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Exploring the Effects of Medicinal Plant Extracts on Tomato (*Solanum lycopersicum* L.) Morphology, Biochemistry, and Plant Growth Regulators under Greenhouse Conditions

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ABSTRACT

Irresponsible applications of chemical fertilizers and pesticides in agricultural production have caused significant environmental impacts a nd damage to human health. This study aimed to explore the effects of extracts from several Iranian medicinal plants on tomato growth under greenhouse conditions. Within a completely randomized design, the treatments included methanolic extracts of *Taraxacum officinale* L., *Conocarpus erectus* L., *Allium jesdianum* L., *Rheum ribe*s L., *Dorema aucheri* L., and *Juniperus sabina* L. at three concentrations of 1000, 5000 , and 8000 mg L^{-1}. The measured variables were stem length (SL), internode distance (ID), branch number (BN), auxin content (AC), cytokinin content (CC), auxin/cytokinin ratio, total protein, total phenol content (TPC), total flavonoid content (TFC), and antioxidant potential (FRAP). Under greenhouse conditions, foliar spraying with *Dorema aucheri* plant extract (8000 mg L^{-1}) significantly increased the stem length by 15% compared to the control. *Rheum ribes* extract (8000 mg L^{-1}) increased internode distance (19%). However, the number of branches decreased by 34% in response to the plant extracts. Compared to the control, the amount of cytokinin decreased after spraying, whereas the auxin content showed a sinusoidal pattern of change. In response to all treatments, the auxin/cytokinin ratio increased, with the highest value observed in plants treated with *Allium jesdianum* extract (5000 mg L^{-1}). According to the results, the plant extracts proved effective and assisted in tomato growth. Future research may aim to identify effective biological compounds in these extracts.

Abbreviations: Stem length (SL), Internode distance (ID), Branch numbe r (BN), Auxin content (AC), Cytokinin content (CC), Total phenol conten t (TPC), Total flavonoid content (TFC), Plant growth regulator (PGR), To tal protein (TP), Specialized metabolites (SM).

Introduction

Humans have used plant-based products historically in various fields, such as food, clothing, and medicine. Medicinal plants are one of the most promising aspects of commercial development and can generate revenues for most countries (Dehghanpour and Dehghanizad, 2013). With technological advances, new findings have added to the discovery of the positive effects of natural compounds, so the approach to medicinal plants is gradually replacing conventional chemical applications. A reason behind this approach is that chemicals can have harmful effects on the environment and human

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populations. Therefore, by recognizing the benefits of these medicinal plants and discoveries by scientists, there is increasing attention to medicinal plants (Vasisht et al., 2016; De Saeger et al., 2020). Medicinal plants have a wide range of compounds that have many positive and negative physiological and non-physiological effects. In this regard, natural growth inhibitors and stimulants in agriculture have generated healthy, natural approaches to organic production, primarily because using chemical toxins can accelerate the destruction of many ecosystems. In line with the benefits of herbal compounds, allelopathy has been a fundamental concept in defining the relationship between plants. Unique chemical compounds, called allelopathic compounds, are used by plants to succeed in their competition with other plants. Today, allelopathic compounds are known as Specialized Metabolites (SM), specific in plant families and even species in a genus. Most plants are harvestable for specialized, medicinal metabolites. Accordingly, medicinal plants can have high allopathic ability because they contain plant metabolites (Mushtaq et al., 2020). From a biochemical viewpoint, these compounds comprise essential oils, phenols, alkaloids, steroids, and glycosides, with a special place in medicine, pesticides, and insecticides (Jakovljevic et al., 2013).

Biocontrol strategies are neither new nor old, protective of the environment (Cazorla and Mercado-Blanco, 2016), well-known and consensual. Therefore, nowadays, researchers are looking for methods with minimal adverse effects on production. Coupled with this fact, plant-based applications, especially medicinal plants, have caused the "plant for plant" concept in organic farming. On the other hand, allelopathy is an ecological phenomenon in which a plant affects the growth, physiology, and growth of another plant living close by while releasing chemical or secondary compounds (Mushtaq et al., 2020). In line with this fact, the alkaloid compound Cylindrospermopsin (CYN) reportedly benefited mitosis in bean plants, resulting in high concentrations of this compound that prevented cell division. Plant compounds at low concentrations can stimulate mitosis and certain phases of mitosis, but they inhibit mitosis at higher concentrations (Garda et al., 2015). Finally, given the potential benefits of medicinal plants in promoting green agriculture through their natural composition, the present study aimed to evaluate how extracts from several medicinal plants can affect tomato growth.

Juniperus sabina (Cupressaceae) has essential oil with significant pharmacological effects besides anticancer properties (Khanavi et al., 2019; Fernandez and Cock, 2016). A secondary compound in *J. sabina* is α-Pinene, known as the main compound, but its importance as a biochemical compound was reportedly absent in *J. sibirica* (Zhang et al., 2019).

Rheum ribes belongs to the family Polygonaceae, which is widely distributed in the Middle East (Keser et al., 2019), a perennial plant that grows as a woody herb. Rhubarb leaves are poisonous due to oxalic acid. Different parts of rhubarb have various medicinal effects. Musa Özcan et al. (2007) and Aynaz et al. (2023) found that rhubarb has valuable compounds such as oxalic acid, gallic acid, rhein, and beta-glucogallin and other different compounds that have medicinal effects.

