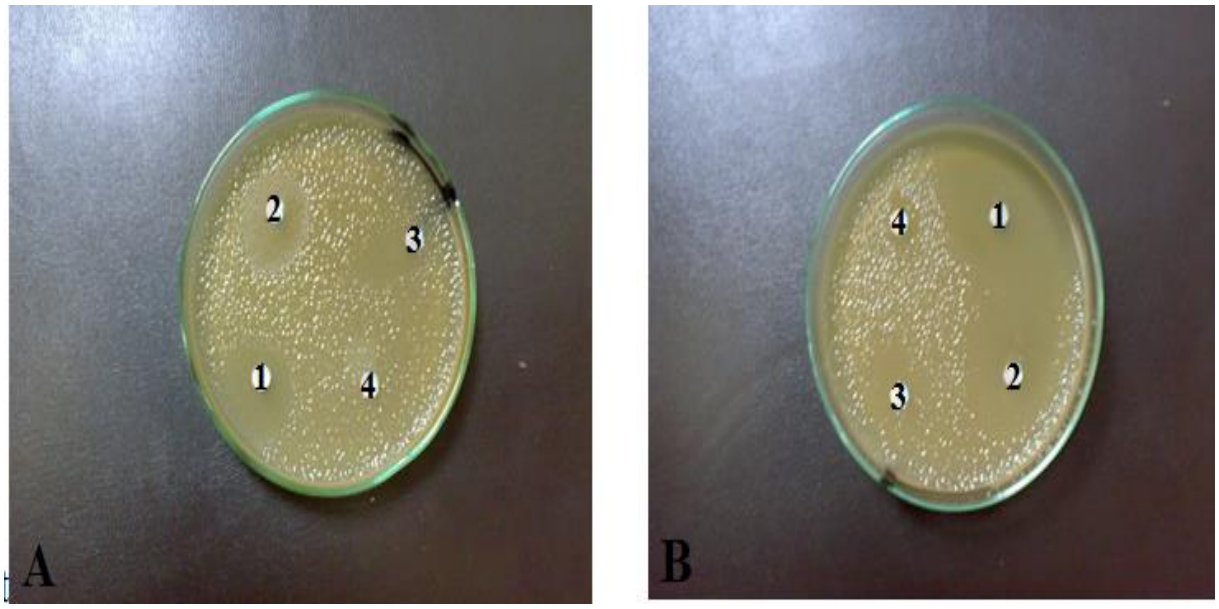

Fig. (1). Inhibition zones produced by different extracts of *Padina pavonica* and *Turbinaria ornata* algae against *Agrobacterium tumefaciens in vitro*.
A: *Padina pavonica*; B: *Turbinaria ornata*. 1- Ethanol extract, 2- Water extract, 3- Benzene extract, and 4- Control.

Estimated minimum effective concentration (MEC)

The MEC of the algal extracts against *A. tumefaciens* 27AS_Pp4 was determined (Figs. 2–4). The lowest MEC was recorded for the ethanol extracts of both algal species (0.0391 mg/ml), followed by the water extracts (0.0781 mg/ml), whereas the benzene extracts showed the highest MEC (0.1563 mg/ml).

