



A Preliminary Experimental Protocol for Enhanced Tomato Callus Formation and Growth via Several Medicinal Plant Extracts

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ABSTRACT

Medicinal plants have been used historically for various treatments and in human nutrition. Due to their natural occurrence and biodegradability, medicinal plants can be an eco-friendly alternative to toxic chemicals such as pesticides, fungicides, and herbicides. An in vitro experiment was conducted to test the impact of six medicinal plant extracts on tomato growth and callus induction. Two concentrations of each extract (50 and 100 mg L⁻¹) were added to the Murashige and Skoog (MS) medium culture. Results showed that *Juniperus sabina* extract (50 mg L⁻¹) accelerated tomato seed germination by 100% in vitro, compared to the control group in MS basal medium. Callus growth index (CGI) and callus weight (CW) increased by 50% and by more than 200% when using *Taraxacum officinale* (100 mg L⁻¹) and *Conocarpus erectus* (50 mg L⁻¹) extracts, respectively. Biochemical analysis revealed that the extracts were rich in phenolic compounds (348 mg g⁻¹ of total phenol), flavonoids (162 mg g⁻¹), antioxidants (61%), and auxin. A rise in antioxidant activity, plant growth regulators (PGR), and plant defense elicitation probably contributed to these outcomes. Plant extracts also affected the biochemical content of calluses, except for their total phenol. We recommend using these plant extracts to increase growth, accelerate seed germination, and promote callus induction. Optimizing concentrations and combinations of medicinal plant extracts require further research to maximize their benefits to different plant species and their growth-related values.

Abbreviation: Auxin content (AC), Callus growth index (CGI), Callus weight (CW), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Indole acetic acid (IAA), Murashige and Skoog culture medium (MS), Seedling fresh weight (SFW), Stem length (SL), Total flavonoid (TF), Total phenol (TP)

Introduction

In recent years, an increase in global populations has been concomitant with the prevalence of diseases caused by unintentional consumption of synthetic chemicals by humans, thus leading to a greater emphasis on natural materials in agriculture. As a result, using biological stimulants such as microorganisms, humic acid,

amino acids, and algae extracts can become a fundamental approach to agricultural safety and sustainability (De Saeger et al., 2020). For centuries, common knowledge was that plants can affect the growth and development of their surrounding plants through the production and secretion of specific substances (Pan et al., 2015). This phenomenon is called allelopathy, and it

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refers to the ability of plants to stimulate or inhibit the growth of other plants (Mahboobi and Heidarian, 2016). Plants produce allelopathic or allelochemical substances, both known and unknown, but primarily specific compounds such as tannins, phenolic acids, lignins, alkaloids, flavonoids, coumarins, and terpenoids (Da Silva et al., 2015). Allelopathy can be negative (Santonja et al., 2019) or positive when considered a phenomenon and as hormesis or positive allelopathy (Da Silva et al., 2015). Previous research showed that seaweed extract application through foliar spraying can increase anthocyanins (hydrocinnamic acid) and flavonols (Salvi et al., 2019). Today, plant growth control is a leading topic of discussion in crop production, especially in marketing management and ripening uniformity (Rietveld, 1983). The effect of allelopathic compounds on plant growth is diverse and depends on many factors. For instance, juglone, the dominant allelopathic compound of walnut, reportedly limited the growth of cucumber by stimulating the synthesis of abscisic acid (ABA) in cucumber as a growth inhibitor or by preventing the production of growth-promoting hormones such as auxin, cytokinin, and gibberellin (Terzi et al., 2003). Parallel to allelopathy, an alkaloid compound, cylindrospermopsin, reportedly halted mitosis in bean plants when used at high concentrations (Garda et al., 2015). *Ziziphus mauritiana* plant extract decreased mitotic indices in onion (Owolarafe et al., 2020). *Ricinus communis* methanolic extract significantly affected corn seed (*Zea mays* L.) germination at different levels; the extract concentration at 10 mg mL⁻¹ resulted in zero germination (Saddiqe et al., 2020).

Some allelopathic compounds can have a positive effect on plant growth. For example, a low concentration (2.5-5%) of chia (*Salvia hispanica* L.) and wormwood (*Artemisia absinthium* L.) extract had a positive effect on lettuce (*Lactuca sativa* L.) germination (Erhatic et al., 2023). Priming safflower seeds with fennel, peppermint, and cumin extracts changed GA3 content in safflower (*Carthamus tinctorius* L.) and had a positive effect on seed germination (Alivand and Farajzadeh, 2017). It is also worth noting that the allelopathic properties of a plant can vary depending on extract concentration. For example, garlic extract (*Allium sativum* L.) showed both stimulatory and inhibitory effects on plant growth, with low concentrations having a stimulatory effect and high concentrations completely stopping growth (Cheng et al., 2016). Thus, understanding how allelopathic compounds affect plant growth can help us

develop more efficient and sustainable agricultural practices.

Tomato belongs to the Solanaceae family and is cultivated worldwide as a fruit. It is a source of bioactive, antioxidant, and antimicrobial compounds (Silva-Beltran et al., 2015). Given the benefits of medicinal plants, we aimed to apply six medicinal plant extracts on tomato seeds *in vitro* and assess callus formation. The six medicinal plants were *Juniperus sabina* (Adams and Wingate, 2008; Dahmane et al., 2015), *Rheum ribes* (Keser et al., 2019), *Allium jesdianum* (Amiri, 2007), *Conocarpus erectus* (Nascimento et al., 2016), *Taraxacum officinale* (Faria et al., 2019), and *Doroma aucheri* (Ajani and Claßen-Bockhoff, 2021; Mianabadi et al., 2015). Thus, the present work aimed to investigate the impact of six medicinal plant extracts on tomato growth and callus induction compared to a regular protocol for tomato culture that uses MS with plant hormones.

Materials and Methods

Plant materials and extraction

All medicinal plants were collected from the Kermanshah region, except for *Conocarpus erectus* and *Juniperus sabina*. All plants were identified based on available sources (Nemati and Jalilian, 2011; Sohrabi, 2022). The plant material was washed with water and dried in the shade. Then, the plants were extracted in a ratio of 1:10 (plant material to 85% methanol solvent) using a cold maceration method for two periods of 24 h (Sohrabi, 2022). After preparing the powder (Fig. 1), the methanolic extract was obtained. Extracts from the two periods were mixed and used for the next steps. The extracts were concentrated using an IKA HB10 rotary evaporator (Fig. 1B). The concentrated extracts were then placed in a freeze-dryer (Christ Beta 2-8 LB plus, made in Germany) for 90 h. This was followed by preparing the pure and dry powder of the plant extracts (Fig. 1C).

Preparing tissue culture medium and samples

To prepare plant samples *in vitro*, seeds of the CH cultivar (Fallat Company) were initially placed in 70% ethanol for 1 min. Then, they were disinfected by immersing them in 5% sodium hypochlorite for 10 min and rinsing them with sterile distilled water.

The seeds were then grown under a laminar hood on MS culture medium containing 8% agar and 3% sucrose, 2 mg L⁻¹ BA and 0.1 mg L⁻¹ IAA (Gerszberg et al., 2016), with ten seeds in each glass. To produce callus, 1 cm stem explants were

obtained from the resulting seedlings.

