Development of a novel protocol for enhanced tomato callus formation and growth through the use of medicinal Plant extracts

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Abstract

Medicinal plants and their derivatives have been used for centuries in various sectors beyond treatment and nutrition. However, the use of toxic chemicals such as pesticides, fungicides, and herbicides has led to environmental pollution, necessitating the search for eco-friendly alternatives. Medicinal plants and their compounds offer a promising solution due to their naturalness and biodegradability. An in-vitro experiment was conducted at Gorgan University in 2022 to test the impact of six medicinal plant extracts on tomato growth and callus induction. Two concentrations of 50 and 100 mg/L were added to the Murashige and Skoog (MS) medium culture. Results showed a 100% increase in germination speed and an increasing trend in stem length (SL), with the 50 mg/L *Juniperus sabina* treatment yielding the highest amount. The amount of callus growth (CG) and weight (CW) increased by 50% and over 200%, respectively, with the *Taraxacum officinale* 100 and *Conocarpus erectus* 50 mg/L treatments producing the best results. More than 90% callus formation was observed compared to the control. Biochemical analysis revealed that the extracts were rich in phenolic compounds (348 mg/g of total phenol), flavonoids (162 mg/g), antioxidants (61%), and auxin. Antioxidant and plant growth regulators (PGR) content and the stimulation of the plant's defense system likely contributed to the results. Plant extracts also affected the biochemical content of calluses, with the exception of total phenol. Using plant extracts to increase growth and reduce time for callus induction is recommended in tomato tissue culture. However, optimal concentration and combination of medicinal plant extracts for different plant species and growth conditions require further research.

Introduction

In recent years, the increasing global population and the emergence of diseases caused by the consumption of synthetic chemicals have led to a greater emphasis on natural materials in agriculture. As a result, the use of biological stimulants such as microorganisms, humic acid, amino acids, and algae extracts has become an increasingly important and fundamental approach (De Saeger et al. 2020). By using these natural substances, we can avoid harmful chemicals and promote healthier and more sustainable agricultural practices. The expansion of these natural approaches is a positive development that can benefit both farmers and consumers alike. Iran's diverse plant species provide an excellent potential for expanding research in the field of natural growth inhibitory compounds. For centuries, it has been known that plants can affect the growth and development of surrounding plants through the production and secretion of certain substances (Pan et al. 2015). This phenomenon is called allelopathy, and it refers to the ability of plants to stimulate or inhibit the growth of other plants (Mahboobi et al. 2016). Plants produce allelopathic or allelochemical substances, both known and unknown, as specialized compounds such as tannins, phenolic acids, lignins, alkaloids, flavonoids, coumarins, and terpenoids (Da Silva et al. 2015). By studying the allelopathic properties of plants, we can identify novel bioactive compounds that can help control growth and increase crop yield without relying on synthetic chemicals. Allelopathy can prevent or delay seed germination and inhibit plant growth through changes in physiological processes. This phenomenon is known as negative allelopathy (Santonja et al. 2019).

However, allelochemicals do not always have a negative effect on other plants. They may also have a positive effect, a phenomenon known as hormesis or positive allelopathy (Da Silva et al. 2015). For example, research on the effects of seaweed extract on grape plants and its specialized compounds has shown that the application of seaweed extract through foliar spraying can increase the content of anthocyanin (hydrocinnamic acid) and flavonols (Salvi et al. 2019). This example demonstrates the potential for using the allelopathic effects of plants and their specialized compounds to produce herbicides and growth inhibitors, as excessive use of synthetic chemicals has become a major biological challenge. Today, plant growth control is a major 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, juglan, the dominant allelopathic compound of walnut, has been reported to reduce the growth of cucumber by stimulating the synthesis of abscisic acid (ABA) as a growth inhibitor or by preventing the production of growth-promoting hormones such as auxin, cytokinin, and gibberellin (Terzi et al. 2003). Other allelopathic compounds can have negative effects on plant growth as well. For example, the alkaloid compound cylindrospermopsin has been shown to stop the mitosis process of bean plants at high concentrations (Garda et al. 2014), and the *Ziziphus mauritiana* plant extract decreased mitotic indices of onion by increasing concentration (Owolarafe et al., 2020). Similarly, the *Ricinus communis* methanolic extract has a significant effect on corn seed (*Zea mays* L.) germination at different levels, with a concentration of 10 mg/ml resulting in zero germination and 100% germination inhibition potential (Saddiqe et al. 2020). On the other hand, 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 plant (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 the concentration of the extract. For example, garlic extract (*Allium sativum* L.) has been shown to have 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). Understanding the effects of allelopathic compounds on plant growth can help us develop more efficient and sustainable agricultural practices. Tomato belongs to the Solanaceae family and are cultivated worldwide as fruits. This plant is a rich source of lycopene, beta-carotene, flavonoids, and vitamin C. Moreover, the tomato plant has significant potential as a source of bioactive, antioxidant, and antimicrobial compounds (Silva-Beltrán et al. 2015). Tissue culture cultivation of tomatoes is used for clonal propagation of commercial cultivars, production of virus-free cultivars, and genetic modification. Given the benefits of tomato plants, the effects of six medicinal plant extracts, namely *Juniperus sabina* (Adams 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 *Dorama aucheri* (Ajani et al. 2021; Mianabadi et al. 2015), were investigated in seed tissue culture and callus formation of tomato plants. Present work aimed to investigate the impact of six medicinal plant extracts on tomato growth and callus induction.

Material and methods

The experiment was conducted from 2020 to 2021 using a completely randomized design with three replications under in vitro conditions at both Guilan University and Gorgan University of Agricultural Sciences and Natural Resources. Data analysis was performed using SAS version 9.4 software, and mean values were compared using the least significant difference (LSD) test at a one percent probability level. Figures were created using Microsoft Excel. Each replication consisted of three explants, and the samples were mixed from all three explants in each replication to obtain a final sample. The extracts were sterilized using a 45-micron filter and added to Murashige and Skoog (MS) culture medium at two levels of 50 and 100 mg/L (Sohrabi et al. 2022). All plants, except *Conocarpus erectus* and *Juniperus sabina*, were collected from the Kermanshah region and identified based on available sources (Nemati and Jalilian 2011; Sohrabi et al. 2022). The collected plants were 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 the cold maceration method for two periods of 24 hours (Sohrabi et al. 2022). Finally, the powder was prepared (Fig. 1).

To prepare plant samples in vitro, seeds of the CH cultivar of tomatoes (Fallat Company) were initially placed in 70% ethanol for 1 minute. Following this, they were disinfected by immersing them in 5% sodium hypochlorite for 10 minutes 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, with ten seeds in each glass. To produce callus, a 1 cm stem explant was obtained from the resulting seedlings. To add plant extracts to the culture medium, the culture medium was cooled, and the extracts were passed through a 45-micrometer filter before being added. The effect of plant extracts on seed germination was investigated by measuring traits such as stem length (SL) and seedling fresh weight (SFW) Additionally, the rate of callus growth (CG) and callus weight (CW) in cultured samples under the influence of plant extract treatment was also investigated (Alizadeh 2013). The amount of total phenol (TP) and total flavonoid (TF) was measured using the method described by Chang et al. (2002), and the percentage of inhibition of free radicals (DPPH) was determined using the method of 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 of gallic acid using the equation $y = 0.0005x + 0.0257$ ($r2 = 0.9925$), and Quercetin was used to determine the standard curve for total flavonoid measurement, which was expressed as mg/g using the equation $y = 0.002x - 0.0282$ ($r2 = 0.99$). The amount of auxin in the plants was measured using the method described by Gang et al. (2019). In this method, Salkowski's reagent was used to measure 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. Next, 1 ml of the resulting extract was mixed with 2 ml of Salkowski's reagent. To prepare the Salkowski reagent, a 0.5 M solution of $FeCl3$ was prepared, and 1 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 minutes to complete the reaction. The presence of auxin was indicated by a pink color, and the absorbance of the samples was read at a wavelength of 530 nm using a spectrophotometer.