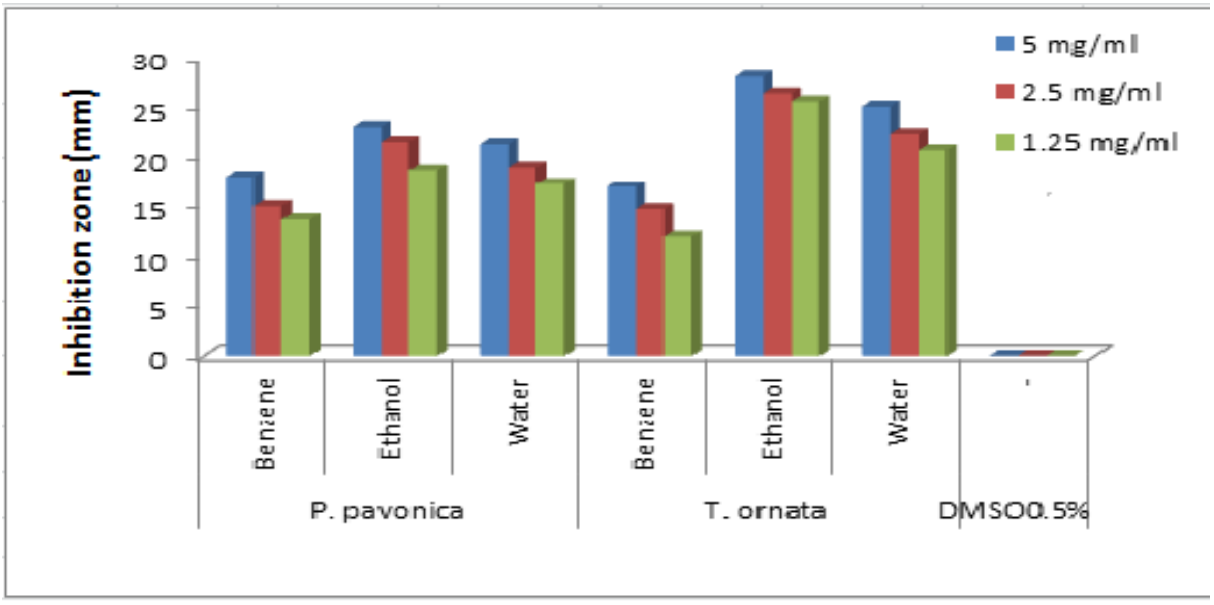

Fig. (2). Inhibition zones (mm) produced by different concentrations of algal extracts (5.00, 2.50, and 1.25 mg/ml). *P. pavonica (Padina pavonica)*. *T. ornata* (*Turbinaria ornata)*. DMSO (dimethyl sulfoxide)

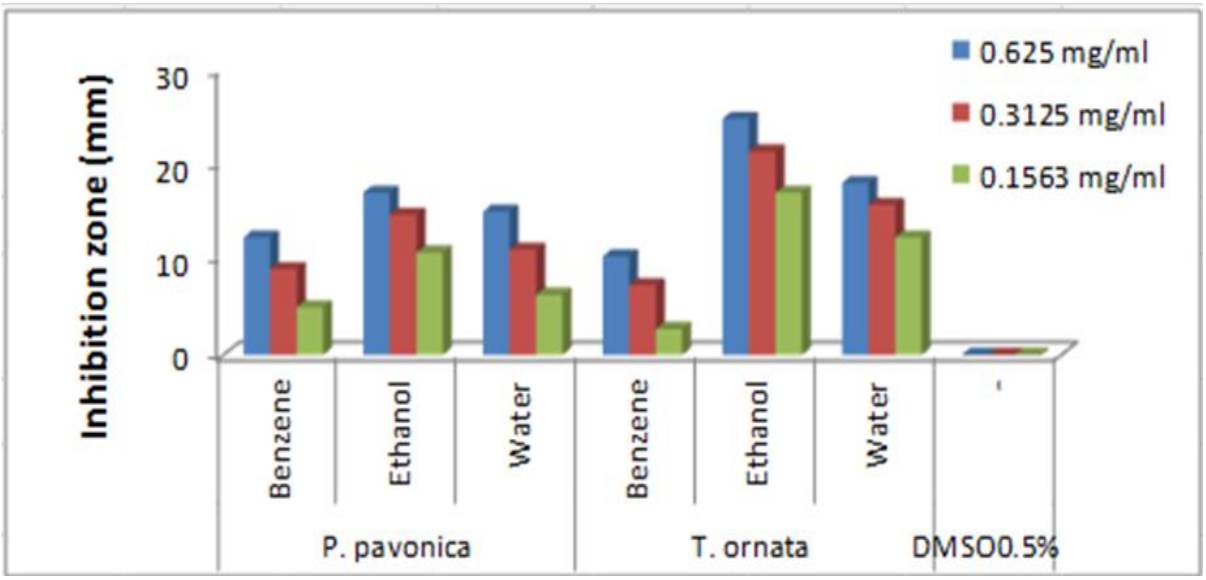

Fig. (3). Inhibition zones (mm) produced by different concentrations of algal extracts (0.6250, 0.3125, and 0.1563 mg/ml). *P. pavonica (Padina pavonica)*. *T. ornata* (*Turbinaria ornata)*. DMSO (dimethyl sulfoxide)

