SHORT COMMUNICATION

Utilization of vegetable extracts for biostimulation and enhancement of medicinal properties in *Aloe vera* cultivation

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ABSTRACT

The rising demand for natural and sustainable agricultural inputs has spurred interest in plant-based biostimulants to boost crop productivity and phytochemical quality. Aloe vera (Aloe barbadensis Miller), valued for its medicinal and commercial applications, owes its utility to bioactive compounds like polysaccharides, phenolics, and antioxidants. This study investigates the effect of aqueous and hydroalcoholic extracts from spinach (Spinacia oleracea), carrot (Daucus carota), and beetroot (Beta vulgaris) on the growth and phytochemical profile of Aloe vera. Over an 8-week greenhouse trial, plants received foliar and soil applications of these extracts. Treated plants showed significant gains in vegetative growth, chlorophyll levels, and leaf biomass versus controls. Biochemical analysis revealed increased phenolic content, flavonoids, and antioxidant activity, especially in beetroot- and carrot-treated groups. Notably, carrot extracts also led to higher polysaccharide yields, enhancing Aloe vera's therapeutic value. These findings suggest vegetable extracts act as natural elicitors, promoting secondary metabolite biosynthesis. As low-cost, eco-friendly biostimulants, they hold promise for improving the vitality and commercial quality of medicinal plants.

Keywords: Antioxidant activity, foliar application, polysaccharides, secondary metabolites, sustainable agriculture

Aloe vera is a perennial succulent belonging to the family Asphodelaceae, widely cultivated for its gel-rich leaves that are used in pharmaceuticals, nutraceuticals, cosmetics, and functional foods. The gel is composed primarily of water (~98–99%) but contains a diverse array of biologically active compounds that confer its medicinal value (Savvas and Ntatsi, 2015; Bhowmik, 2019). The phytochemical profile of *Aloe vera* is highly sensitive to environmental conditions, cultivation practices, and stress factors such as drought, salinity, and nutrient deficiency (Yakhin *et al*., 2017). These stresses often lead to suboptimal growth and reduced concentrations of valuable secondary metabolites. Biostimulants offer a sustainable, eco-friendly strategy to address these challenges. Defined as substances or microorganisms that stimulate natural processes to improve nutrient uptake, stress tolerance, and crop quality (du Jardin, 2015), they differ from fertilizers in that their primary action is physiological rather than purely nutritional. Plant-derived biostimulants, including vegetable extracts, are rich in amino acids, phenolics, phytohormone-like molecules, and organic acids, which can act as elicitors—triggering secondary metabolism and enhancing phytochemical accumulation (Ertani *et al*., 2014).

While previous studies have shown that seaweed extracts and compost teas improve biomass, chlorophyll content, and antioxidant activity in *Aloe vera* (Chow *et al.*, 2005; Yakhin *et al*., 2017), the potential of vegetable-derived extracts—especially from spinach (*Spinacia oleracea*), carrot (*Daucus carota*), and beetroot (*Beta vulgaris*)—remains underexplored. These vegetables are abundant in bioactive compounds such as betaines, betalains, carotenoids, phenolic acids, and nitrates, which could synergistically boost growth and enhance the medicinal quality of *Aloe vera*.

Therefore, this study evaluates the effects of aqueous extracts from spinach, carrot, and beetroot on the growth, physiology, and phytochemical profile of *Aloe vera*. By integrating detailed phytochemical analysis with agronomic assessment, it aims to demonstrate how targeted biostimulant applications can sustainably increase both yield and medicinal value, meeting the growing demand for high-quality *Aloe vera* products in the pharmaceutical and cosmetic sectors.

The trial was performed in spring 2025 within a climate-controlled greenhouse at the Landscaping Plants and Nursery Research Unit, affiliated with the Italian Council for Agricultural Research and Economics (CREA) in Pescia (PT), Italy. Aloe vera (*Aloe barbadensis Miller*) plants, approximately four months old and confirmed to be uniform and free from disease symptoms, were selected for the study. Each plant was potted in a 25 cm diameter plastic container filled with a sterilized growth medium comprising sandy loam soil blended with compost in a 3:1 proportion. Before the treatments commenced, the plants were allowed to acclimate for 14 days under ambient conditions with regular watering to ensure uniform physiological status. The experimental layout followed a completely randomized design (CRD) with four treatment groups and five replicates per treatment, totaling 20 pots. The groups were: T0 (Control): Distilled water only; T1: Aqueous spinach extract; T2: Aqueous carrot extract; T3: Aqueous beetroot extract. Each treatment involved both foliar spray and soil drenching, applied weekly for 8 consecutive weeks.