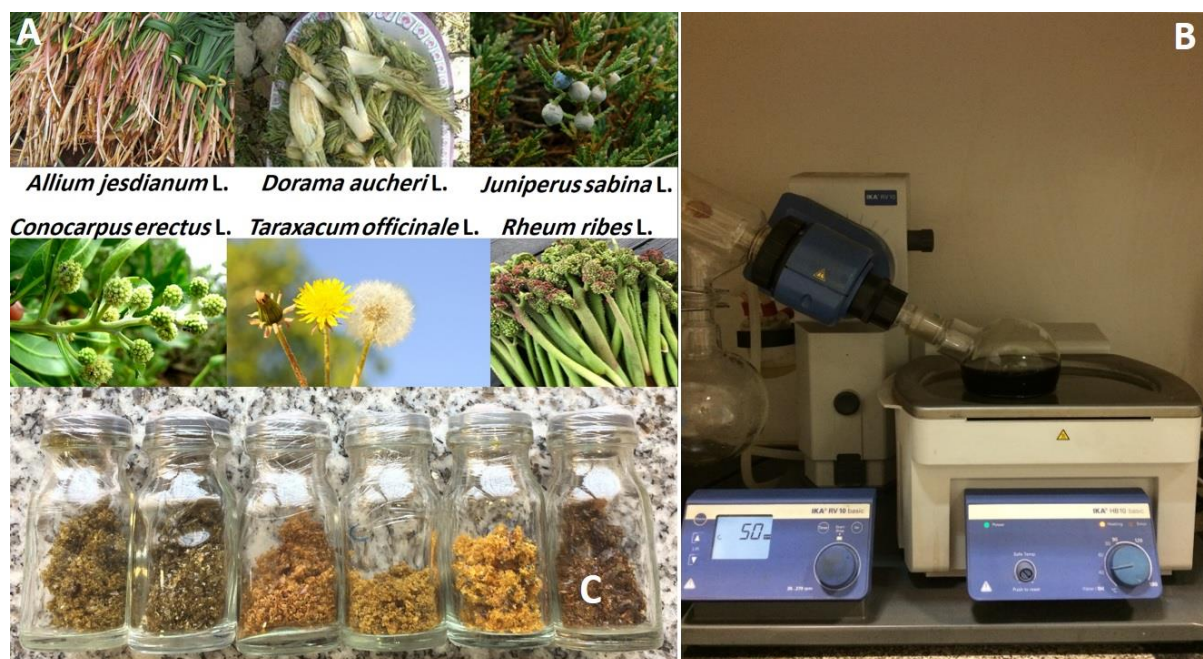


Fig. 1. (A) Medicinal plants as a natural compound source. (B) Concentration of herbal extracts. (C) Pure extract powders of the medicinal plants.

Applying treatments in culture medium

Before adding plant extracts to the culture medium, the culture medium was cooled and the extracts were sterilized using a 45-micron filter. Then, they were added to Murashige and Skoog (MS) culture medium at two concentrations (50 and 100 mg L⁻¹) (Sohrabi, 2022). Each replication consisted of three explants, and each sample was a mixture of all three explants in each replication to obtain a final sample.

Morphological measurement

The effect of plant extracts on seed germination was investigated by measuring traits such as stem length (SL) and seedling fresh weight (SFW). Callus growth index (CGI) and callus weight (CW) were evaluated in the cultured samples under the influence of plant extract treatment (Alizadeh, 2013).

Biochemical measurement

Total phenols (TP) and total flavonoids (TF) were measured using a method described by Chang et al. (2002). The percentage of inhibition of free radicals (DPPH) was determined using a method described by Brand-Williams et al. (1995). Different concentrations of gallic acid were used as a standard for the standard curve. The total phenol was expressed as mg g⁻¹ of gallic acid using the equation $y = 0.0005x + 0.0257$ ($R^2 = 0.9925$). Quercetin was used for determining the

standard curve for total flavonoid measurement, which was expressed as mg g⁻¹ using a relevant equation:

$$y = 0.002x - 0.0282 \quad (R^2 = 0.99)$$

Plant growth hormone measurement (auxin)

Auxin content in the plants was measured using a method described by Gang et al. (2019). In this method, a Salkowski reagent was used for measuring auxin levels. For this purpose, one gram of callus tissue was boiled in 10 mL of 80% ethanol. The callus tissue was then ground and the solution was filtered using Whatman filter paper. Then, 1 mL of the resulting extract was mixed with 2 mL of the Salkowski reagent. To prepare the Salkowski reagent, a 0.5 M solution of FeCl₃ was prepared. One mL of it was mixed with 50 mL of 35% perchloric acid. The resulting mixture was placed in a steam bath at 40-50 °C for 15 min to complete the reaction. The presence of auxin was indicated by a pink color. The absorbance of each sample was read at a wavelength of 530 nm using a spectrophotometer.

Statistical analysis

The experiment comprised a completely randomized design with three replications under in vitro conditions in laboratories of the Agricultural Sciences and Natural Resources Department at Guilan University and Gorgan

University (2020-2021). Data analysis was performed using SAS version 9.4 software, and mean values were compared using the least significant difference (LSD) ($p \leq 0.01$). Figures were illustrated using Microsoft Excel.

Results

Stem length

Table 1 indicates that the effect of treatments on stem length was significant ($p \leq 0.01$). In the case of some extracts, an increase in extract concentration resulted in a decrease in seedling

length, whereas this relationship had a linear pattern in other cases (Fig. 2). The results largely depended on plant species and extract concentration. For example, as seen in Figure 2, *Juniperus sabina*, *Rheum ribes*, and *Taraxacum officinale* treatments led to a decrease in growth when using them at high extract concentrations. However, *Allium jesdianum*, *Dorema aucheri*, and *Conocarpus erectus* treatments resulted in enhanced growth when using them at high extract concentrations (Fig. 2).

Table 1. Statistical analysis of the effect of treatments on the morphological and biochemical properties of tomato callus.

S.O.V	df	SL	SFW	CW	CGI	TP	TF	Anti-oxidant	Auxin
Treatments	12	1.54**	0.0025**	0.0078**	1.22**	271.1 ^{ns}	6.44**	123.22**	0.0023**
Error	26	0.06	0.0004	0.0014	0.34	144.05	1.63	37.09	0.00047
CV		3.46	11.58	25.45	39.56	18.68	13.73	7.88	21.83

^{ns} non-significant and * and ** are significant at 5 and 1 percent probability levels, respectively.

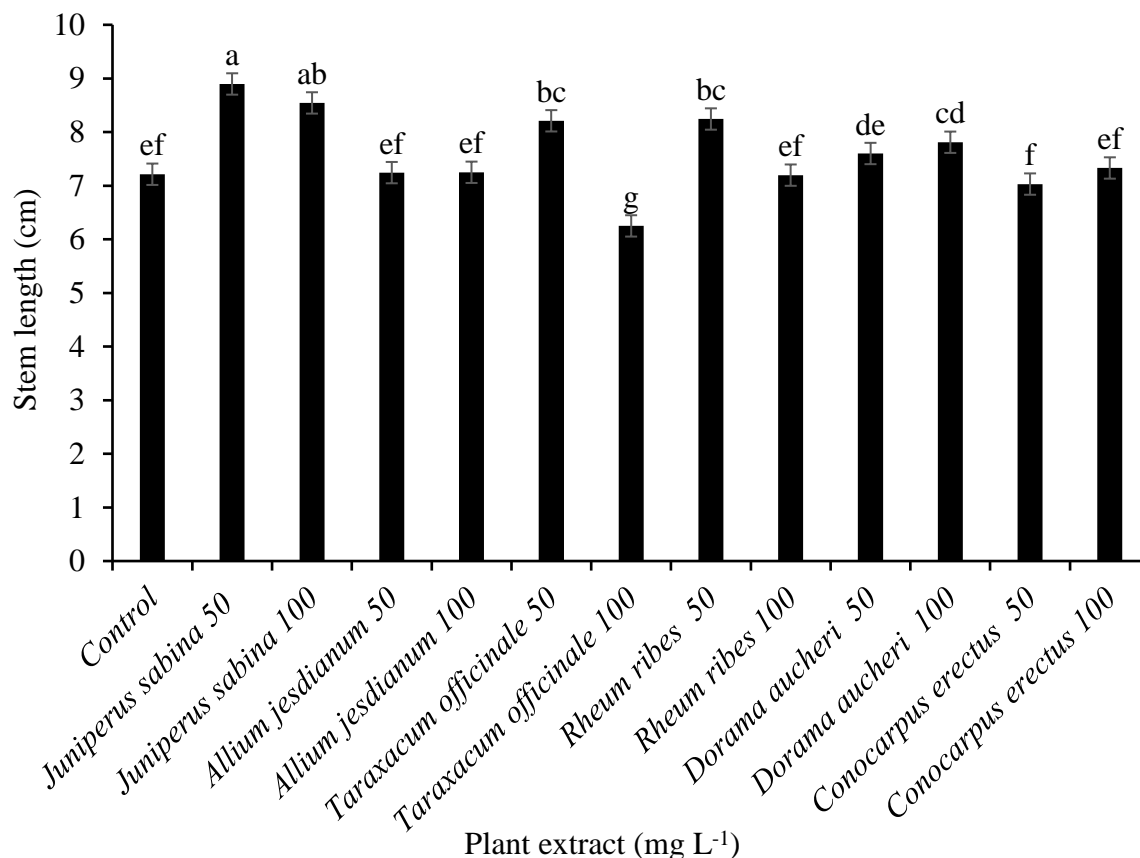


Fig. 2. Effects of medicinal plant extracts on tomato seedling stem length *in vitro*. Control was MS medium without any plant extract.