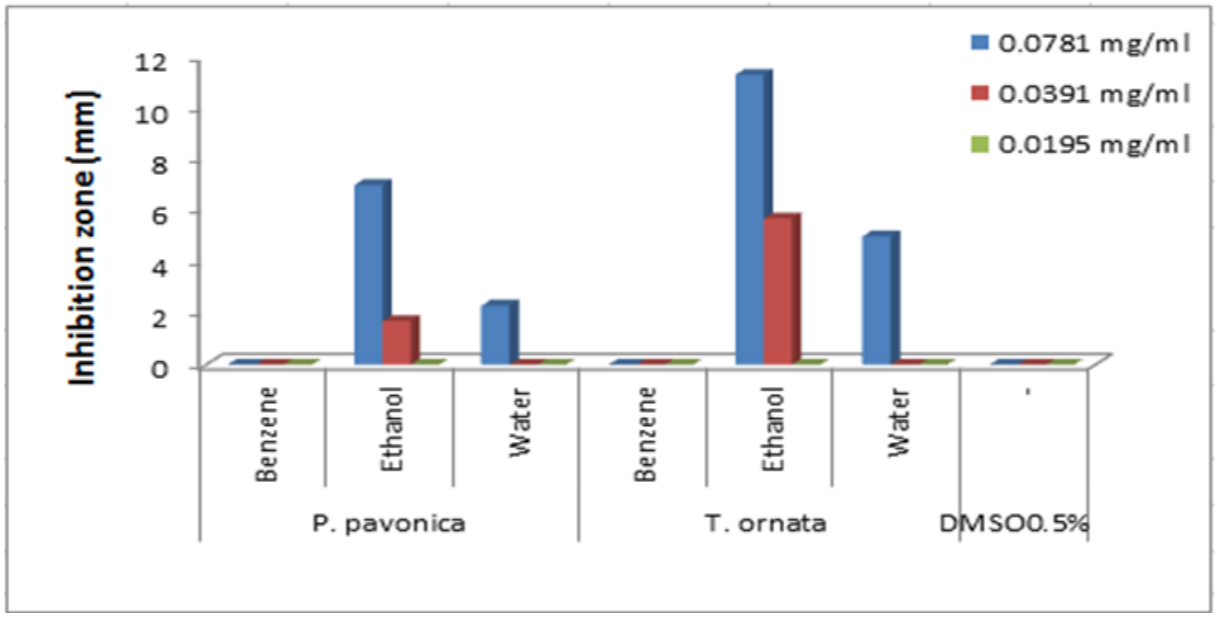

Fig. (4). Inhibition zones (mm) produced by different concentrations of algal extracts (0.0781, 0.0391, and 0.0195 mg/ml). *P. pavonica (Padina pavonica)*. *T. ornata* (*Turbinaria ornata)*. DMSO (dimethyl sulfoxide)

Inhibitory activity of algal extracts on *A. tumefaciens* 27AS_Pp4 *in vivo*

No gall formation was observed in plants treated with the ethanol extract of *T. ornata*, resulting in a response comparable to that of the negative control (Table 2). Additionally, the average number of galls/plant was decreased in all extract treatments, ranging from 0.2 ± 0.2 to 1.0 ± 0.45, compared with the positive control (3.2 ± 0.49). The ethanol extracts of both algae and the water extract of *T. ornata* showed significantly lower gall numbers than the positive control ($P < 0.05$), whereas the benzene extracts and the water extract of *P. pavonica* did not differ significantly from the positive control. The average weight of galls/plant (g) was also decreased in all extract treatments, ranging from 0.05 ± 0.05

to 0.19 ± 0.08 g for fresh weight and from 0.02 ± 0.02 to 0.06 ± 0.05 g for dry weight, compared with the positive control treatment (1.53 ± 0.39 g fresh weight and 0.76 ± 0.10 g dry weight). The same statistical pattern was observed for gall fresh and dry weights ($P < 0.05$).