Results and discussion

SL

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

S.O.V	df	SL	SFW	CFW	CG	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.

Table 1 indicates that the effect of treatments on stem length was significant at a 1% level. As shown in Fig. 2, for some extracts, an increase in concentration resulted in a decrease in seedling length, while for others, this relationship had a linear effect. Typically, chemicals have a stimulating effect at low concentrations and a suppressive effect at higher concentrations (Qasem and Foy 2001). However, our results showed different outcomes. For example, as seen in Fig. 2, *Juniperus sabina*, *Rheum ribes*, and *Taraxacum officinale* treatments led to a decrease in growth with increasing concentration, while *Allium jesdianum*, *Dorema aucheri*, and *Conocarpus erectus* treatments resulted in an increase in growth with increasing concentration (Fig. 2). These findings suggest that plants can exhibit various effects at different concentrations. The highest growth in stem length (8.9 cm ± 0.117 cm) was observed in *Juniperus sabina* extract at 50 mg/L compared to the control (7.2 cm ± 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 (e.g., *Taraxacum officinale* treatment resulted in a length of 6.25 ± 0.117 cm compared to the control of 7.2 ± 0.117 cm).

Biological stimulants are substances of natural origin that have beneficial effects on the growth and development of plants, as well as on their resistance to stress and 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 garden crops have shown that seaweed extract can enhance the growth strength of bean seeds (Carvalho et al. 2013). Bio-stimulants can include a variety of compounds such as amino acids, saponins, vitamins, PGR, and plant compounds, as demonstrated in various studies (Paradikovic et al. 2019). However, the effect of caffeic acid has been shown to decrease stem length with increasing concentration (Batish et al. 2008).

As mentioned earlier, plant height is influenced by growth, which is determined by the processes of cell division and cell enlargement. These processes are strongly related to the amount of 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 others during 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. 2012).

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

Medicinal plant	Total phenol (mg/g)	Total Flavonoid (mg/g)	Antioxidant potential (%)	Auxin content (mg/g)
J.sabina	311.870	158.93	74.285	0.3838
A. jesdianum	210.870	158.98	12.780	0.3260
T. officinale	225.95	147.57	80.483	0.2832
C. erectus	625.45	185.6	67.568	0.2553
D. aucheri	316.63	170.43	58.46	0.3567
R. ribes	402.69	155	80.805	0.1483

The use of medicinal plants to investigate their impact on the growth of other plants has been limited to in-vitro culture. Previous studies have mainly focused on investigating the effect of different plant compounds, such as the addition of allelopathic plant powder to agar (ECAM (equal compartment agar medium)) (Mushtaq et al. 2020). In another study, a comparison was made between plant compounds and growth regulators in improving the micro-propagation process in acacia plants (*Robinia pseudoacasia* L.) through tissue culture. The levels of 0, 1000, 2000, and 3000 mg/L of *Ascophyllum nodosum* brown algae extract were investigated in MS medium. The results showed that *Ascophyllum nodosum* extract at a concentration of 1000 mg/Liter or 1 g/liter led to the highest number of roots among other treatments (Kaviani et al. 2016). 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. In addition to differences in morphology, the germination rate of seeds cultivated in the culture medium containing plant extracts was much higher than the control. Figure 2 shows the results of the plant extracts.

A study showed that the special compound Cylindrospermopsin (CYN) had varying effects at different concentrations, which is consistent with our results (Garda et al. 2015). Other studies on the effect of plants on other plants have shown that the aqueous extract 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.), botrytis variety (Gulzar and Siddiqui 2017). This observation is crucial because it reveals that herbal compositions can have different effects on different plants, which is also evident in our study. For instance, the *Juniperus sabina* extract (at both concentrations) stimulated growth, while *Taraxacum officinale* at 100 mg/L reduced stem length growth. Furthermore, another study showed that aqueous extracts of the leaves, stems, and flowers of *Celosia argentea* L. increased the activity of α-amylase on lentils (*Lens culanaris* Medic) (Kengar and Patil 2018). Based on our results, tomato seedlings showed significant growth differences under the influence of the herbal treatment extracts. As shown in Fig. 2, the *Taraxacum officinale* plant extract at 100 mg/L (6.25 ± 0.117 cm) caused a decrease in growth compared to the control (7.21 ± 0.117 cm). It can be concluded that plant extracts have different effects due to variations in chemical substances and different levels of PGR at different concentrations.

SFW

Seedling fresh weight was also significantly affected by the treatments at a 1% level, according to Table 1. Furthermore, based on the average data comparison, it was evident that plant extracts had varying effects on seedling fresh weight (Fig. 3). Some treatments showed an increasing effect, while others showed a decreasing effect. These results further confirm that different extracts have varying effects on plant growth and development. It is important to note that plant growth and development are complex processes that are influenced by a variety of factors, including the genetic makeup of the plant, environmental conditions, and the presence of various signaling molecules and growth regulators. Therefore, studies investigating the effects of medicinal plant extracts on tomato plant growth and development need to take into consideration these various factors and carefully design experiments that can accurately capture the effects of these extracts on plant growth. Overall, our study provides valuable insights into the potential use of herbal plant extracts in promoting plant growth and highlights the need for further research in this area.

The highest fresh weight of the seedlings was observed in the treatment of 100 mg/L *Juniperus sabina* plant (0.231 ± 0.0052 g), while the lowest weight was observed in the treatment of 50 mg/L *Rheum ribes* (0.129 ± 0.0052 g) (Fig. 3). This finding is consistent with previous studies that have demonstrated the reduction of sesame (*Sesamum indicum* L.) fresh weight with the application of aqueous extract of cloud flower (*Ageratum conyzoides* L.) (Natarajan et al. 2014). Additionally, the reduction of mustard plant seed germination, root length, branch length, chlorophyll content, fresh weight (FW), dry weight (DW), and relative amount of water (RWC) with the application of aqueous extract of root, stem, and leaf of wild senna plant (*Cassia tora* L.) was observed (Sarkar et al. 2012). The differences in growth observed in our study can be attributed to the variations in biochemical and phytochemical profiles of the extracts

(Table 2), such as differences in phenolic, antioxidant, and hormonal substances. Research has also shown that the use of 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 important to carefully select and evaluate the appropriate plant extracts for specific plants and growth conditions to achieve optimal growth results. Overall, our study contributes to the growing body of research on the potential use of plant extracts as natural and sustainable alternatives to traditional chemical fertilizers in promoting plant growth and development.

CG

The treatment of *Taraxacum officinale* at a concentration of 100 mg/L resulted in the highest growth of callus (2.5 ± 0.126) (Fig. 4).

During cell division and growth, plant cells produce free radicals that can target intracellular organelles, thereby reducing their efficiency. This effect is particularly evident during the preparation of explants in tissue culture (Taiz et al. 2015). However, the high levels of phenolic and antioxidant compounds present in the applied plant extracts (Table 2) neutralize the effect of these free radicals and promote better activity and growth. Additionally, the compounds in the extracts serve as biological stimulators for growth, as they contain a high level of precursors for plant growth regulators, leading to increased callus formation. The balance of auxin and cytokinin hormone levels is crucial for callus formation (Taiz et al. 2015), and significant differences in callus growth are evident in Figs. 5 and 6. An interesting finding is the difference in the *Rheum ribes* plant extract, where growth reduction is observed with an increasing concentration of the extract (Fig. 6C and D). In the current study, it was observed that the culture medium supplemented with plant growth regulators (auxin, cytokinin) did not yield significant findings after a two-week period. However, the culture medium supplemented with medicinal plant extract demonstrated a remarkable and novel outcome (Figs. 5 and 6).

AC

Based on the variance table results (Table 1), a significant difference at a 1% level was observed in the amount of callus auxin due to the application of plant extracts in tissue culture. As shown in Fig. 7, the control treatment had the highest amount of internal auxin in the plant (0.16 ± 0.0052 µg/g), while the *Allium jesdianum* treatment at 50 mg/L had the lowest amount (0.062 ± 0.0052 µg/g).