Additionally, the biochemical potential of the medicinal plant extracts revealed their hormonal

potential, particularly with the presence of auxin (Table 2).

Table 2. Biochemical profile and amount of auxin in medicinal plant extracts.

Medicinal plant	TP (mg g ⁻¹)	TF (mg g ⁻¹)	Antioxidant potential (%)	Auxin (mg g ⁻¹)
<i>J. sabina</i>	31.1870	15.893	74.285	0.3838
<i>A. jesdianum</i>	21.0870	15.898	12.780	0.3260
<i>T. officinale</i>	22.595	14.757	80.483	0.2832
<i>C. erectus</i>	62.545	18.56	67.568	0.2553
<i>D. aucheri</i>	31.663	17.043	58.46	0.3567
<i>R. ribes</i>	40.269	15.5	80.805	0.1483

Highest growth in stem length (8.9 cm \pm 0.117 cm) was observed in response to *Juniperus sabina* extract at 50 mg L⁻¹ compared to the control (7.2 cm \pm 0.117). Moreover, no significant difference was observed between seedlings treated with *Juniperus sabina*, *Dorema aucheri*, *Allium jesdianum*, and *Conocarpus erectus* extracts (Fig. 2). On the other hand, in some treatments, a high concentration resulted in suppression. For example, using *Taraxacum officinale* extract at 100 mg L⁻¹ decreased the stem length to 6.25 \pm 0.117 cm compared to the control (7.2 \pm 0.117 cm). In addition to differences in morphology, the germination rate of seeds cultivated in the culture medium with plant extracts was much higher than the control. Figure 2 shows the results of using the plant extracts. For instance, the *Juniperus sabina* extract (at both concentrations) stimulated growth, while *Taraxacum officinale* at 100 mg L⁻¹ reduced stem length. Based on our results, tomato seedlings showed significant growth differences under the influence of the medicinal treatment extracts. As shown in Figure

2, the *Taraxacum officinale* plant extract at 100 mg L⁻¹ (6.25 \pm 0.117 cm) decreased stem growth compared to the control (7.21 \pm 0.117 cm).

Seedling fresh weight

Seedling fresh weight was also significantly affected by the treatments ($p \leq 0.01$) (Table 1). Furthermore, it was evident that the plant extracts had various effects on seedling fresh weight (Fig. 3). Some treatments had stimulatory effects, while others had inhibitory effects. As revealed in Figure 3, some treatments such as *Juniperus sabina* extract increased SFW through a linear pattern of changes in extract concentration. In contrast, a negative relation was observed in SFW by increasing the *Allium jesdianum* extract concentration.

The highest seedling fresh weight was observed in response to 100 mg L⁻¹ *Juniperus sabina* extract (0.231 \pm 0.0052 g), while the lowest weight was observed in response to 50 mg L⁻¹ *Rheum ribes* (0.129 \pm 0.0052 g) (Fig. 3).

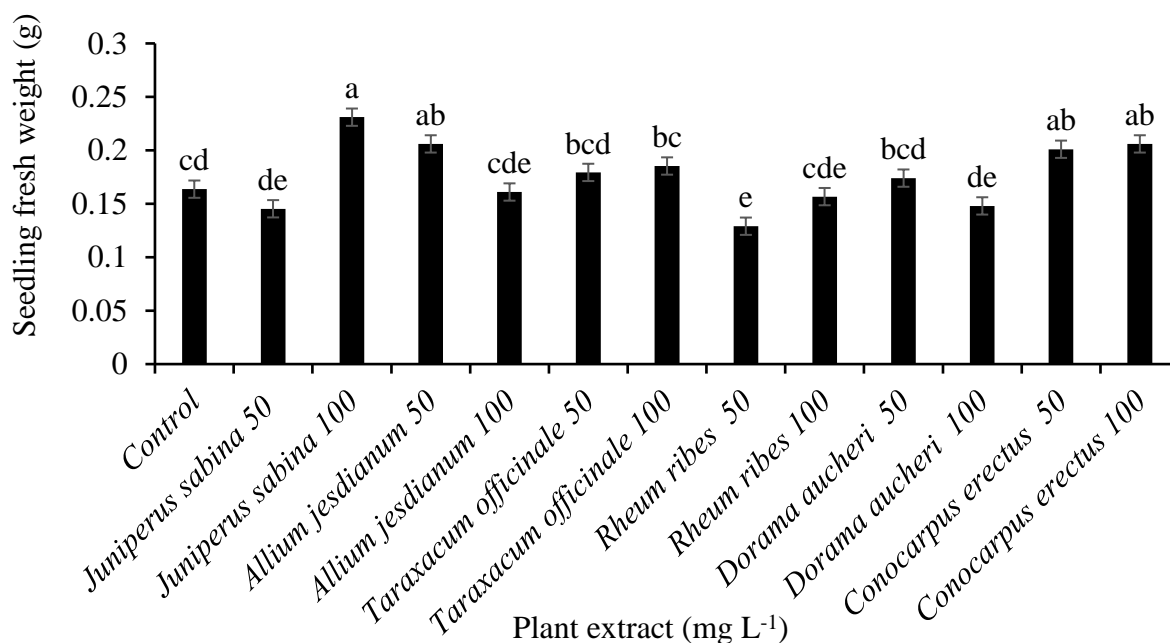


Fig. 3. Effects of medicinal plant extracts on tomato seedling fresh weight *in vitro*. Control was MS medium without any plant extract.

Callus growth index

Table 1 indicates that the effect of treatments on CGI was significant ($p \leq 0.01$). *Taraxacum officinale* extract at 100 mg L⁻¹ resulted in the

highest CGI (2.5 ± 0.126). In contrast, the lowest CGI was observed in control plants (0.5 ± 0.126). All treatments showed significant differences with the control group (Fig. 4).

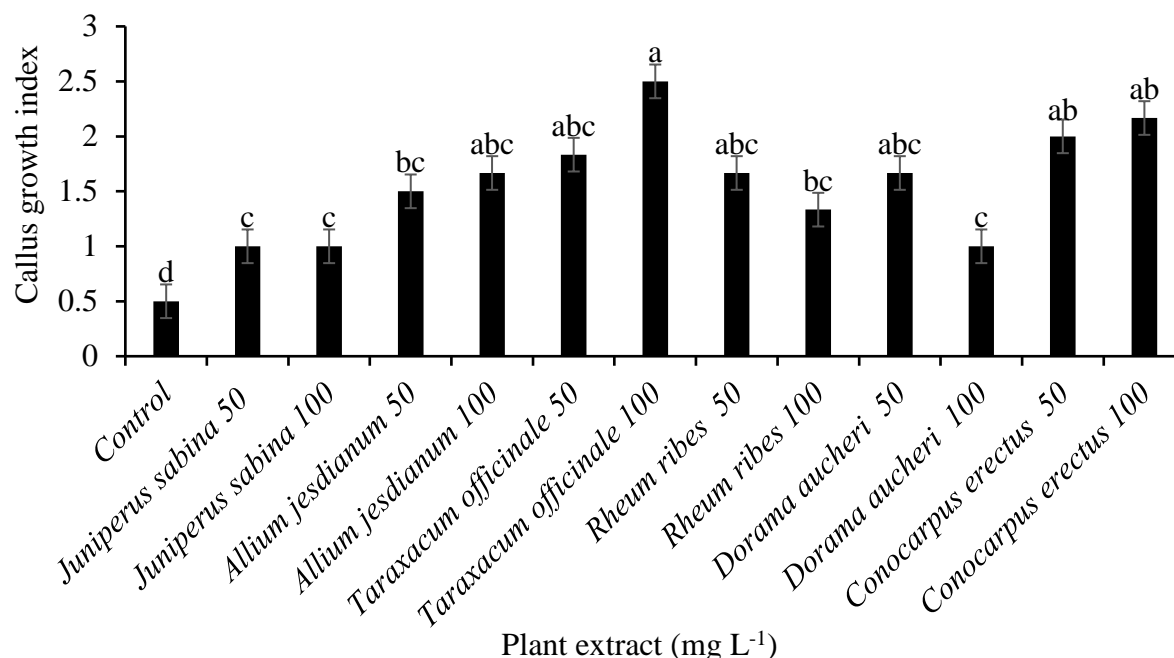


Fig. 4. Effects of medicinal plant extracts on tomato callus growth index *in vitro*. Control was MS medium without any plant extract.