In the same table, disease reduction reached 100.00% in plants treated with the ethanol extract of *T. ornata*, which was comparable to the negative control, followed by 93.75% for the ethanol extract of *P. pavonica*. Furthermore, disease reduction values for the water extracts of *T. ornata* and *P. pavonica* were 87.50% and 81.25%, respectively, whereas the benzene extracts of *T. ornata* and *P. pavonica* resulted in disease reductions of 75.00% and 68.75%, respectively.

Table (2). Inhibitory activity of different extracts of *Padina pavonica* and *Turbinaria ornata* on the development of *Agrobacterium tumefaciens* 27AS_Pp4 in pot experiments (pathological parameters)

Treatment		Average number of galls/plant	Average weight of galls/plant (g)		Disease Reduction %
			Fresh	Dry	
P. pavonica	**Benzene**	1.0 ± 0.45 ab	0.18 ± 0.08 ab	0.05 ± 0.04 ab	68.75%
	Ethanol	0.2 ± 0.2 b	0.05 ± 0.05 b	0.02 ± 0.02 b	93.75%
	Water	0.6 ± 0.4 ab	0.09 ± 0.56 ab	0.03 ± 0.03 ab	81.25%
T. ornata	**Benzene**	0.8 ± 0.37 ab	0.19 ± 0.08 ab	0.06 ± 0.05 ab	75. 00%
	Ethanol	0.0 ± 0.00 b	0.0 ± 0.00 b	0.0 ± 0.00 b	100.00%
	Water	0.4±0.4 b	0.07 ± 0.07 b	0.02 ± 0.02 b	87.50%
dimethyl sulfoxide (DMSO) 0.5% Healthy control		0.0 ± 0.00 b	0.0 ± 0.00 b	0.0 ± 0.00 b	100.00%
***A. tumefaciens* Infected control**		3.2 ± 0.49 a	1.53 ± 0.39 a	0.76 ± 0.10 a	0.0%

Values are presented as mean ± standard deviation (SD) (n = 5). * Analyzed using Dunn's post hoc test with Bonferroni correction to adjust for multiple comparisons. A P-value < 0.05 was considered statistically significant.

On the other hand, data in Table 3 revealed that the fresh average weight of shoots/plant (g) was lower in most extract treatments (2.79 ± 0.39 to 4.55 ± 0.27 g) than in the *A. tumefaciens* treatment (4.62 ± 0.40 g), except for the *T. ornata* ethanol extract, which resulted in a higher fresh shoot weight (5.66 ± 0.29 g). For dry shoot weight, values ranged from 1.30 ± 0.02 to 1.79 ± 0.11 g, compared with 1.93 ± 0.14 g in the positive control (*A. tumefaciens*), except for the ethanol extracts of *P. pavonica* and *T. ornata*, which resulted in dry shoot weights of 2.42 ± 0.35 and 2.91 ± 0.22 g, respectively. The fresh and dry shoot weights of plants treated with the *T. ornata* ethanol extract were significantly higher than those of the positive control ($P < 0.05$).

Also, data revealed that the average weight of roots/plant (g) was numerically higher in plants treated with the ethanol and water extracts of *T. ornata*, with fresh and dry root weights of 2.80 ± 0.29 and 1.46 ± 0.10 g, respectively, for the ethanol extract, and 2.68 ± 0.19 and 1.35 ± 0.14 g, respectively, for the water extract, compared with 2.55 ± 0.29 and 1.23 ± 0.10 g, respectively, in plants treated with *A. tumefaciens*. Significant differences were observed between the *A. tumefaciens* treatment and the benzene extract treatments of both algae.

Plant height was generally higher in the extract-treated plants than in the *A. tumefaciens* treatment, except for the benzene extracts. The highest plant height was recorded in plants treated with the *T. ornata* ethanol extract (29.6 ± 0.68 cm), which was numerically higher than that of the *A. tumefaciens* treatment (27.4 ± 0.37 cm)

From Tables 2 and 3, the ethanol extracts of both algae showed the greatest overall effects on disease suppression and plant growth, followed by the water extracts, whereas the benzene extracts were the least effective. In general, *T. ornata* extracts showed greater effects than the corresponding *P. pavonica* extracts.