Organic fresh spinach (*Spinacia oleracea*), carrot (*Daucus carota*), and beetroot (*Beta vulgaris*) were obtained from a local farm. After washing and removing non-edible parts, each vegetable was chopped and homogenized using a blender with distilled water at a 1:10 (w/v) ratio. The homogenates were filtered using muslin cloth followed by Whatman No. 1 filter paper. The filtrates were considered 100% stock solutions. These were applied directly as aqueous extracts without any dilution. For foliar application, 100 ml of each extract was sprayed evenly on the leaves using a hand sprayer. For soil drenching, 200 ml per pot was poured around the plant base. Control plants received the same volume of distilled water. All extracts were freshly prepared 24 hours before application and stored at 4°C to prevent microbial degradation (Gómez-Merino and Trejo-Téllez, 2015).

After 8 weeks of treatment, morphological data were recorded. Parameters included: plant height (cm): measured from the soil surface to the tip of the tallest leaf using a measuring scale; number of leaves per plant; leaf length and width (cm): averaged from three randomly selected leaves per plant; fresh weight (g): harvested whole plant weight measured using a digital balance; dry weight (g): to assess dry biomass, plant samples were dried in a hot air oven at 65 °C for 72 hours and then weighed. All observations were made during morning hours to minimize the influence of daily temperature and light fluctuations on the measurements.

Fresh leaf tissue (0.5 g) was homogenized in 80% acetone, and the resulting extract was centrifuged at 10,000 rpm for 10 minutes to obtain a clear supernatant. Absorbance readings were taken at 645 nm and 663 nm with a UV–Visible spectrophotometer. Chlorophyll a, chlorophyll b, and total chlorophyll concentrations were calculated using Arnon's standard equations (Yakhin *et al*., 2017).

The Folin–Ciocalteu assay was used to estimate total phenolic content in dried *Aloe*

vera gel extract. After mixing with sodium carbonate and incubating at room temperature, absorbance was measured at 765 nm. Gallic acid served as the standard, and results were expressed as GAE per gram of dry extract (Saa *et al*., 2015).

Total flavonoids in *Aloe vera* extract were measured using an aluminum chloride-based colorimetric method. The mixture was incubated for 30 minutes, and absorbance was measured at 415 nm. Results were calculated as quercetin equivalents per gram of dry sample. Antioxidant Activity (DPPH Assay). The antioxidant activity of *Aloe vera* gel extract was assessed using the DPPH radical scavenging method. After mixing the extract with a methanolic DPPH solution, the reaction was kept in the dark at room temperature for 30 minutes (Chow *et al.*, 2005). Absorbance was then measured at 517 nm to calculate the scavenging efficiency by using following formula:

$$\text{Inhibition } (\%) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

The antioxidant activity of Aloe vera gel extracts was evaluated using the DPPH free radical scavenging method described by Chow et al. (2005) with minor modifications. Briefly, 1 ml of *Aloe vera* extract (1 mg ml^{-1}) was added to 3 ml of a freshly prepared 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in methanol. The mixture was vortexed and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm against a methanol blank using a UV–Vis spectrophotometer.

The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\text{Antioxidant activity } (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 = absorbance of control (DPPH + methanol) and A_1 = absorbance of sample (DPPH + extract).

High-performance liquid chromatography (HPLC) with photodiode array detection at 254 nm following Keem *et al.* (2023). C18 reverse-phase column, mobile phase of methanol:water (70:30 v/v), flow rate 1 mL/min. HPLC quantification as per Souza *et al*., (2017), using detection at 430 nm.

Vitamin C estimated via 2,6-dichlorophenolindophenol titration (AOAC, 2016; Pegg and Eitenmiller, 2017); β-carotene measured spectrophotometrically at 450 nm after hexane extraction Raman *et al.* (2023).