An interesting finding was the versatility of the *Rheum ribes* plant extract, where CGI decreased in response to the increase in extract concentration (Figs. 6C and D). A significant difference in CGI between the control and other treatments are evident in Figures 5 and 6. In the control group, the MS basal medium that contained some amounts of plant growth regulators (auxin and cytokinin) did not yield significant results after a two-week period. However, the culture medium supplemented with medicinal plant extracts demonstrated a remarkable outcome in this regard (Figs. 5 and 6).

Auxin content

Based on the analysis of variance (Table 1), a significant difference ($p \leq 0.01$) was observed in the auxin content of callus due to the application of plant extracts *in vitro*. As shown in Figure 7, the control treatment led to the highest amount of

internal auxin in callus ($0.16 \pm 0.0052 \mu\text{g g}^{-1}$), whereas the *Allium jesdianum* extract (50 mg L⁻¹) caused the lowest auxin content in callus ($0.062 \pm 0.0052 \mu\text{g g}^{-1}$).

As shown in Figure 7, a downward trend was observed in the amount of auxin in all treatments compared to the control. An increase in *Juniperus sabina* and *Dorema aucheri* extract concentrations led to a decrease in internal auxin content, whereas *Conocarpus erectus*, *Allium jesdianum*, *Taraxacum officinale*, and *Rheum ribes* extracts increased the internal auxin content. Table 2 shows that extracts of medicinal plants contain high amounts of specific compounds and auxin. The sensitivity of callus cells to auxin may be higher compared to mature plants, which could explain the relationship between the lowest CGI and the highest internal auxin content in each sample (Figs. 5 and 6).

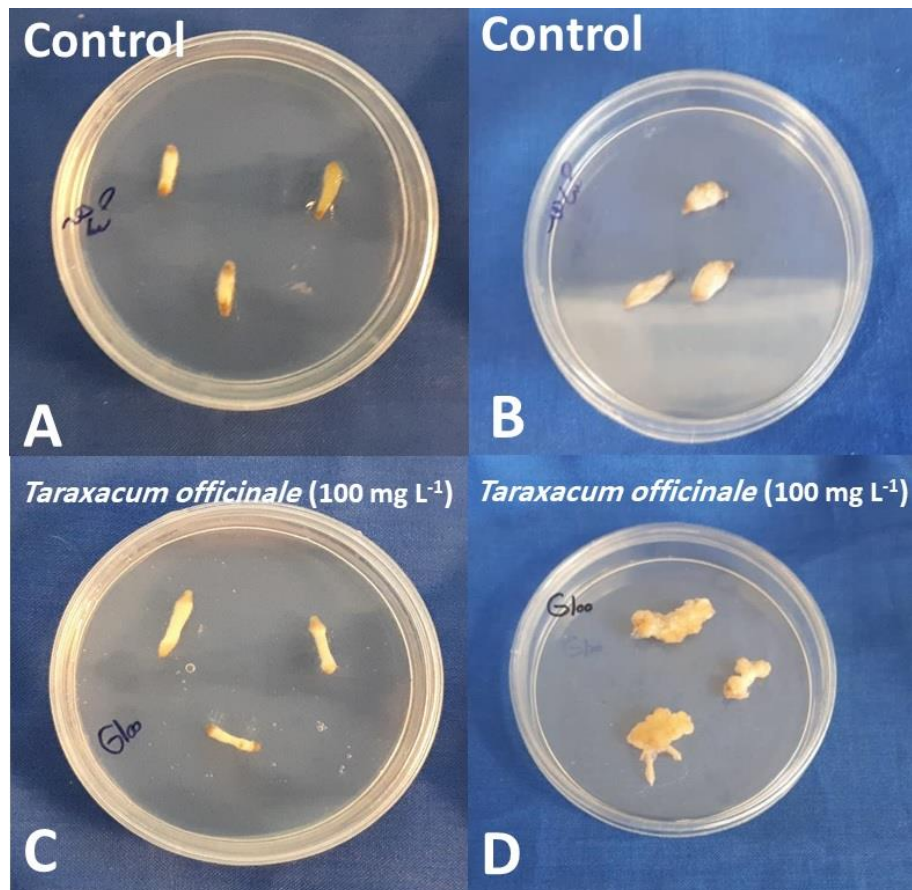


Fig. 5. (A) Control sample on the first day. (B) Control sample after two weeks. (C) Medium with *Taraxacum officinale* extract on the first day. (D) Medium with *Taraxacum officinale* extract after two weeks. The control was MS medium without any plant extract.

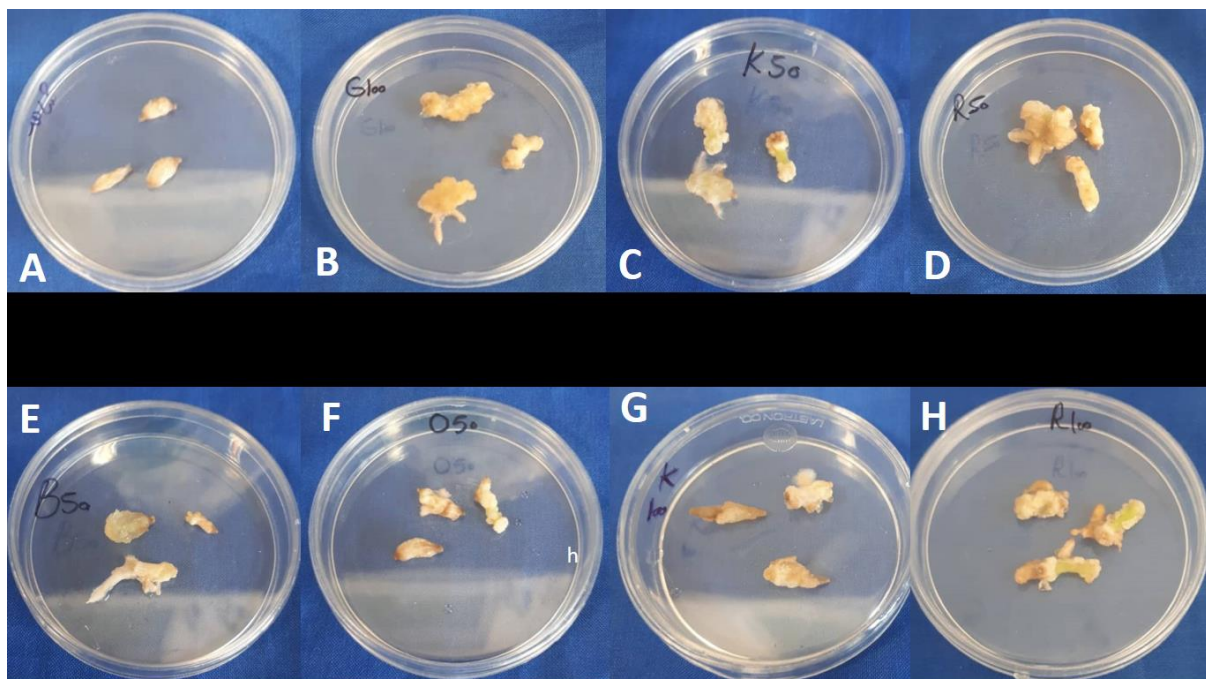


Fig. 6. Effects of different medicinal plant extracts on tomato callus formation compared to the control group. (A) Control, (B) *Taraxacum officinale* 100 mg L⁻¹, (C) *Conocarpus erectus*, (D) *Rheum ribes* 50 mg L⁻¹, (E) *Allium jesdianum* 50 mg L⁻¹, (F) *Juniperus sabina* 50 mg L⁻¹, (G) *Conocarpus erectus* 100 mg L⁻¹, (H) *Rheum ribes* 100 mg L⁻¹. The control was MS medium without any plant extract.