According to the basic document, high levels of each of the two plant growth regulators (PGR), auxin and cytokinin, can direct cell division in a specific direction. However, the ratio between these two regulators is the final determining factor (Taiz et al. 2015). As shown in Fig. 7, a downward trend was observed in the amount of auxin in all treatments compared to the control. Additionally, in some plants, an increase in the extract concentration led to a decrease in the amount of internal auxin (*Juniperus sabina* and *Dorema*

aucheri), while in extracts of *Conocarpus erectus*, *Allium jesdianum*, *Taraxacum officinale*, and *Rheum ribes*, the amount of internal auxin increased. 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 the process of cell division. Therefore, in discussions of 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 the cells of the tobacco plant were cultured and the auxin source was removed in the culture medium, the cells performed DNA centration, but they 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 the expression of arc A gene was induced (Ishida et al., 1993). It has also been determined that auxin acts as a growth stimulant in low concentrations and as a growth inhibitor and herbicide (2-4-D) in high concentrations (Taiz et al. 2015). Medicinal plants contain a wide range of compounds (Vermerris and Nicholson 2007), which 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 upregulating 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. 2012). 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). In this study, it was shown that sinapic and ferulic acids can increase the growth regulator by preventing IAA decarboxylation, while monophenols can do the opposite and increase IAA decarboxylation. Overall, phenolic compounds can affect the amount of growth regulators (Tomaszewski and Thimann 1996). Studies of different plant compounds confirm that these compounds are effective in inducing hormonal changes in plants. For instance, some flavonoid aglycones can disturb the polar transport of auxin (Brunn et al. 1992). In another study, it was shown that 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 concentrations of auxin stimulate ethylene production (Taiz et al. 2015). Therefore, if we attribute the role of auxin analogue to 3,4-DHB, the suppressive effect is proven at higher concentrations of this compound. This 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. 1996; Cvikrova et al. 1996). Table 2 shows that extracts of medicinal plants contain a high amount of special 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 callus growth and the highest amount of internal auxin in the sample, as shown in Figs. 5 and 6. However, gene studies currently under investigation in other studies will provide stronger

confirmations. Growth is not solely under the control of a plant growth regulator, and it has been proven that 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). This 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 has the ability to transform into salicylic acid in its biochemical pathway (Taiz et al. 2015). Salicylic acid can act as a stimulant in low concentration and as a growth inhibitor in high concentration (Horvath et al. 2007).

Various studies have shown that plant compounds (plant extracts) can suppress or stimulate the 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 HM et al. 2016) or the production of ethylene and ABA increases with increasing allelopathy (Bogatek et al. 2005). In another study, *Artemisia argyi* L. water extract caused auxin accumulation in roots (Li et al. 2022). Considering that the growth conditions (light, temperature, culture medium, samples) were identical, the difference in the level of internal auxin is definitely related to plant extracts. Enzymatic and molecular investigations will be carried out in future studies to gain a deeper understanding of this process.

CFW

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) reported that allelochemicals and secondary metabolites in plant extracts can affect plant growth and development by regulating gene expression and metabolic pathways. The results of the present study are consistent with these findings, as all treatments resulted in an increase in callus weight (Fig. 8), likely due to amplified cell growth and division. The observed increase in callus weight is supported by other traits such as callus volume, growth, and internal auxin levels. The callus samples treated with *Conocarpus erectus* extract at a concentration of 50 mg/L exhibited the highest callus weight, while the control samples had the lowest weight (Fig. 8). These results suggest that plant compounds in the extracts can affect cell growth and division, leading to changes in callus weight. The findings of the present study are 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 medicine.

TP

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). The study by Singh et al. (2006) demonstrated that alpha-pinene, a major compound in *Juniperus sabina*, increased the activity of antioxidant enzymes. This finding is supported by a recent study by Singh et al. (2006), which 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 on 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 stimulates while a high concentration inhibits growth, is a common occurrence in plant biochemistry (Batish et al. 2008). The study by Desoky et al. (2020) demonstrated the differential effects of different plant extracts on the enzymatic and non-enzymatic defense system in *Vigna unguiculata* L. This finding is consistent with a recent study by Bingol et al. (2022), which showed that extracts of *Xanthoparmelia somloensis* had varying effects on the growth and physiology of tomato plants. The study by Laureys et al. (1998) showed that lovastatin, a compound against mevalonic acid, prevents the production of cytokinin. This finding highlights the importance of studying the effects of plant compounds on other plants, as observed in the present study. The study by Hassanzadeh (2014) suggests that the stimulation of the plant immune system (SAR) may be the main factor responsible for the observed effects in the present study. This finding is consistent with a recent study by Li et al. (2021), which showed 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 the specific mechanisms underlying the observed effects in the present study. Overall, the paragraph provides a comprehensive and informative discussion of the results of the experiment, linking them to previous studies and highlighting the importance of investigating the specific compounds responsible for the observed effects. The new references further support the findings of the present study and provide additional insights into the mechanisms underlying the effects of plant extracts on neighboring plants.

TF

The analysis of variance table reported in Table 1 indicates that there was a significant difference at the 1% level in the effect of plant extracts. The highest amount of total flavonoids (12.16 ± 0.284 mg/g) was observed in the 50 mg/L *Conocarpus erectus* treatment, while the lowest amount was observed in the 50 mg/L *Rheum ribes* treatment (6.58 ± 0.284 mg/g), as shown in Fig. 10. This finding is 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 tomato. The present study showed that the amount of total flavonoids increased with increasing concentration from 50 to 100 mg/L in all treatments except the *Conocarpus erectus* treatment. This finding is supported by 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* plant. 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, in the *Conocarpus erectus* treatment, the amount of flavonoids decreased with increasing concentration from 50 to 100 mg/L. 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. The study by Kengar and Patil (2018) demonstrated that the aqueous extracts of the leaves, stems, and flowers 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 the specific compounds responsible for the observed effects in future studies.

Antioxidant activity (DPPH)

The analysis of variance table (Table 1) revealed a significant difference at the 1% level between the treatments and the control for antioxidant activity (DPPH). The lowest amount of antioxidant activity (63.97 ± 1.283%) was observed in the control plant, while the application of plant extracts showed an upward and increasing trend in antioxidant activity (Fig. 11). 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, 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 sunflower. These findings suggest that plant extracts can cause changes in specialized compounds in plants, leading to increases in antioxidant activity. Further enzyme studies will be conducted in the next 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 the application of plant extracts can increase antioxidant activity in plants. This finding is consistent with previous studies and highlights the potential of plant extracts as a natural and sustainable means of enhancing plant growth and development.

Conclusion

In conclusion, the use of natural plant extracts is a promising approach 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 has caused significant damage to the environment, 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 the desired results for different applications. However, as the results of this study demonstrated, the effect of a plant extract on plant growth and development varies 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. 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 highlight 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.

Abbreviations

Stem length (SL), Seedling fresh weight (SFW), Callus growth (CG), Callus fresh weight (CFW), Total phenol (TP), Total flavonoid (TF), Auxin content (AC).

Declarations

Author contribution statement

Author contributions as mentioned below: **OS**: Methodology, Formal analysis, Investigation, Data curation, Writing original draft. **AGH**: Project advisor in sabbatical leave, Validation, Writing-review & editing. **A H**: Project administration, Validation, Writing-review & editing. **HS**: Consulting in data analysis. **V EM**: Consulting in data analysis.

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Data availability

The authors declare that the data supporting the findings of this study are available within the article.

Conflict of interest

The authors declare that they have no competing interests.