Table (3). Effects of different extracts of *Padina pavonica* and *Turbinaria ornata* on plant growth in tomato plants inoculated with *Agrobacterium tumefaciens* 27AS_Pp4 in pot experiments (plant growth parameters)

Treatment		Average weight of shoots/plant (g)		Average weight of roots/plant (g)		Average length of plant (cm)
		Fresh	Dry	Fresh	Dry	
P. pavonica	Benzene	2.79 ± 0.39 d	1.41 ± 0.13 c	1.45 ± 0.23 c	0.88 ± 0.13 cd	26.6 ± 0.60 bc
	Ethanol	4.55 ± 0.27 b	2.42 ± 0.35 ab	2.53 ± 0.20 ab	1.13 ± 0.08 bc	27.6 ± 1.12 ab
	Water	3.98 ± 0.24 bc	1.79 ± 0.11 c	1.89 ± 0.28 bc	0.92 ± 0.06 cd	27.4 ± 0.81 ab
T. ornata	Benzene	3.52 ± 0.39 cd	1.6 ± 0.26 c	1.36 ± 0.11 c	0.79 ± 0.06 d	24.6 ± 0.87 c
	Ethanol	5.66 ± 0.29 a	2.91 ± 0.22 a	2.80 ± 0.29 a	1.46 ± 0.10 a	29.6 ± 0.68 a
	Water	4.20 ± 0.14 bc	1.69 ± 0.23 c	2.68 ± 0.19 a	1.35 ± 0.14 ab	27.8 ± 0.37 ab
Dimethyl sulfoxide (DMSO) 0.5% Healthy control		2.98 ± 0.09 d	1.30 ± 0.02 c	1.4 ± 0.09 c	0.75 ± 0.07 d	24.8 ± 0.96 c
***A. tumefaciens* Infected control**		4.62 ± 0.40 b	1.93 ± 0.14 bc	2.55 ± 0.29 ab	1.23 ± 0.10 ab	27.4 ± 0.37 ab

Values are presented as mean ± standard deviation (SD) (n = 5). * Analyzed using Tukey's Honestly Significant Difference (HSD). P-value < 0.05.

Estimation of total phenols, free phenols, flavonoids, tannins, saponins, and alkaloids in extracts of *P. pavonica* and *T. ornata*

As shown in Fig. 5, the contents of total phenols, free phenols, flavonoids, tannins, saponins, and alkaloids varied among the extracts depending on both the algal species and the extraction solvent. The highest contents of all the estimated compounds were obtained from the ethanol extracts of both algae, with *T. ornata* showing higher contents than *P. pavonica*.

The phytochemical profiles of the water extracts also differed between the two algal species. The *T. ornata* water extract contained higher amounts of total phenols, saponins, and alkaloids than the corresponding *P. pavonica* extract, whereas the contents of free phenols, flavonoids, and tannins were higher in the *P. pavonica* water extract than in the *T. ornata* water extract. A similar variation was observed between the benzene extracts. The contents of flavonoids, tannins, and alkaloids were higher in the *T. ornata* benzene extract, whereas the contents of total phenols, free phenols, and saponins were higher in the *P. pavonica* benzene extract.

Overall, the ethanol extracts contained the highest levels of the estimated phytochemical constituents, followed by the water extracts, whereas the benzene extracts generally contained the lowest levels. This pattern was consistent with the higher antibacterial activity observed for the ethanol extracts, particularly the *T. ornata* ethanol extract, suggesting that differences in phytochemical composition may be associated with the observed variation in antibacterial activity among the algal extracts.