To ensure consistency, all extractions, biochemical assays, and measurements were performed in triplicate. All instruments were calibrated prior to use. Blank controls and standards were included in every batch of biochemical assays.

Polysaccharide content in *Aloe vera* gel was measured using the phenol sulfuric acid method. After reaction with phenol-sulfuric acid and 30-minute incubation, absorbance was measured at 490 nm. Results were expressed as glucose equivalents per gram of dry sample (Saa *et al*., 2015).

Acemannan was quantified using the colorimetric periodic acid–Schiff (PAS) method with commercial acemannan standard (Sigma-Aldrich, USA) following Eberendu *et al.* (2005). Lyophilized gel samples were enzymatically digested with cellulase to release polysaccharides. Periodic acid oxidation followed by Schiff reagent reaction produced a pink chromophore measured at 550 nm.

One-way ANOVA was used to evaluate treatment effects, and Tukey's HSD test was applied for pairwise comparisons where significant differences were found ($p < 0.05$). Data are presented as mean ± standard deviation. Statistical analysis was conducted using SPSS or R, while GraphPad Prism and Excel were used for graph preparation.

Vegetable extracts significantly enhanced the vegetative growth of *Aloe vera* compared to the control group (Table 1). The highest plant height was recorded in the beetroot extract treatment (40.3 ± 1.6 cm), followed by carrot (38.1 ± 1.4 cm) and spinach (35.7 ± 1.5 cm). These increases in plant stature are consistent with previous reports indicating

that bioactive compounds in vegetable extracts can promote cell elongation and division (Calvo *et al*., 2014.).

Similarly, leaf number and fresh weight improved significantly. Plants treated with beetroot extract produced the most leaves (15.5 ± 0.6), indicating a strong biostimulatory effect, possibly due to the high betaine and nitrate content that supports nitrogen metabolism and shoot formation (Nardi *et al*., 2016). The fresh biomass gain in beetroot-treated plants (205.8 ± 6.5 g) was 41% higher than control, aligning with findings from Rouphael *et al.* (2018) on natural biostimulants enhancing biomass. Leaf morphological parameters followed similar trends to plant height and leaf number (Table 1). The longest leaves were recorded in the beetroot extract treatment (49.6 ± 2.2 cm), followed by carrot (46.8 ± 2.0 cm) and spinach extract (44.2 ± 2.1 cm). The control group had the shortest leaves (37.8 ± 1.8 cm). Likewise, leaf width increased significantly, from 5.8 ± 0.3 cm in the control to 7.3 ± 0.4 cm under beetroot treatment. These increases suggest that vegetable extract biostimulants enhanced cell expansion and water retention capacity in leaf tissues, promoting overall leaf growth. The improvement in laminar dimensions may be attributed to enhanced photosynthetic activity and nitrogen assimilation stimulated by compounds such as betaine and nitrate in beetroot and carrot extracts (Nardi *et al*., 2016). Similar findings were reported by Calvo *et al*. (2014) and Rouphael *et al*. (2018), where biostimulant applications enhanced leaf area and thickness in horticultural crops.

The dry weight of leaves was also significantly increased with all treatments (Table 1), further supporting the role of vegetable extracts in improving biomass accumulation through improved water and nutrient utilization (Rouphael *et al*., 2017).

Chlorophyll content showed a marked increase in all treatment groups **(**Table 2**).** The control group had the lowest chlorophyll content (1.25 ± 0.05 mg/g FW), while beetroot-treated plants recorded the highest (2.01 ± 0.08 mg/g FW). This suggests enhanced photosynthetic capacity due to biostimulant activity. These results are consistent with prior studies indicating that phenolic-rich extracts enhance photosynthetic pigment biosynthesis by reducing oxidative stress and improving enzymatic activity (Saa *et al*., 2015). The improved pigment concentration under beetroot treatment may be due to betacyanin-mediated photoprotection, stabilizing chlorophyll under greenhouse light fluctuations.

As shown in Table 2, total phenolic content was highest in beetroot-treated plants (11.2 ± 0.4 mg GAE/g DW), representing a 65% increase over control. Carrot and spinach treatments also significantly improved phenolic accumulation. This is indicative of the elicitor effect of bioactive molecules in vegetable extracts, which stimulate secondary metabolite pathways such as phenylpropanoid metabolism (Yakhin *et al*., 2017).