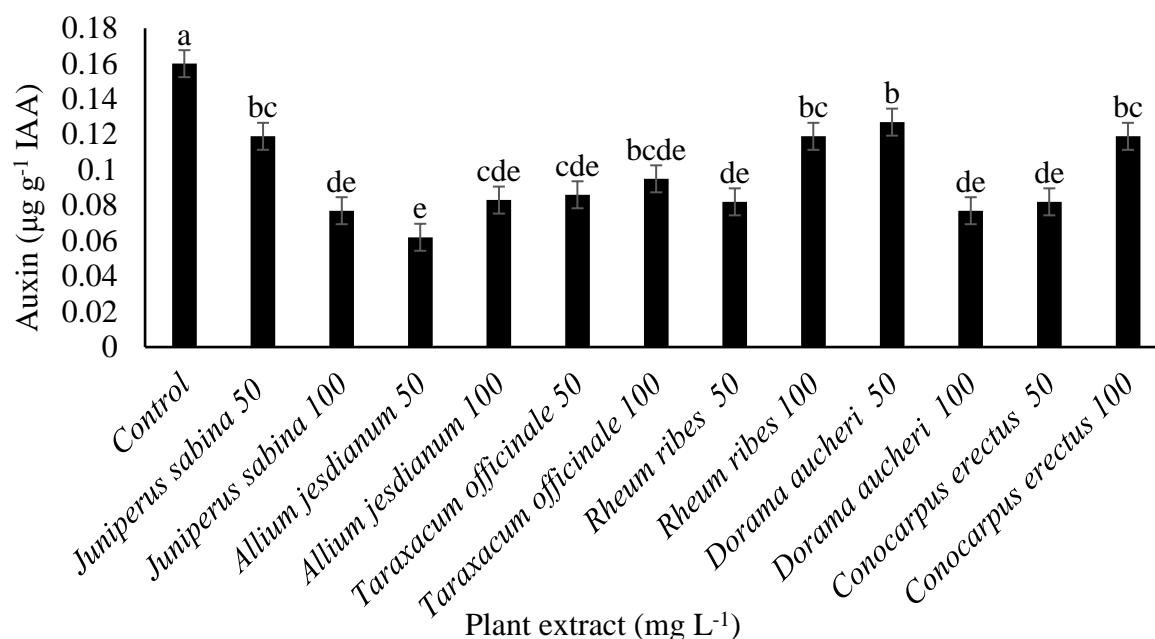


Fig. 7. Effects of medicinal plant extracts on tomato callus auxin content *in vitro*. Control was MS medium without any plant extract.

Callus weight

Plant extracts showed significant effects ($p \leq 0.01$) on CW *in vitro* (Table 1). As shown in Figure 8, the control group showed the lowest CW (0.05 ± 0.0052 g). *Conocarpus erectus* extract at 50 mg L^{-1} caused the highest CW (0.24 ± 0.0052 g). All treatments resulted in an increase in CW

(Fig. 8), which is probably due to amplified cell growth and division. The observed increase in CW is consistent with the increase in other traits such as callus volume, growth, and internal auxin levels. As mentioned, callus samples treated with *Conocarpus erectus* extract at 50 mg L^{-1} exhibited the highest CW, whereas the control samples had the lowest CW (Fig. 8).

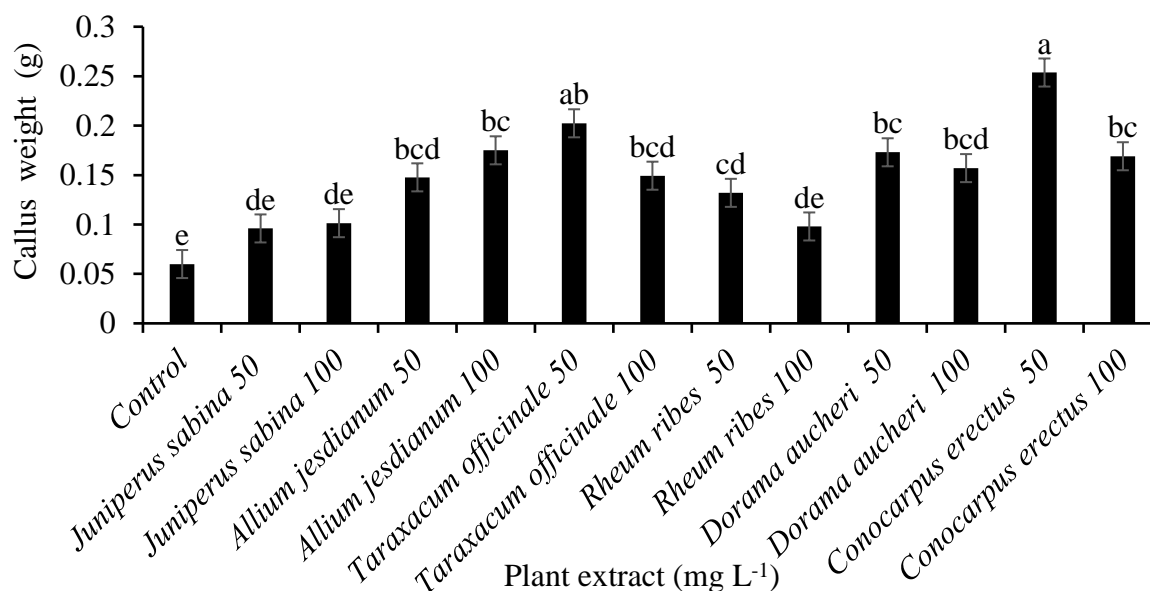


Fig. 8. Effects of medicinal plant extracts on tomato callus weight *in vitro*. The control was MS medium without any plant extract.

Total phenol

Plant extracts caused no significant difference in

total phenol content of tomato callus *in vitro* (Fig. 9).

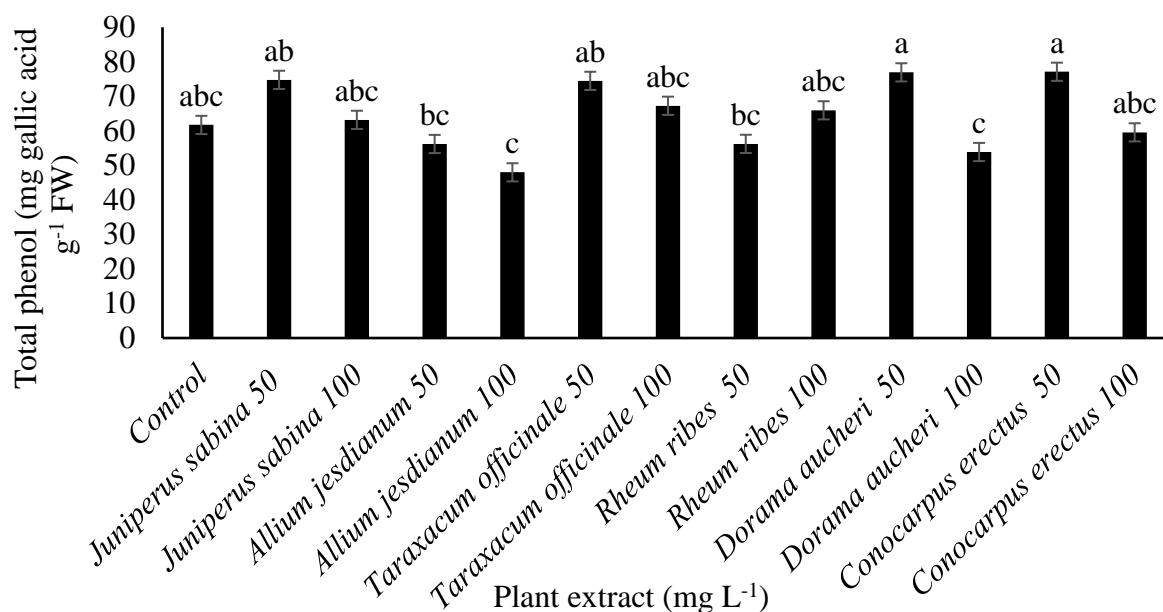


Fig. 9. Effects of medicinal plant extracts on tomato phenol content *in vitro*. The control was MS medium without any plant extract.