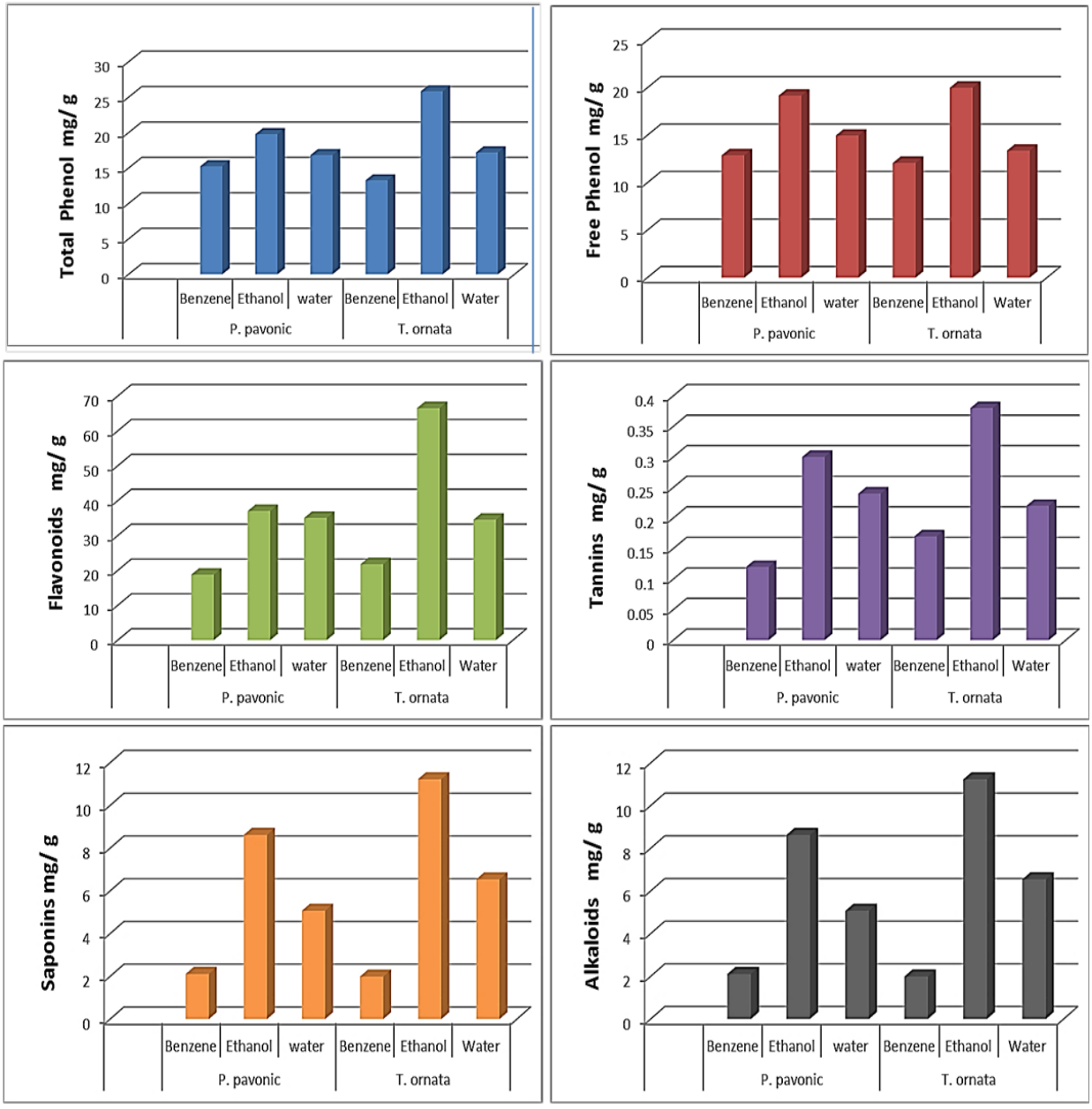

Fig. 5. Contents of selected phytochemical constituents in different algal extracts. *P. pavonica (Padina pavonica), T. ornata* (*Turbinaria ornata*).

4. Discussion

Crown gall disease is one of the most destructive diseases affecting a wide range of plant families, and several methods have been used to control this disease, particularly biological control, which is considered an environmentally save friendly approach **(Amusa, 2005; Pulawska, 2010)**.