Antioxidant activity (measured by DPPH radical scavenging) showed similar trends, with the highest values observed under beetroot extract ($65.4 \pm 2.5\%$) **(**Table 2**).** This finding aligns with reports by Nardi *et al*. (2016), who observed significant increases in antioxidant defense under natural elicitor treatments in medicinal plants.

Additionally, the flavonoid content of the gel **(**Table 2) increased significantly under carrot and beetroot extract treatments, suggesting the upregulation of flavonoid biosynthesis genes such as CHS and FLS, commonly triggered under stress signaling and elicitation (Gómez-Merino and Trejo-Téllez, 2015).

The total flavonoid content of *Aloe vera* gel showed a pronounced increase following vegetable extract treatments (Table 2). The control group exhibited the lowest flavonoid concentration (3.25 ± 0.15 mg QE g^{-1} DW), while beetroot extract achieved the highest level (5.21 ± 0.22 mg QE g^{-1} DW), representing a 60% increase over control. Carrot and spinach extracts also resulted in significant enhancements. This improvement can be attributed to elicitor-like compounds such as phenolics, ca-

rotenoids, and nitrates that stimulate chalcone synthase (CHS) and flavonol synthase (FLS) gene expression, key enzymes in the flavonoid biosynthetic pathway (Yakhin *et al*., 2017).

The elevated flavonoid content corresponds with enhanced antioxidant activity, suggesting a coordinated activation of phenolic and flavonoid metabolism under biostimulant influence. Similar findings have been reported in medicinal plants treated with natural extracts, where elicitor compounds promote flavonoid accumulation as part of stress adaptation and defense priming (Gómez-Merino and Trejo-Téllez, 2015; Rouphael *et al*., 2018).

Polysaccharides, especially acemannan, are considered critical for the medicinal efficacy of *Aloe vera*. Table 2 shows a significant enhancement in polysaccharide concentration across all treatments. Beetroot extract resulted in the highest increase (194.3 ± 5.4 mg/g DW), supporting the hypothesis that certain vegetable extracts stimulate carbohydrate biosynthesis. Polysaccharide accumulation may be attributed to enhanced carbon assimilation, as well as hormonal stimulation of sugar metabolism. This biochemical enrichment reinforces the potential of vegetable extracts in improving both yield and functional value of *Aloe vera* gel.

Application of vegetable extracts significantly enhanced *Aloe vera*-specific bioactives in the gel and latex fractions.

Control plants recorded 5.8 ± 0.3 mg/g fresh gel, whereas beetroot extract treatment increased this to 8.9 ± 0.4 mg/g ($p < 0.05$) (Table 3). This 53% increase suggests upregulation of polysaccharide biosynthesis, likely due to carbohydrate precursors and elicitor compounds in the extract (Chow *et al*., 2005).

Highest concentrations were observed in spinach extract treatment (2.85 ± 0.09 mg/g dry latex), representing a 32% increase over control (Table 3). Elevated anthraquinone glycosides may reflect stress-related secondary metabolism triggered by biostimulants (Yakhin *et al*., 2017).

Carrot extract notably enhanced aloe-emodin content by 27% compared to control, potentially linked to phenolic precursors in carrot-derived biostimulants (Table 3) (Keem *et al*., 2023).

All treatments improved ascorbic acid content (Table 3), with beetroot extract yielding the highest value (23.4 ± 0.8 mg/100 g fresh gel), possibly due to its nitrate content promoting antioxidant biosynthesis (Gómez-Merino and Trejo-Téllez, 2015).

Spinach extract increased β-carotene content by 41%, consistent with its own carotenoid profile and potential priming effect on the terpenoid pathway (Table 3). These results demonstrate that vegetable-derived biostimulants can enhance *Aloe vera*'s unique medicinal compounds particularly acemannan and anthraquinones beyond the general phenolic/flavonoid profile (Saa *et al*., 2015).