Total flavonoids

A significant difference ($p \leq 0.01$) was observed from the effects of plant extracts on total flavonoids (Table 1). The highest amount of total flavonoids ($12.16 \pm 0.284 \text{ mg g}^{-1}$) was observed in response to 50 mg L^{-1} *Conocarpus erectus* treatment, whereas the lowest was observed in response to 50 mg L^{-1} *Rheum ribes* treatment

($6.58 \pm 0.284 \text{ mg g}^{-1}$) (Fig. 10). The present study showed that the total flavonoids increased, parallel to the increase in extract concentration from 50 to 100 mg L^{-1} in all treatments except the *Conocarpus erectus* treatment. In some treatments, no statistically significant differences were observed. Total flavonoids decreased, parallel to the increase in *Conocarpus erectus* extract concentration from 50 to 100 mg L^{-1} .

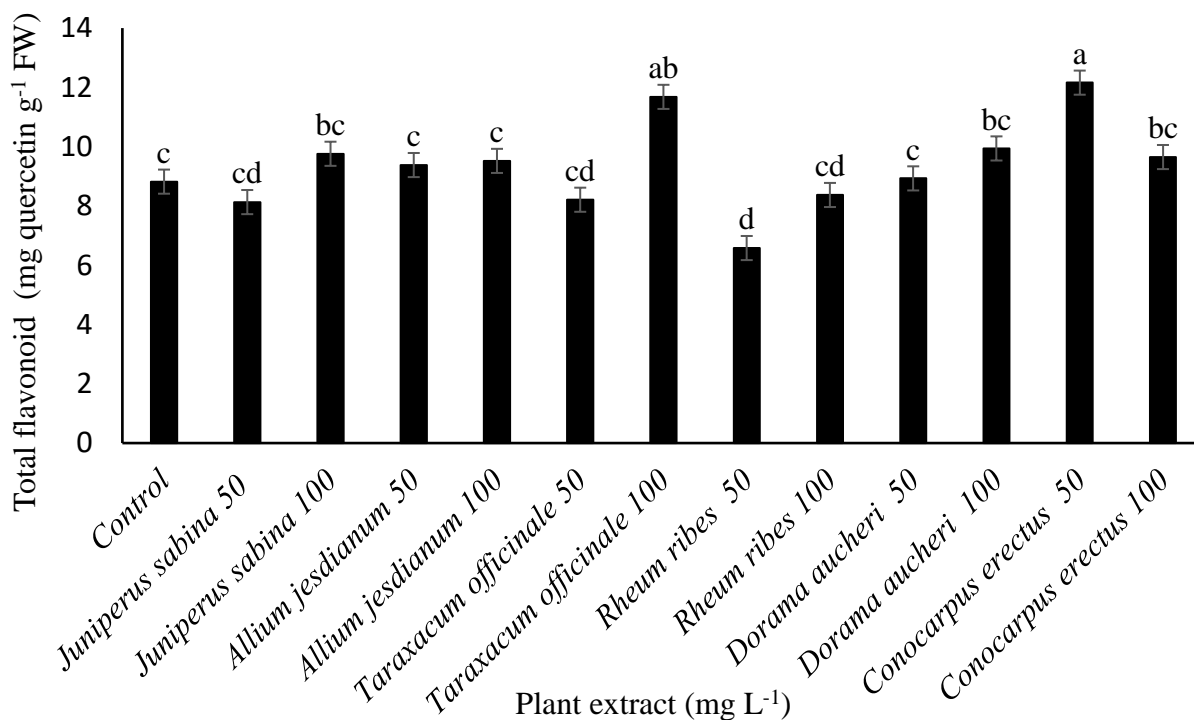


Fig. 10. Effects of medicinal plant extracts on total flavonoid content of tomato callus *in vitro*. The control was MS medium without any plant extract.

Antioxidant activity (DPPH)

A significant difference ($p \leq 0.01$) was observed in antioxidant activity (DPPH) among the treatment groups and the control (Table 1). The lowest

amount of antioxidant activity ($63.97 \pm 1.283\%$) was observed in the control group, while the application of plant extracts improved the antioxidant activity to various extents (Fig. 11).

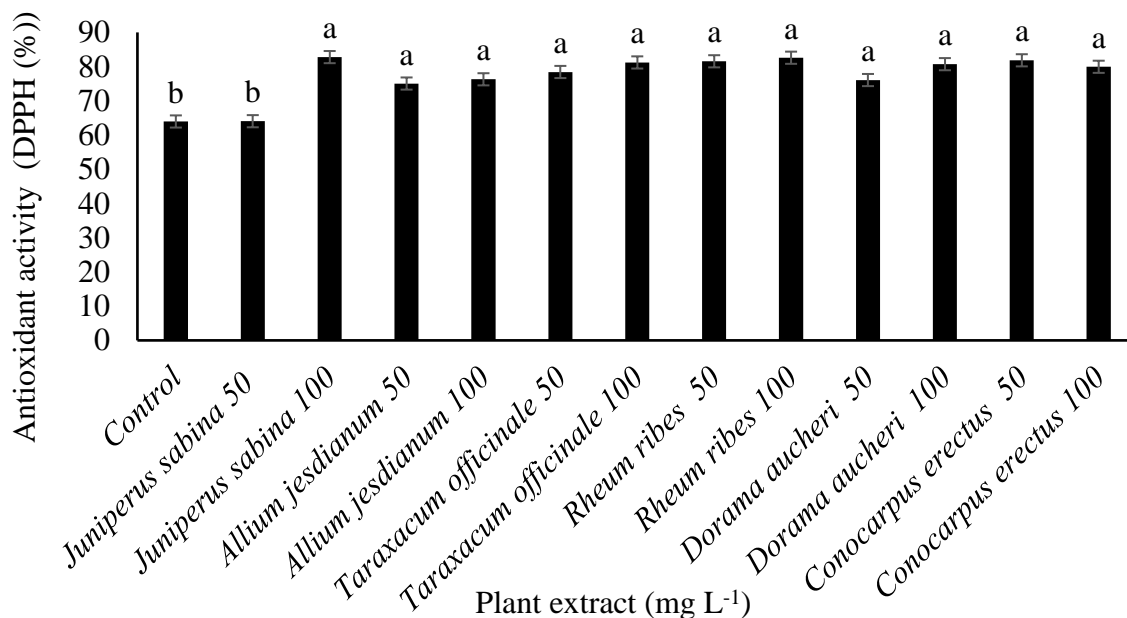


Fig. 11. Effects of medicinal plant extracts on antioxidant activity of tomato callus *in vitro*. Control was MS medium without any plant extract.

Discussion

Effect of pure plant extract on stem length

Typically, chemicals have a stimulatory effect on plants at low concentrations and a suppressive effect at higher concentrations (Qasem and Foy, 2001). However, the current findings about stem length suggest that plant extracts can exhibit various effects at different concentrations. Biological stimulants are substances of natural origin that have beneficial effects on the growth and development of plants, their resistance to stress, their yield, and product quality. The physiological effects of these stimulants depend on their composition, which includes various organic and inorganic compounds that plants can use as metabolites and growth regulators (Paradikovic et al., 2019). Previous studies on crops have shown that seaweed extract can enhance growth vigor in bean seeds (Carvalho et al., 2014). Bio-stimulants can include a variety of compounds, such as amino acids, saponins, vitamins, PGR, and plant compounds, as demonstrated in previous research (Paradikovic et al., 2019). However, caffeic acid reportedly decreased stem length at high concentrations (Batish et al., 2008). As mentioned earlier, plant height is influenced by growth and determined by