Marine macroalgae are a rich source of diverse bioactive compounds with various biological activities **(Hamed *et al*., 2018; Ishmayana *et al*., 2025)**. These naturally occurring substances produced by the algae have been reported to be effective against several plant-pathogenic bacteria **(Ara *et al*., 2002; Abd-El-Aziz, 2020)**. In addition, marine macroalgae contain cell-wall polysaccharides and other bioactive compounds that can stimulate plant defense mechanisms against various plant pathogens, including bacteria, fungi, and viruses, as well as pests and nematodes. These properties support the potential use of algae and their compounds as plant protectants **(Shukla *et al*., 2016; Asimakis *et al*., 2022)**. In the present study, extracts of *P. pavonica* and *T. ornata* contain

antibacterial product activity against *A. tumefaciens*, suppressed crown gall development, and improve plant growth. In this respect *T. ornata* generally showing greater activity than *P. pavonica*. Ethanol extracts showed the greatest overall effects on disease suppression and plant growth, followed by water extracts, whereas benzene extracts were the least effective. The superior performance of the ethanol extracts as shown in the present study may be partly associated with their higher contents of phenolic compounds and flavonoids, together with other bioactive constituents such as tannins, saponins, and alkaloids. Differences among the three tested extracts may therefore be related to the types and concentrations of bioactive compounds recovered by each solvent. Overall, the antibacterial and crown gall-suppressive activities of the algal extracts, as well as their effects on plant growth, may be result from the combined effects of several bioactive compounds rather than from a single constituent.

Brown algae are recognized as important sources of diverse bioactive compounds with antibacterial and other biological activities **(Zerrifi *et al*., 2018)**. Their potential in the management of plant pathogens has received increasing attention because of the various biologically active metabolites produced by these algae **(Hamed *et al*., 2018; Shukla *et al*., 2021)**. Several studies have reported that antibacterial activity of brown algal extracts against different Gram-positive and Gram-negative bacteria, has been attributed to their diverse bioactive constituents **(Nagayama *et al*., 2002; Zerrifi *et al*., 2018)**. Brown algae are known to contain relatively high levels of phenolic compounds, which have been associated with antimicrobial activity **(Suleria *et al*., 2015)**. Marine macroalgal products are also recognized as rich sources of phenolic compounds with various biological activities **(Machu *et al*., 2015)**. In addition, extracts of brown algae have demonstrated antibacterial activity against a range of Gram-positive and Gram-negative bacteria **(Ara *et al*., 2002)**. The presence of phenolic compounds may therefore contribute to the antibacterial activity of brown algal extracts. Marine algae contain diverse groups of secondary metabolites, including phenolic compounds, alkaloids, tannins, saponins, glycosides, flavonoids, and steroids **(Cornish and Garbary, 2010; Asimakis *et al*., 2022)**. The biological activities of algal extracts may result from the combined effects of several of these compounds rather than from a single constituent **(Holdt and Kraan, 2011)**.

Species of the genus *Turbinaria* produce a variety of bioactive compounds, including alginic acid, fucoidan, mannitol, and laminarin, which have been reported to possess various biological activities **(Zubia *et al.*, 2003; Kayalvizhi *et al.*, 2014; Alka *et al.*, 2017)**. In particular, *T. ornata* contains more diverse secondary metabolites, including tannins, saponins, terpenoids, phenolic compounds, flavonoids, and coumarins, which may contribute to its biological activities **(Rajkumar and Bhavan, 2017; Alka *et al.*, 2017; Ishmayana *et al.*, 2025)**. Previous studies have also demonstrated antibacterial activity of *T. ornata* extracts against a range of pathogenic bacteria, supporting its potential as a source of natural antibacterial compounds **(Vijayabaskar and Shiyamala, 2011)**.

P. pavonica has been reported to contain other variety of bioactive compounds, including phenolic compounds such as kaempferol, ellagic acid, delphinidin-3-O-glucoside, ferulic acid, and naringenin, as well as flavonoids, triterpenoids, glycosides, amino acids, tannins, saponins, and coumarins **(Sudha and Balasundaram, 2018; Ishmayana *et al*., 2025)**. The diversity of these secondary metabolites may contribute to the biological activities reported for *P. pavonica*. Flavonoids are polyphenolic compounds with diverse biological activities, including antioxidant and antimicrobial properties, and their presence in marine macroalgae has been widely reported **(Ren *et al.*, 2003; Svobodová *et al.*, 2003; Kim *et al.*, 2005; Gul *et al.*, 2017; Ruslin *et al.*, 2018)**. *Padina* species contain diverse bioactive compounds, particularly phenolic compounds and flavonoids, which may contribute to their biological activities **(Jose *et al.*, 2015; Isnansetyo *et al.*, 2017; Ruslin *et al.*, 2018; Al-Enazi *et al.*, 2018; Benita *et al.*, 2018)**.