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Figures

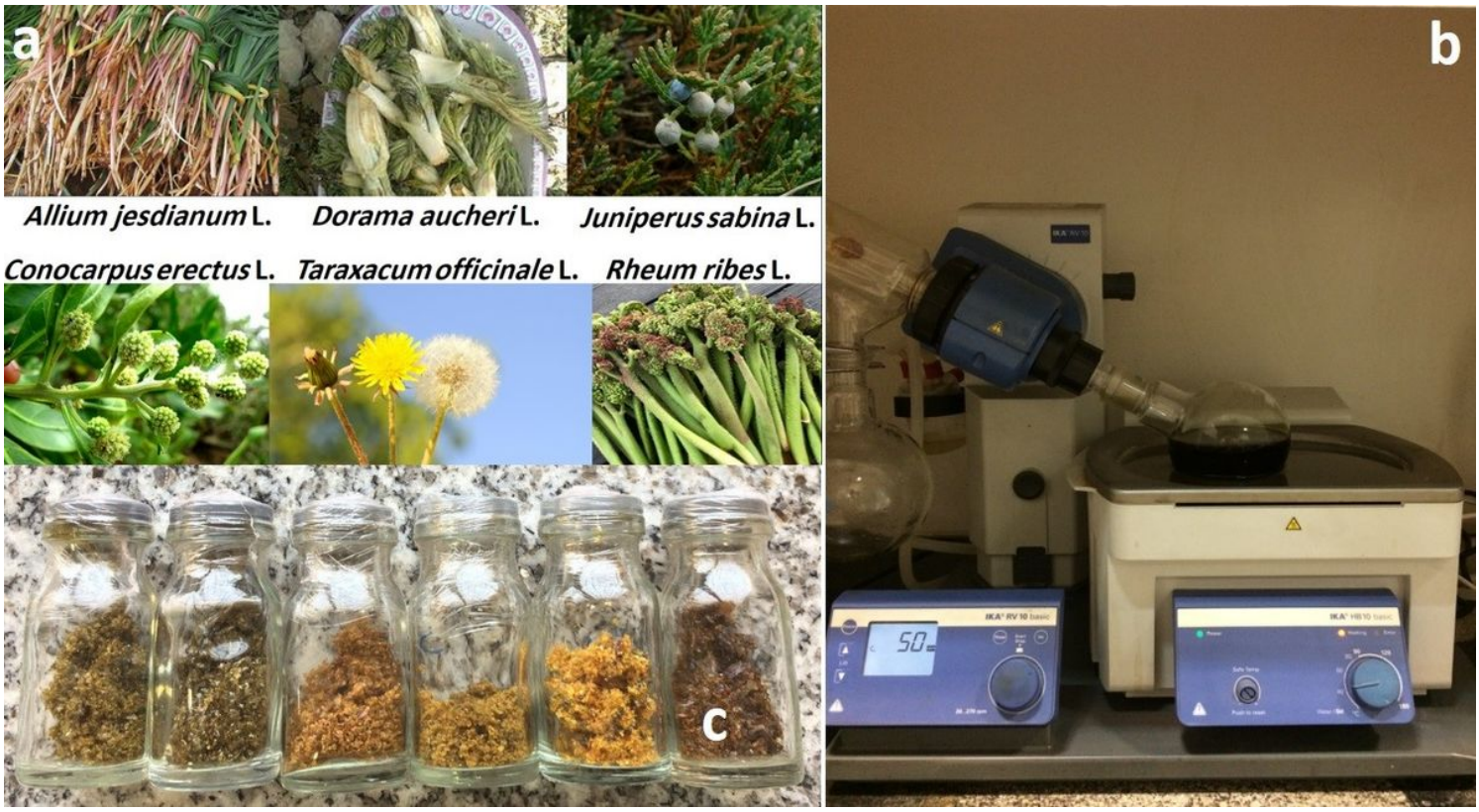

Figure 1

a Collected medicinal plant as a natural compound source **b** Concentration of herbal extracts **c** Pure extract powder of medicinal plants.

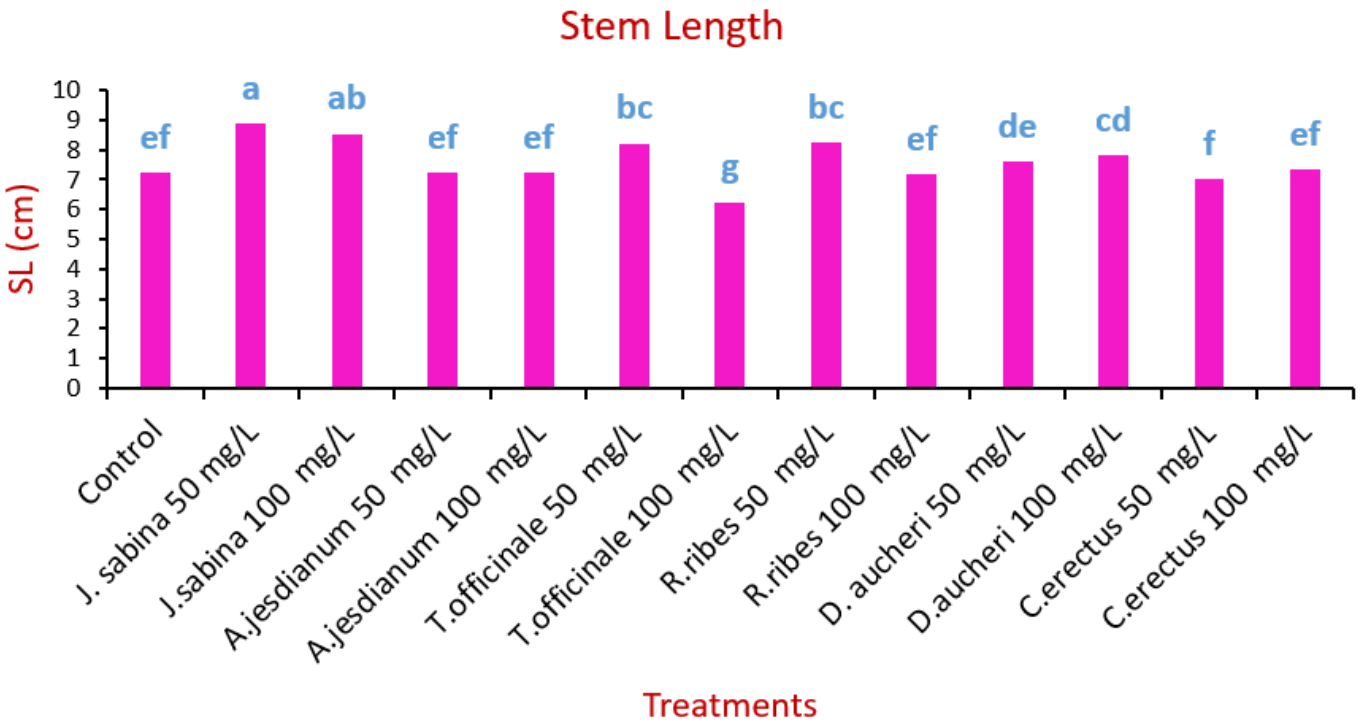

Figure 2

Effect of medicinal plants extract on seedling stem length change in tomato (*Solanum lycopersicum*).