cell division and enlargement. These processes depend strongly on internal regulators in plants, including auxin and cytokinin (Taiz et al., 2015). According to Table 2, plant extracts contain numerous compounds such as phenols, phenolic acids, terpenes, and auxin. As previously mentioned, plants have various compounds that can transform into other compounds through biological pathways (Vermerris and Nicholson, 2007). These substances can become precursors of plant regulators during intracellular processes, following the biosynthesis pathway of growth regulators (Negi et al., 2005; Peer and Murphy, 2007; Nagata et al., 1992). Using medicinal plants to evaluate their impact on the growth of other plants has been limited to *in vitro* culture experiments. Previous studies have mainly focused on investigating the effects of different plant compounds, such as adding allelopathic plant powder to agar (ECAM (equal compartment agar medium)) (Mushtaq et al., 2020). In another study, researchers compared plant compounds and growth regulators that improved the micro-propagation process in acacia plants (*Robinia pseudoacacia* L.) through tissue culture. Plant samples were supplemented with 0, 1000, 2000, and 3000 mg L⁻¹ *Ascophyllum nodosum* brown

algae extract in MS medium. The results showed that the *Ascophyllum nodosum* extract (1000 mg L⁻¹) led to the highest number of roots among other treatments (Kaviani et al., 2016), which confirms the stimulatory effects observed in our findings. It should be noted that during the process of starch decomposition and germination, seeds produce free radicals within themselves due to biochemical and physiological interactions (Taiz et al., 2015). These free radicals can affect growth through their impact on cell organelles. However, medicinal plants are rich in compounds that counteract these radicals, which means that seedlings exposed to these specialized compounds absorb fewer radicals during seed germination and growth, leading to safer and faster growth. Similar to the current research, previous findings showed that cylindrospermopsin (CYN) had various effects at different concentrations, thus being consistent with our results (Garda et al., 2015). Other studies on plant extracts affecting other plants have shown that aqueous extracts of *Calotropis procera* L. can increase germination and seedling growth in corn (Naz and Bano, 2014). Interestingly, the same extract had an inhibitory effect on wild cabbage (*Brassica oleracea* L.) and botrytis variety (Gulzar and Siddiqui, 2017). This observation is crucial because it reveals that herbal compositions can affect plants differently, which was also evident in our study. For instance, the *Juniperus sabina* extract (at both concentrations) stimulated growth, while *Taraxacum officinale* at 100 mg L⁻¹ decreased stem growth and length. Furthermore, another study showed that aqueous extracts of *Celosia argentea* obtained from its leaf, stem, and flower increased α -amylase activity in lentils (*Lens culinaris* Medic) (Kengar and Patil, 2018). It can be concluded that plant extracts have different effects due to variations in chemical substances and their various levels of PGRs.

Effect of pure plant extract on seedling fresh weight

The changes in SFW further confirmed that different extracts have varying effects on plant growth and development. It is important to note that plant growth and development are complex processes and can change in response to multiple factors, including the genetic base of a plant, environmental conditions, and the presence of various signaling molecules and growth regulators. Therefore, research on the effects of medicinal plant extracts on tomato plant growth and development needs to consider these factors and carefully design experiments that can

accurately capture the effectiveness of these extracts on plant growth.

Our study provided valuable insights into the potential use of herbal plant extracts in promoting plant growth and highlighted the need for further research in this field. Our findings are consistent with previous studies that have demonstrated a decrease in sesame (*Sesamum indicum* L.) fresh weight in response to an aqueous extract of billygoat weed (*Ageratum conyzoides* L.) (Natarajan et al., 2014). Additionally, a decrease occurred in mustard plant seed germination, root length, branch length, chlorophyll content, fresh weight (FW), dry weight (DW), and relative water content (RWC) in response to aqueous extracts of senna tora roots, stems, and leaves (*Cassia tora* L.) (Sarkar et al., 2012). The differences in growth in our study can be attributed to variations in the biochemical and phytochemical profiles of the extracts (Table 2). These differences relate to phenolic, antioxidant, and hormonal substances. Research has also shown that using aqueous seaweed extract can increase plant biomass in various plants (De Saeger et al., 2020). These findings are consistent with our results and suggest that the application of different plant extracts can have varying effects on plant growth and development. Therefore, it is crucial to carefully select and evaluate appropriate plant extracts as they affect specific plants and as researchers aim to achieve optimal growth. Overall, our study contributes to the current knowledge on potential plant extract usage as a natural and sustainable alternative to traditional chemical fertilizers in promoting plant growth and development.

Effects of pure plant extract on callus growth index

During cell division and growth, plant cells produce free radicals that can target intracellular organelles, thus reducing their efficiency. This effect is particularly evident when preparing explants for tissue culture (Taiz et al., 2015). However, high levels of phenolic and antioxidant compounds in the plant extracts (Table 2) can neutralize the effects of these free radicals and promote better cellular activity and growth. Additionally, compounds in the extracts serve as biological stimulators for plant growth as they contain abundant precursors of plant growth regulators, leading to increased callus formation. A correct balance between auxin and cytokinin hormone levels is crucial for callus formation (Taiz et al., 2015).

Effects of pure plant extract on auxin content

High levels of auxin and cytokinin can direct cell division in specific ways. However, the ratio between these two regulators is the final determining factor in growth (Taiz et al., 2015). Cell division and cell enlargement are two important mechanisms that influence growth, and they are influenced by growth regulators such as auxin, cytokinin, gibberellin, ethylene, abscisic acid, jasmonic acid, brassinosteroids, and salicylic acid. Research over many years has demonstrated that two regulators, auxin and cytokinin, play a vital role in cell division. Therefore, regarding the developmental process, these two regulators can be relative indicators for physiological studies (Taiz et al., 2015). Auxin, a regulator of cell growth, is a basic factor in the cell cycle. A study demonstrated that when tobacco plant cells were cultured without an auxin source in the culture medium, the cells performed DNA centration but could not complete the cell cycle and stopped in the G1 phase. Later, auxin was added to the culture medium, and the expression of genes was checked, revealing that *arcA* gene expression was induced (Ishida et al., 1993). Auxin acts as a growth stimulant in low concentrations, a growth inhibitor, and a herbicide (2-4-D) in high concentrations (Taiz et al., 2015). Medicinal plants contain many compounds (Vermerris and Nicholson, 2007) that can affect auxin content in various ways. Some compounds can act as inhibitors of auxin biosynthesis, while others can stimulate auxin production. For example, flavonoids, a type of plant pigment, can increase auxin levels in some plants by up regulating the expression of auxin biosynthesis genes (Peer and Murphy, 2007). In contrast, some alkaloid compounds, such as cinchonine and quinine, can inhibit auxin biosynthesis in plants (Nagata et al., 1992). The relationship between plant compounds and auxin content in plants is complex and varies depending on the specific compound and plant species involved. Plant extracts can change auxin content depending on the type of plant or concentration used (Fig. 7). For instance, past studies with elevated carbon dioxide have demonstrated that polyphenols increase growth by intensifying the effects of auxin by preventing IAA decarboxylation (Tomaszewski and Thimann, 1996). Sinapic and ferulic acids can increase growth regulators by preventing IAA decarboxylation, while monophenols can do the opposite and increase IAA decarboxylation. Phenolic compounds can change growth regulator contents (Tomaszewski and Thimann, 1996). Studies on different plant compounds

confirmed that these compounds effectively induce some hormonal changes in plants. For instance, some flavonoid aglycones can disturb the polar transport of auxin (Brunn et al., 1992). In another study, benzoic acid and its derivatives (such as 3, 4-dihydroxybenzoic acid (DHB)) stimulate callus formation at low concentrations but have a suppressive effect on root growth at high concentrations in tobacco plants (Mucciarelli et al., 2000). It is important to note that higher auxin concentrations stimulate ethylene production (Taiz et al., 2015). Therefore, if we attribute the role of auxin analog to 3, 4-DHB, the suppressive effect is proven at higher concentrations of this compound. This fact may explain the concentration-dependent effect of allelochemicals that mimic or affect auxin synthesis. In contrast, other studies have reported that some phenolic compounds can prevent auxin degradation, leading to auxin accumulation (Mato et al., 1994; Cvikrova et al., 1996). However, gene studies currently under investigation in other studies may provide stronger confirmations. Growth is not solely under the control of a plant growth regulator, and the ratio of auxin to cytokinin is an important factor for growth regulation (Taiz et al., 2015). Some compounds can affect the biosynthesis pathway and production of a compound, such as lovastatin, which inhibits the production of mevalonic acid and is a significant anti-cytokinin that prevents normal plant growth (Laureys et al., 1998). It is valuable because it highlights that compounds in plant extracts can affect the biosynthesis pathway of plant growth regulators in specific concentrations, as shown in the results of the present study. For example, coumaric acid is one of the dominant and important compounds in dandelion (Piccolella et al., 2023), and it can transform into salicylic acid in its biochemical pathway (Taiz et al., 2015). Salicylic acid can act as a stimulant at low concentrations and a growth inhibitor at high concentrations (Horvath et al., 2007). Various studies have shown that plant compounds (plant extracts) can suppress or stimulate enzymes involved in the biosynthesis of plant compounds. For instance, the production of IAA is suppressed and stimulated by different concentrations of different plant extracts (ALHaithloul et al., 2022; Abou El-Ghit et al., 2016), or the production of ethylene and ABA increases with an increase in allelopathy (Bogatek et al., 2005). In another study, *Artemisia argyi* water extract caused auxin accumulation in roots (Li et al., 2022). Considering that growth conditions (light, temperature, culture medium, samples) were identical, the differences in internal auxin content were attributable to plant

extracts. Further enzymatic and molecular investigations can gain a deeper understanding of this process.