The used extraction solvent plays an important role in determining the type and quantity of bioactive compounds recovered from algae. Previous studies have reported that alcoholic solvents can be effective for extracting phenolic compounds, flavonoids, and tannins compared with other solvents **(Kumar *et al.*, 2008; Sahayaraj and Kalidas, 2011; Generali´c Mekini´c *et al.*, 2021)**. Water and ethanol are also commonly preferred for extraction because of their economic, toxicological, and environmental advantages **(Zubia *et al.*, 2009)**. Differences in the antibacterial activity of algal

extracts obtained using different solvents may therefore be related to the ability of each solvent to extract specific bioactive compounds, thereby influencing the composition and antibacterial activity of the resulting extracts **(Kausalya and Narasimha, 2015; Generali´c Mekini´c *et al.*, 2021)**. Moreover, the antibacterial activity of algal extracts may vary depending on the bacterial species tested, as different bacterial species can respond differently to the same algal extract **(Hamed *et al.*, 2018; Ibrahium *et al.*, 2020)**.

Previous studies have demonstrated that brown algal extracts can reduce crown gall disease, particularly methanolic extracts, and have suggested that induced resistance may contribute to the protective effect of these extracts **(Esserti *et al.*, 2017; Asimakis *et al.*, 2022)**. In the present study, the observed antibacterial activity of the algal extracts against *A. tumefaciens*, together with their suppression of crown gall development, suggests that direct antibacterial activity may have contributed to disease reduction. However, the involvement of induced resistance or other plant defense mechanisms cannot be excluded and requires further investigation. In addition to their antibacterial and disease-suppressive effects, brown algal extracts may positively influence plant growth through several physiological mechanisms. They may enhance nutrient uptake, regulate phytohormone signaling, strengthen antioxidant defense, support osmotic regulation, and stimulate stress-responsive genes and pathways. These effects may contribute to improved seed germination, increased plant biomass through enhanced cell division and elongation, and ultimately greater crop productivity **(Sariñana-Aldaco *et al.*, 2025; Kumar *et al.*, 2026)**. Therefore, the effects of the algal extracts observed in the present study on plant growth may be associated not only with disease suppression but also with their potential growth-promoting and stress-mitigating properties.

5. Conclusion

The present study demonstrated that extracts of the brown algae *Turbinaria ornata* and *Padina pavonica* possess antibacterial activity against *Agrobacterium tumefaciens* 27AS_Pp4 and can suppress crown gall development in tomato plants. The antibacterial activity and disease-suppressive efficacy of the extracts varied according to both algal species and extraction solvent, with ethanol extracts showing the greatest overall activity, followed by water extracts, whereas benzene extracts were the least effective. Among the tested treatments, the ethanol extract of *T. ornata* showed the highest antibacterial activity, the lowest MEC, and complete suppression of crown gall development, while also markedly improving plant growth parameters. The ethanol extract of *P. pavonica* also showed considerable antibacterial and disease-suppressive activity and improved several plant growth parameters. The higher levels of phenolic compounds, flavonoids, tannins, saponins, and alkaloids detected in the ethanol extracts, particularly that of *T. ornata*, were associated with their greater antibacterial activity, suggesting that the observed effects may result from the combined activity of multiple bioactive constituents. The observed improvements in plant growth may also reflect the potential growth-promoting and stress-mitigating properties of brown algal bioactive compounds. Overall, the findings highlight *T. ornata*, particularly its ethanol extract, as a promising natural source for the development of environmentally compatible approaches for crown gall management. Further studies are needed to identify the specific active compounds and elucidate the mechanisms underlying disease suppression and their effects on plant defense responses and plant growth.

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