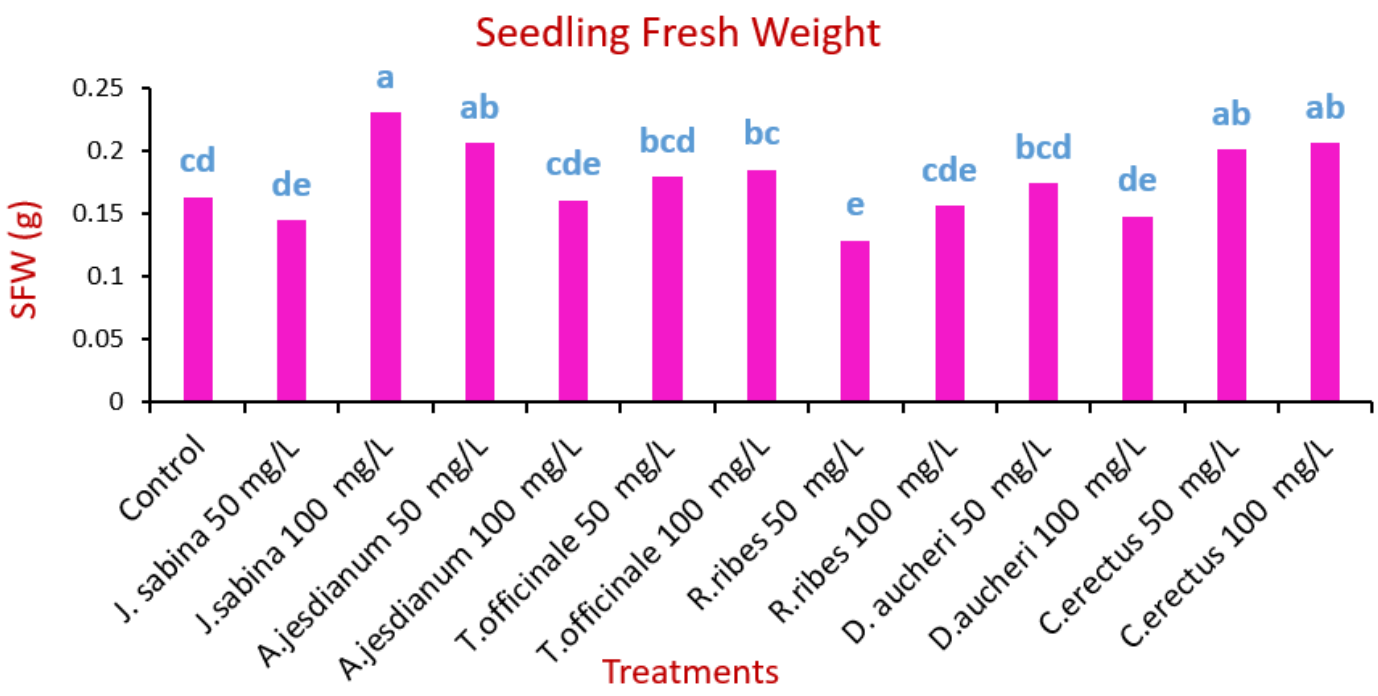

Figure 3

Effect of medicinal plants extract on seedling fresh weight change in tomato (*Solanum lycopersicum*).

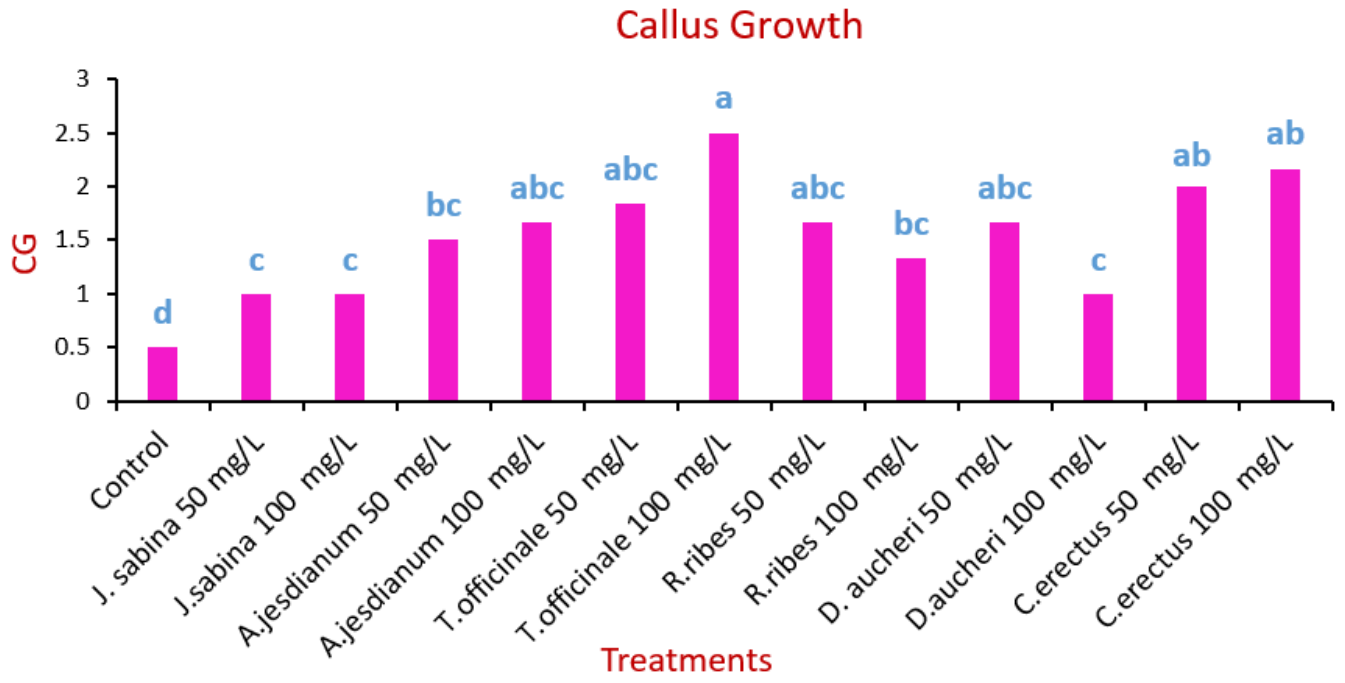

Figure 4

Effect of medicinal plants extract on callus growth change in tomato (*Solanum lycopersicum*).

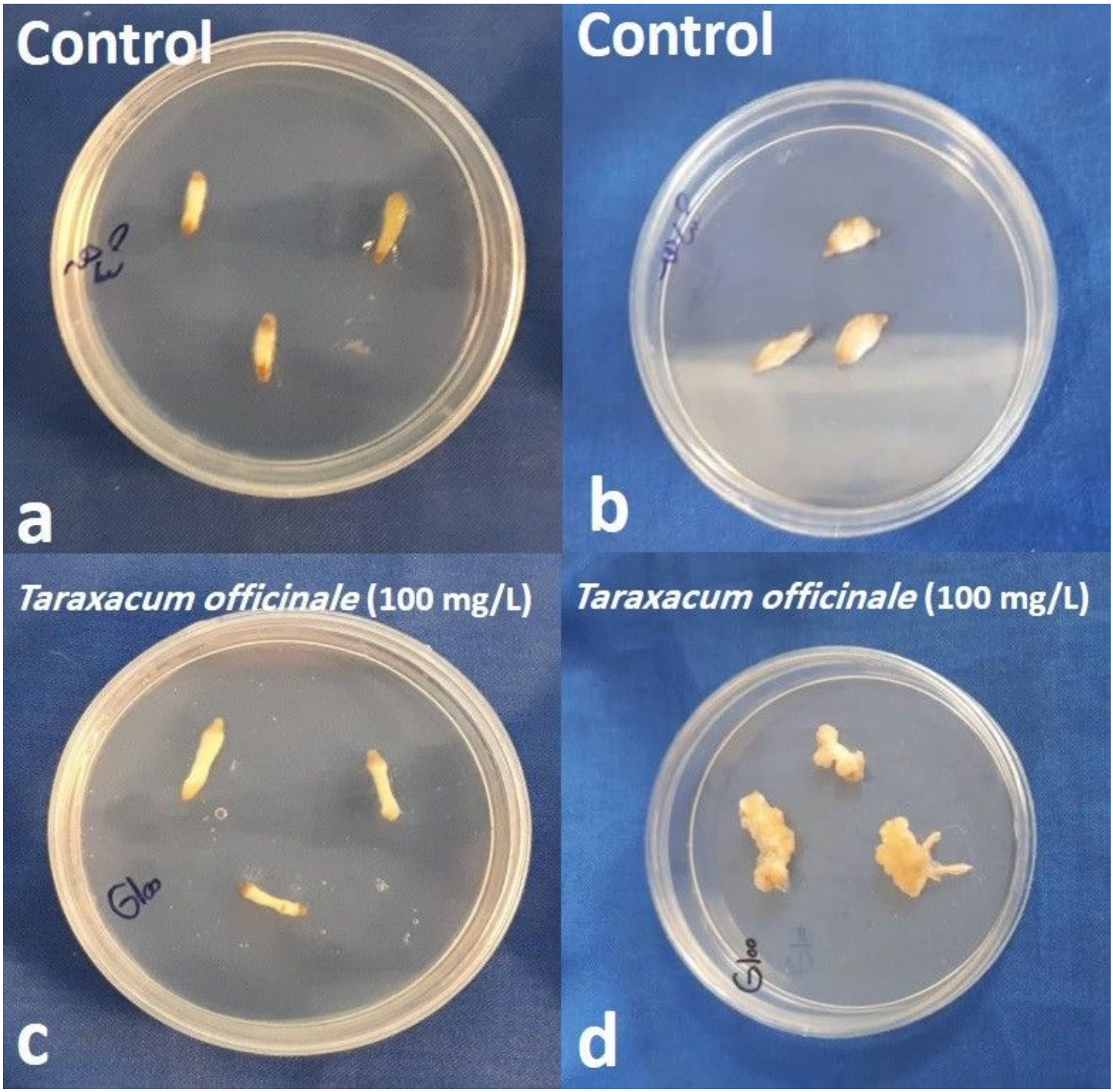

Figure 5

a Control sample at first day **b** Control sample after two weeks **c** Medium with *Taraxacum officinale* extract in first day **d** Medium with *Taraxacum officinale* extract after two weeks.