Effect of pure plant extract on callus weight

Several studies have reported that plant compounds can affect plant growth and development by regulating various physiological processes, including cell growth and division. For example, Alsharekh et al. (2022) suggested that different plant compounds, such as secondary metabolites, can act as growth regulators by influencing cell division and differentiation. Similarly, a study by Bashar et al. (2023) demonstrated that secondary metabolites can serve as allelochemicals and affect plant growth and development by regulating gene expression and metabolic pathways. These results suggest that plant compounds in the extracts can affect cell growth and division, leading to changes in callus weight. The present study is in line with previous research on the effects of plant compounds on growth regulation. The results suggest that plant extracts containing specific compounds can be used as growth regulators to enhance plant growth and development. Further research is needed to explore the mechanisms of action of these compounds and their potential applications in agriculture and medicinal applications.

Effect of pure plant extract on total phenol

The concept of allelopathy, which refers to the ability of plants to influence the growth and development of neighboring plants through the release of chemical compounds, has been well-established in the literature (Mushtaq et al., 2020). A study by Singh et al. (2006) demonstrated that alpha-pinene, a major compound in *Juniperus sabina*, increased the activity of antioxidant enzymes. Singh et al. (2006) showed that alpha-pinene effectively enhanced the activity of antioxidant enzymes in rice plants under drought stress.

Furthermore, a study by Khatun et al. (2023) demonstrated that extracts from *Trewia nudiflora* Linn. had a significant effect on the growth and physiology of lettuce (*Lactuca sativa* L.) and foxtail fescue (*Vulpia myuros* L.). The bell-shaped response observed in the present study, where a low concentration of compounds is stimulatory while a high concentration inhibits growth, is common in plant biochemistry (Batish et al., 2008). A study by Desoky et al. (2020) demonstrated the differential effects of different plant extracts on the enzymatic and non-enzymatic defense systems in *Vigna*

unguiculata L., which is consistent with a recent study by Bingol et al. (2022). It showed that extracts of *Xanthoparmelia somloensis* had varying effects on the growth and physiology of tomato plants. A study by Laureys et al. (1998) showed that lovastatin, a compound against mevalonic acid, prevents cytokinin production. It highlighted the importance of studying the effects of plant compounds on other plants, as observed in the present study. A study by Hassanzadeh (2014) suggested that the stimulation of the plant immune system (SAR) may be the main factor responsible for the observed effects in the present study, which is consistent with a recent study by Li et al. (2021) that the application of plant extracts can activate the SAR pathway and enhance the resistance of plants to various stresses.

However, further studies are needed to elucidate specific mechanisms underlying the observed effects in the present study. Overall, the present study and recent research provide insights into the mechanisms underlying the effects of plant extracts on neighboring plants.

Effect of pure plant extract on total flavonoids

Our findings are consistent with a recent study by Mutale-Joan et al. (2020), which showed that the concentration of microalgae extracts can significantly affect the metabolite profile in tomatoes. Also, this finding is in agreement with a recent study by El-Shora et al. (2022), which demonstrated that the application of *Rumex dentatus* L. plant extracts can increase the production of flavonoids in *Portulaca oleraceae*. An ex vitro study by Salvi et al. (2019) also showed that foliar application of seaweed extract increased the amount of anthocyanin and flavonols in grape plants, further supporting the findings of the present study. However, as mentioned earlier, this may be due to the stimulation and suppression of the plant's immune system, and further enzyme studies are needed to provide stronger evidence. A study by Kengar and Patil (2018) demonstrated that the aqueous extracts of the leaf, stem, and flower of *Celosia argentea* L. increased the α -amylase activity of lentils, which supports the argument that plant extracts can have varying effects on the growth and development of neighboring plants, as observed in the present study.

Finally, the results of the present study suggest that allelopathic substances can have varying effects on different plant species, depending on the type of plant and its concentration. This finding is consistent with previous studies

(Mushtaq et al., 2020) and highlights the importance of investigating specific compounds responsible for the observed effects in future studies.

Effect of pure plant extract on antioxidant activity (DPPH)

Reactive oxygen species (ROS) are compounds with high energy levels that can have destructive effects on cells, causing disruptions in growth processes (Taiz et al., 2015). Antioxidant activity can reduce the harmful effects of these compounds and create a low-stress environment for cell growth. The biochemical profile of herbal treatments shown in Table 2 also indicates that the plant extracts are rich in phenolic compounds and antioxidants, which may contribute to stress-free growth for the plant. The observed increase in antioxidant activity is consistent with the findings of several previous studies. For example, Desoky et al. (2020) reported that foliar application of the extracts of two medicinal plants, fennel (*Foeniculum vulgare* L.) and toothpick (*Ammi visnaga* L.), at a concentration of 2000 mg L⁻¹, increased the antioxidant potential compared to the control plant. Similarly, a study by Singh et al. (2015) showed that the internal chemical compounds of tobacco leaves (*N. plumbaginifolia*) stimulated some enzymes (CAT and SOD) in sunflowers. These findings suggest that plant extracts can cause changes in specialized compounds in plants, leading to an increase in antioxidant activity. Further enzyme studies can be conducted in future stages of the current research to investigate the potential mechanisms underlying the observed effects on antioxidant activity. For example, the activities of antioxidant enzymes such as catalase, peroxidase, and superoxide dismutase can be measured to provide insights into the mechanisms of action of the plant extracts. In conclusion, the present study showed that plant extracts can increase antioxidant activity in plants. The findings are consistent with previous studies highlighting plant extracts as a natural and sustainable means of enhancing plant growth and development.

Conclusion

In conclusion, natural plant extracts are a promising source for various applications due to their compatibility with the environment and potential to replace synthetic chemicals. The indiscriminate use of chemical-based fertilizers and pesticides can have environmental implications, emphasizing the importance of research that focuses on introducing herbal compounds. The high diversity of natural

compounds found in various plant species increases the chance of achieving desirable results by different applications. However, as the results of this study demonstrated, the effects of a plant extract on plant growth and development vary significantly from plant to plant, as well as on the type and concentration of the extract used. The results showed that the plant extracts significantly influenced the growth of seedlings from seeds, sample regeneration, and tomato callus formation efficiency. This relationship is important to mention that the main difference is between the control group and other treatments, although in some treatments there was no significant difference. Therefore, achieving the desired results from plant extracts requires extensive and targeted research, taking into account the type and composition of the extract as well as the plant species. The findings of this study highlighted the potential of natural plant extracts as effective and environmentally friendly biological compounds. However, to fully realize their potential, further research is needed to understand their mechanisms of action and optimize their use in different plant species and applications. Generally, this study underscores the importance of conducting further research to uncover the full potential of natural plant extracts as sustainable alternatives to synthetic chemicals and to promote the development of environmentally friendly approaches to agriculture and crop production. Finally, these plant extracts in a tissue culture medium are advisable for inducing an increase in callus formation.

Author contributions

Author contributions as mentioned below: OS: Methodology, Formal analysis, Investigation, Data curation, writing original draft. AGH: Project advisor in sabbatical leave, Validation, Writing-review and editing. AH: Project administration, Validation, Writing-review and editing. HS: Consulting in data analysis. VEM: Consulting in data analysis.

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Conflict of Interest

The authors indicate no conflict of interest in this

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