Earlier studies using commercial biostimulants (seaweed extracts, humic substances) reported similar benefits for *Aloe vera* and other medicinal plants. However, this is among the first controlled studies using *vegetable-based* extracts, particularly beetroot and carrot, as direct plant growth stimulants and phytochemical enhancers. Comparable findings were recently observed in basil and lettuce treated with onion peel and tomato pulp extracts, which increased chlorophyll, polyphenols, and root length (Ertani *et al*., 2014; Halpern *et al*., 2015). This suggests a broader application of food byproducts and vegetable waste in sustainable agriculture.

CONFLICT OF INTEREST STATEMENT

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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Table 1: Effect of vegetable-derived biostimulants on growth parameters of *Aloe vera*

Treatment	Plant Height (cm)	Leaf Number	Fresh Weight (g)	Leaf Length (cm)	Leaf Width (cm)	Leaves Dry Weight (g)
Control	28.4 ± 1.2 a	10.2 ± 0.6 a	145.3 ± 5.4 a	37.8 ± 1.8 a	5.8 ± 0.3 a	18.6 ± 0.9 a
Spinach Extract	35.7 ± 1.5 b	13.1 ± 0.5 b	178.6 ± 6.2 b	44.2 ± 2.1 b	6.6 ± 0.4 b	23.8 ± 1.0 b
Carrot Extract	38.1 ± 1.4 b	14.2 ± 0.7 c	190.1 ± 5.9 c	46.8 ± 2.0 c	6.9 ± 0.3 b	25.2 ± 1.1 c
Beetroot Extract	40.3 ± 1.6 c	15.5 ± 0.6 d	205.8 ± 6.5 d	49.6 ± 2.2 d	7.3 ± 0.4 c	27.1 ± 1.2 d
CD ($p \leq 0.05$)	1.728	0.694	8.372	2.234	0.382	1.052
CV (%)	12.59	15.50	14.69	4.76	4.95	4.89

Table 2: Effect of vegetable-derived biostimulants on biochemical parameters of *Aloe vera*

Treatment	Chlorophyll (mg/g FW)	Total Phenolics (mg GAE/g DW)	Total Flavonoids (mg QE g^{-1} DW)	Polysaccharide Content (mg g^{-1} DW)	Antioxidant Activity (%)
Control	1.25 ± 0.05 a	6.80 ± 0.40 a	3.25 ± 0.15 a	132.6 ± 4.8 a	41.20 ± 2.10 a
Spinach Extract	1.72 ± 0.07 b	9.10 ± 0.30 b	4.12 ± 0.18 b	168.2 ± 5.6 b	55.60 ± 2.30 b
Carrot Extract	1.85 ± 0.06 b	10.30 ± 0.50 c	4.85 ± 0.20 c	181.7 ± 5.9 c	59.80 ± 2.00 c
Beetroot Extract	2.01 ± 0.08 c	11.20 ± 0.40 d	5.21 ±0.22 d	194.3 ± 5.4 d	65.40 ± 2.50 d
CD ($p \leq 0.05$)	0.086	0.558	0.276	7.934	3.024
CV (%)	15.31	17.44	4.73	4.21	15.94

Table 3: Effect of vegetable extract biostimulants on Aloe vera–specific medicinal compounds

Treatment	Acemannan (mg g^{-1} fresh gel)	Aloin A + B (mg g^{-1} dry latex)	Aloe-emodin (mg g^{-1} dry latex)	Vitamin C (mg 100 g^{-1} fresh gel)	β-Carotene (µg g^{-1} fresh gel)
Control	5.8 ± 0.3 a	2.16 ± 0.07 a	1.12 ± 0.05 a	18.6 ± 0.7 a	7.4 ± 0.4 a
Spinach Extract	7.6 ± 0.4 b	2.85 ± 0.09 c	1.28 ± 0.06 b	21.8 ± 0.6 b	10.4 ± 0.5 c
Carrot Extract	8.1 ± 0.3 b	2.52 ± 0.08 b	1.42 ± 0.07 c	22.6 ± 0.9 b	9.5 ± 0.4 b
Beetroot Extract	8.9 ± 0.4 c	2.44 ± 0.10 b	1.33 ± 0.05 b	23.4 ± 0.8 c	9.8 ± 0.5 b
CD ($p \leq 0.05$)	0.47	0.12	0.09	1.04	0.63
CV (%)	5.21	4.56	5.74	4.18	6.02