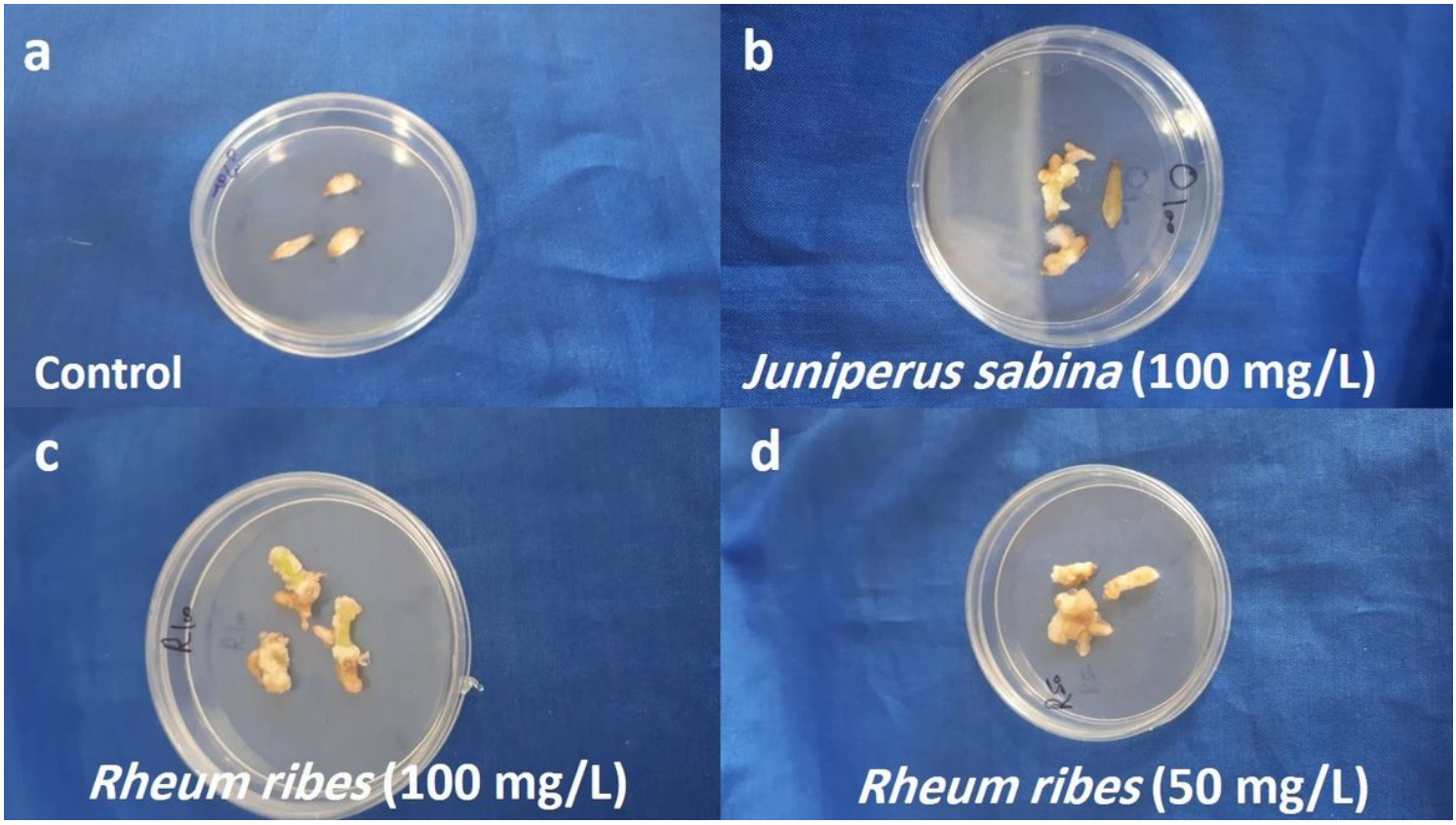

Figure 6

Effect of different medicinal plant extract on callus formation in tomato (*Solanum lycopersicum*) compare to control.

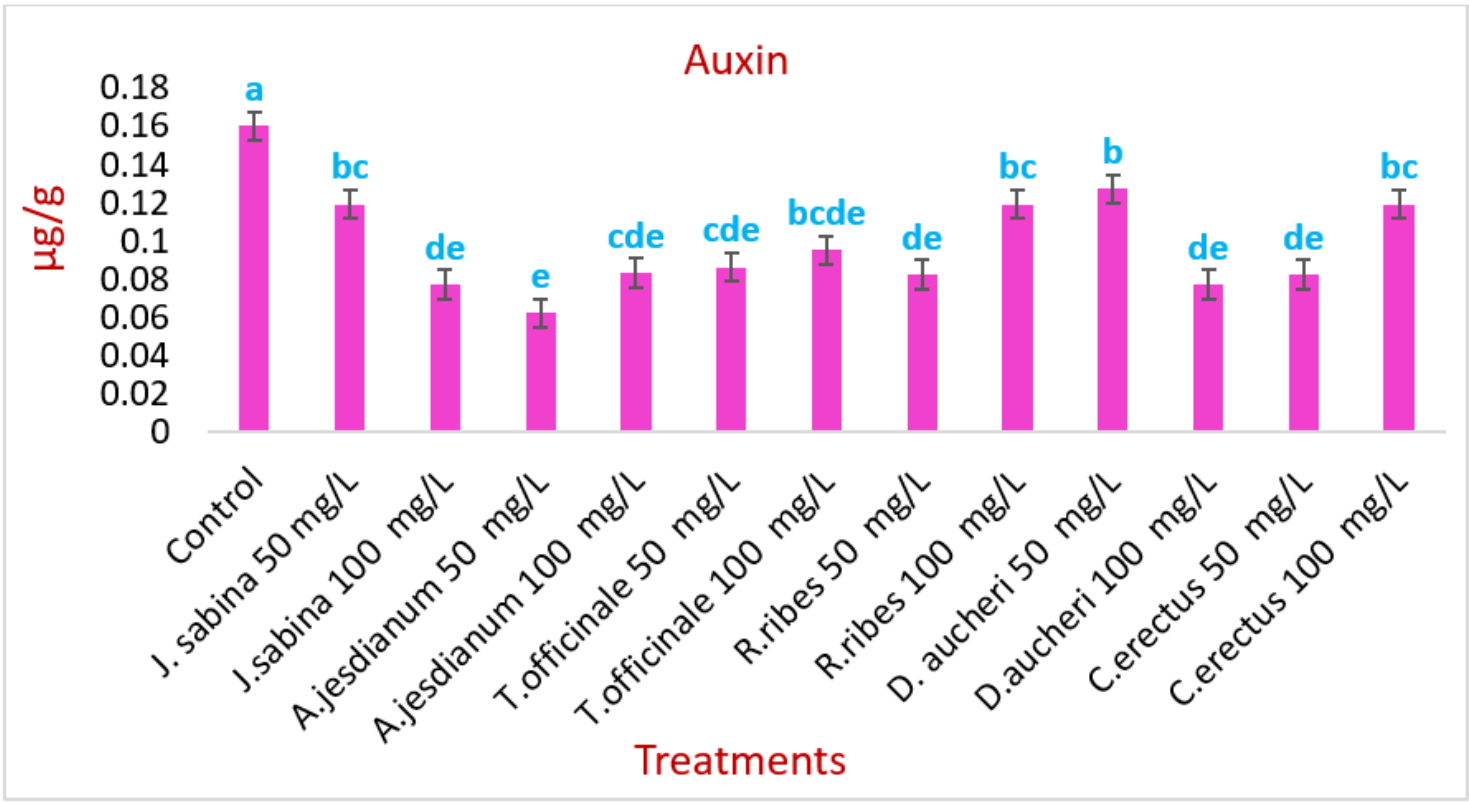

Figure 7

Effect of medicinal plants extract on auxin content change in tomato (*Solanum lycopersicum*).

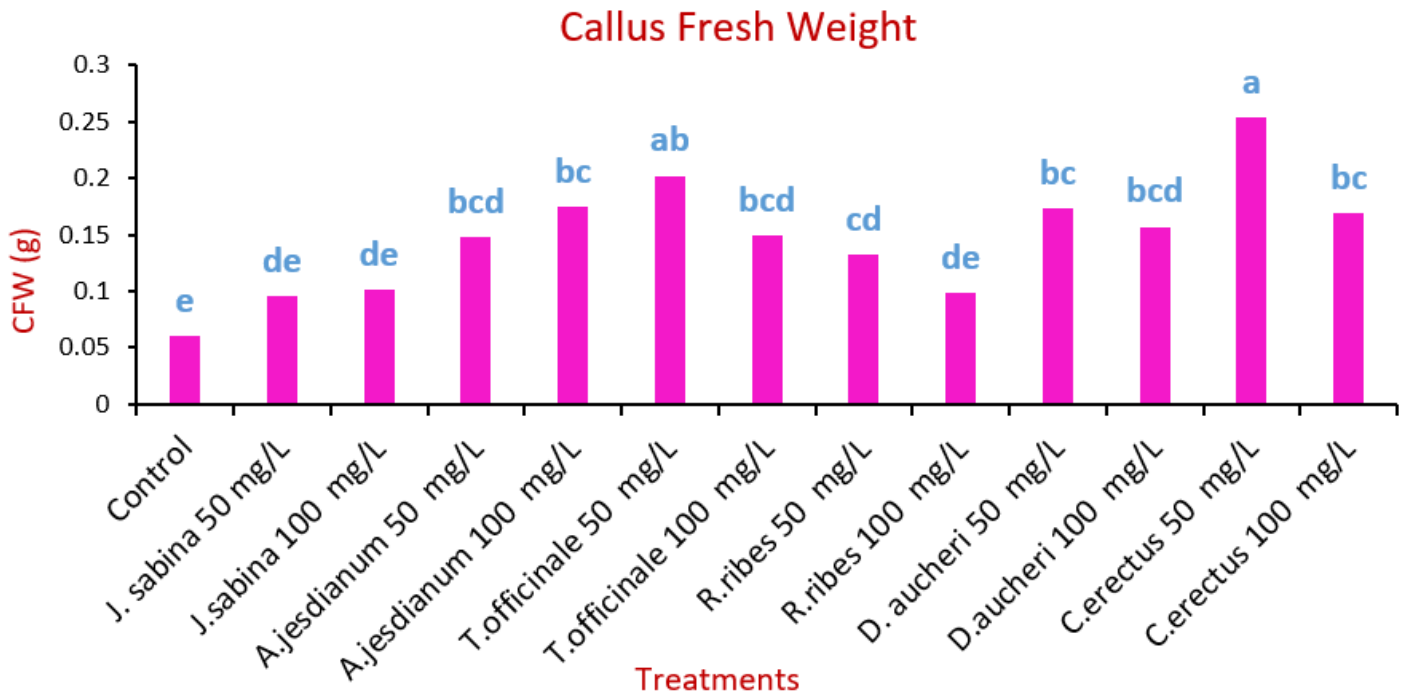

Figure 8

Effect of medicinal plants extract on callus weight change in tomato (*Solanum lycopersicum*).

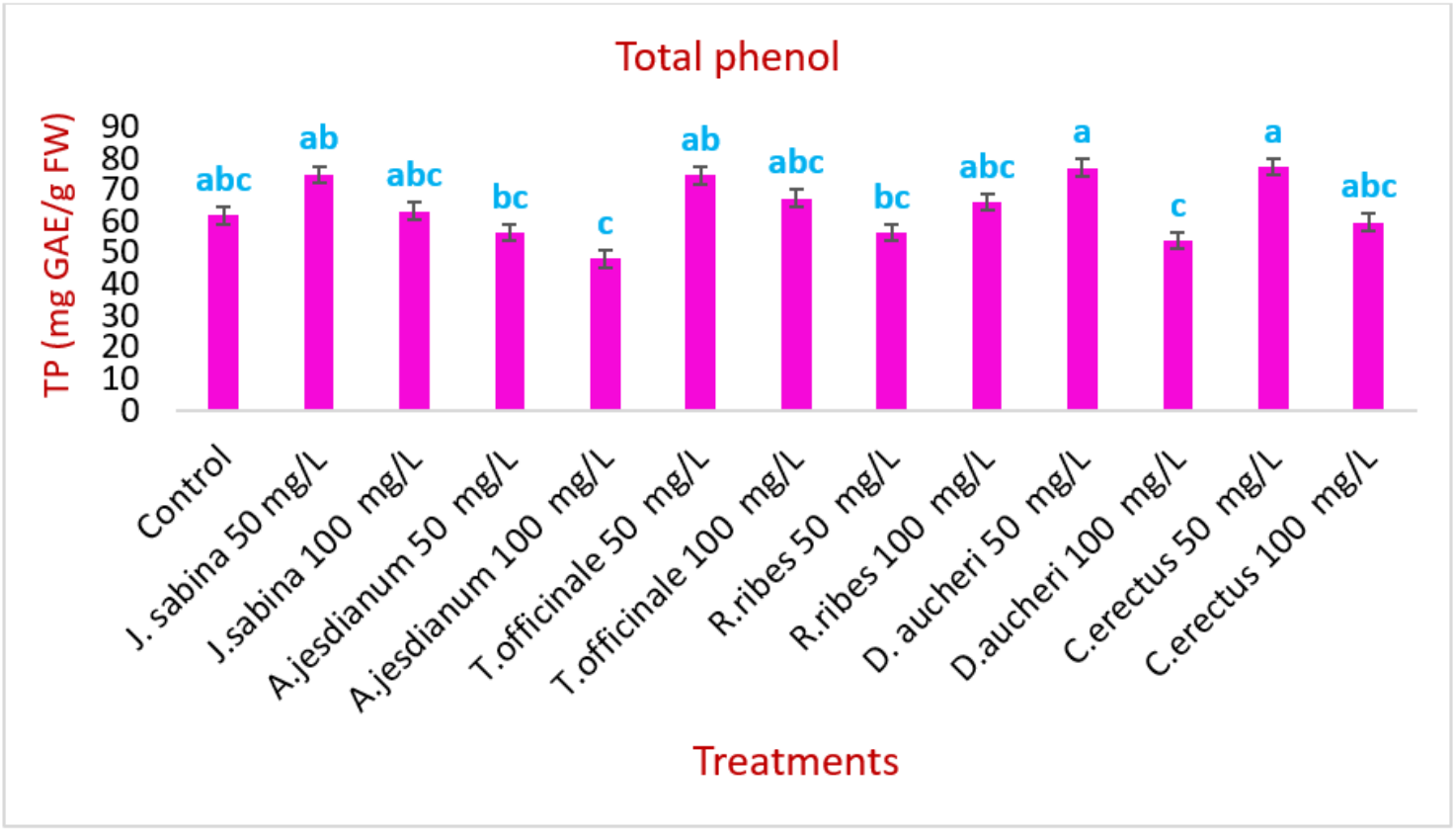

Figure 9

Effect of medicinal plants extract on Total phenol change in tomato (*Solanum lycopersicum*).

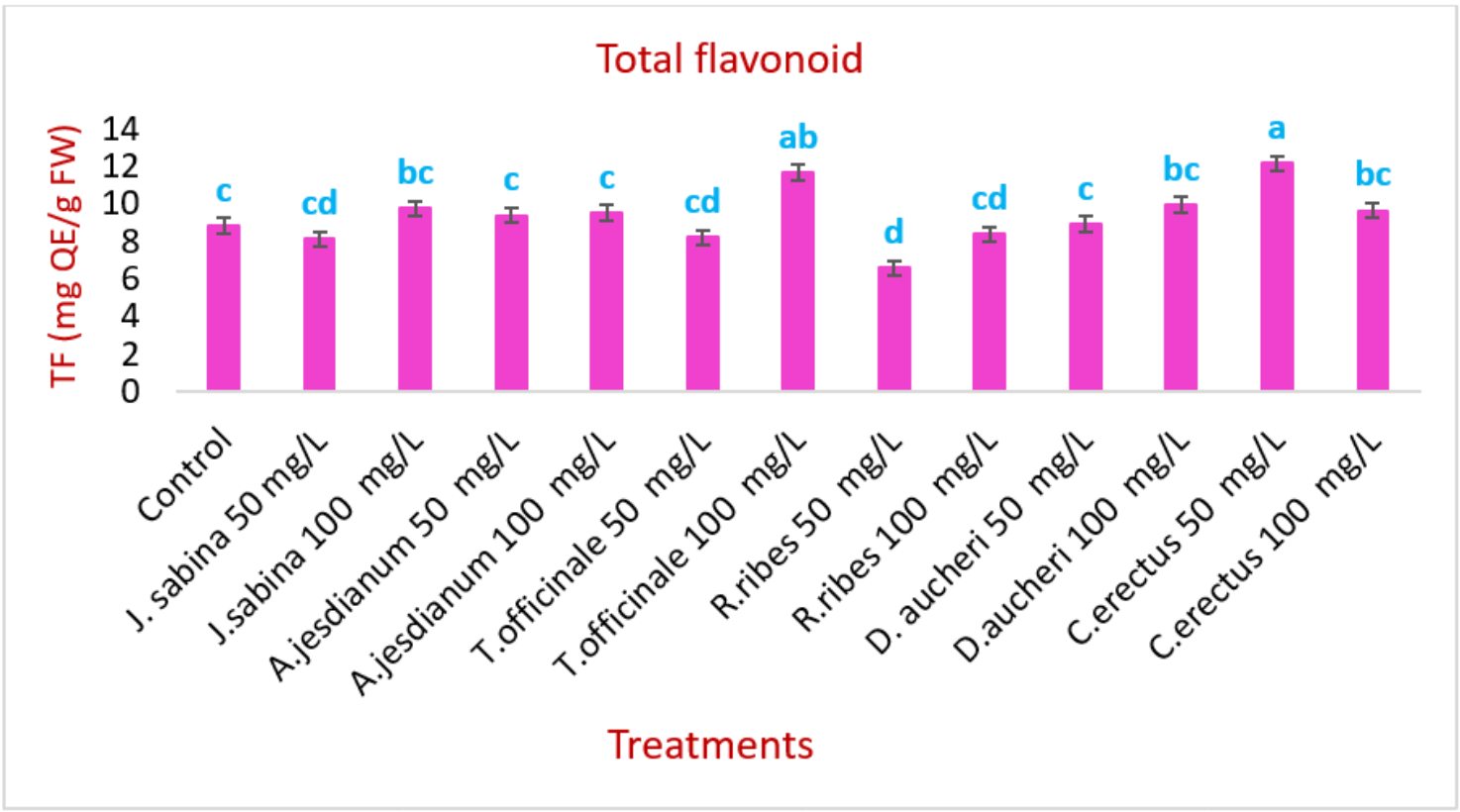

Figure 10

Effect of medicinal plants extract on Total flavonoid change in tomato (*Solanum lycopersicum*).

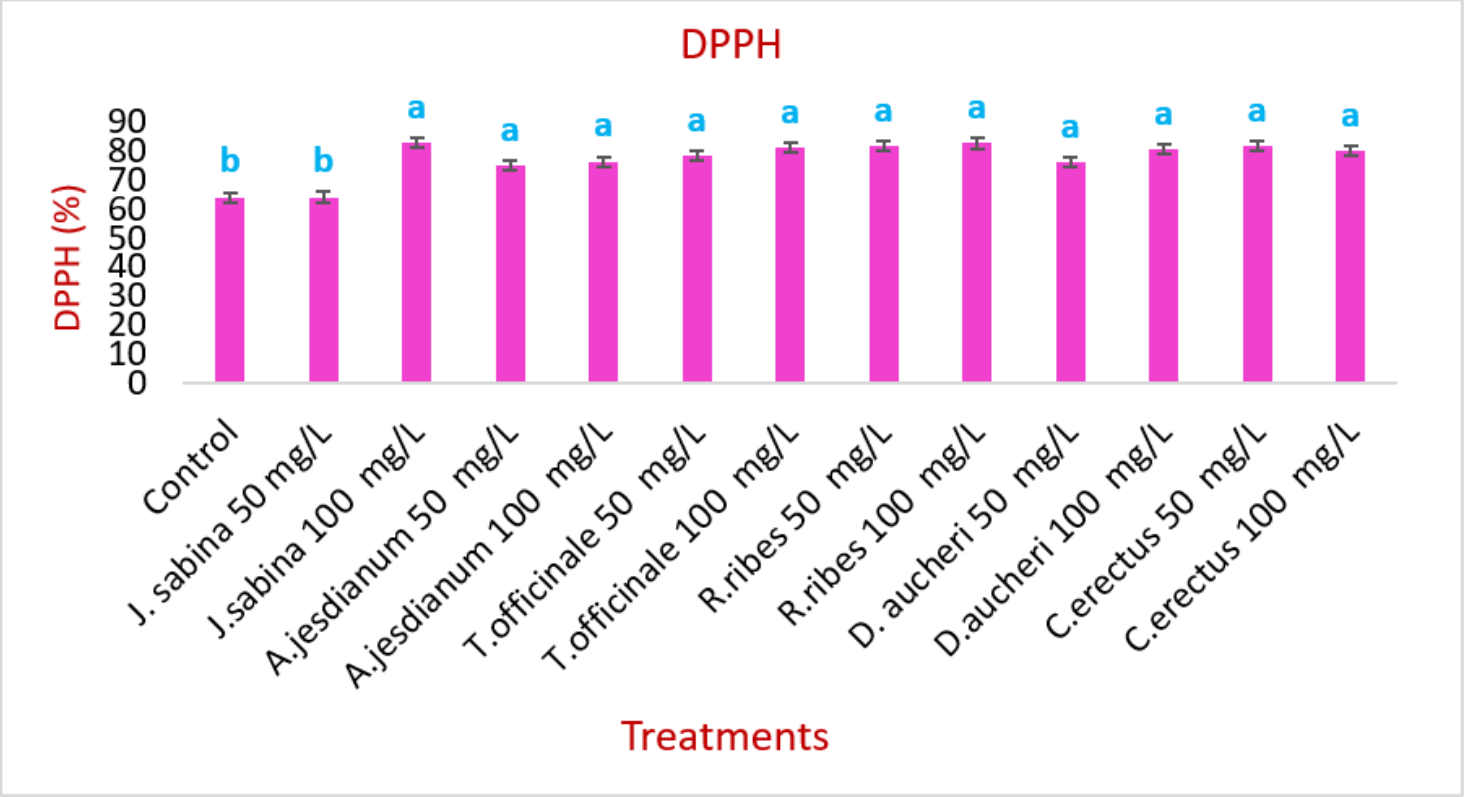

Figure 11

Effect of medicinal plants extract on anti-oxidant potential change in tomato (*Solanum lycopersicum*).

Supplementary Files

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