Allium jesdianum contains different phenolic compounds (Yazdanian et al., 2022). This plant belongs to the Liliaceae family and the category of onion plants (Vahdani et al., 2012). *Allium jesdianum* is a medicinal herb with leek-like leaves and umbelliferous flowers that grows in cold altitudes of Zagros and some tropical mountains (Vajari et al., 2014).

Conocarpus erectus is a perennial tree that belongs to Combretaceae (family) and Conocarpus (genus) (Alshibly et al., 2022). It has many types of phenolic compounds and some biochemical compounds, such as gallic acid, ellagic acid, dimethoxy-ellagic acid, quercetin-3-glucuronide, myristin-3-glucuronide, syringatin-3-glucuronide, and ellagitannin (Ayoub, 2010).

Taraxacum officinale (Dandelion) belongs to Asteraceae. It is a perennial herb, one of the most well-known lawn weeds. Its leaves are rosette, simple, and bayonet (Faria et al., 2019), with a high amount of phenol (Schütz et al., 2006). The analysis of leaf extracts and flowers of this plant showed that the most significant phenolic compounds are hydroxycinnamic and its derivatives, especially caffeic acid esters, such as chlorogenic and chicoric acid (Epure et al., 2023).

Dorema aucheri is dominant in the Kermanshah region. It is a plant of the Apiaceae family (Arabjafari et al., 2022). It is a perennial plant with effective substances such as flavonoids, anthocyanin, carotenoids, gallic acid, chlorogenic acid, caffeic acid, and coumaric acid (Mianabadi et al., 2015).

Previous research considered the tomato a model plant. Tomato (*Solanum lycopersicum*) is a small shrub that bears green fruit with a soapy smell and a very stimulating taste. The length of the plant is approximately 30 cm. It has a weak woody stem (Shukla et al., 2013). In this research, we evaluated the effects of extracts of several Iranian traditional medicinal plants on the growth and

biochemical changes of tomato plants to identify viable alternatives to harmful pesticides and chemical compounds used in agriculture.

Materials and Methods

Plant materials

All medicinal plants, except Conocarpus erectus and Juniperus sabina, were harvested in spring (April) from the Zagros Mountains (Dalahoo) in the western part of Kermanshah province, located between 34°9° to 34°46° N and 53 ° 45 to ´35 ° 46 E. The botanical identification of the collected plants was according to Nemati and Jalilian (2011). We obtained Conocarpus erectus tree leaf samples on the same date (April) from Shiraz (29°36'37.12"N, 52°31'52.07"E). Juniperus sabina was obtained from the forests of Tooskestan, 40 km southeast of Gorgan (36°50'29.94" N, 54°26'37.00"E).

Methanolic extract preparation

To prepare the methanolic extract, the plants were washed with tap water and then dried in the shade. The maceration method was used for extraction. For this purpose, the plant sample was mixed with 75% methanol at a ratio of 1:10. The sample was shaken for 24 hours. After this period, the sample was filtered, and the fresh solvent was added to the remains of the plant and maintained for 24 hours. The extract from the two periods was 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 hours. Then, the pure and dry plant extract powders were prepared (Fig. 1C). Experimental treatments involved spraying 1000, 5000, and 8000 mg L^{-1} of water solvent. An ultrasonic device facilitated extract dissolution (Parsonic 2600s, made in Iran). The prepared extract was stored in a refrigerator (4 °C) in dark conditions until further use.

Fig. 1. (A) Medicinal plant material as a natural compound source. (B) Herbal extract concentrations. (C) Pure extract powder of the medicinal plants. (D) Tomato plants treated with foliar sprays of the medicinal plant extracts.

Growth condition and treatments

In late February, we purchased sterilized seeds of tomatoes, CH variety, from Flat Company. They were sown in culture trays containing perlite, coco-peat, and peat moss in a ratio of 50:30:20%, respectively. Irrigation occurred by considering the field capacity of the pots. The optimal temperature range for growth during the study period was 15-18 °C at night and 23-25 °C during the day. Relative humidity was 55-65% (Testo 174T). A light intensity of 350000 to 45000 Lux was for light intensity with a lux meter (LX-1128SD). Hoagland's solution and 20-20-20 fertilizer with micronutrients supplemented the

plants with nutrients. At the four-leaf stage, the plants received spray treatments of the medicinal extracts once a week. The arrangement of the pots in the greenhouse was random (Fig. 1D). To investigate the effects of plant extracts on growth under greenhouse conditions, six medicinal extracts were foliar sprayed at concentrations of 1000, 5000, and 8000 mg L^{-1} once a week for five weeks (Fig. 1A). The control treatment received water sprays. Evaluations began five weeks from the four-leaf stage.

Extraction to measure biochemical and PGR traits

There were three plants in each replication (pot). The samples were harvested and mixed from all three plants in each replication before the final sampling. The extraction process followed procedures outlined by Enayati et al. (2017). We mixed one gram of leaf tissue with 10 mL of solvent (85% methanol with deionized water) and placed it in a shaker overnight at room temperature in dark conditions. The extract was filtered using Whatman filter paper and kept in the refrigerator until further use.

Quantification of total phenol (TPC)

To measure the total phenol content, the Ordonez method was used (Ordonez et al., 2006). From the prepared methanolic extract stored in the refrigerator, 100 µL of methanolic extract was added to 2.8 mL of deionized water, 2 mL of sodium carbonate (Na_2CO_3) (2%) and 100 µL of 50% Folin reagent ($C_{10}H_5NaO_5S$). The samples were vortexed at room temperature for 30 min. The spectra of the samples were then read at 720 nm using a spectrophotometer. Different concentrations of gallic acid were used as standards for the standard curve. Total phenol content was expressed as mg/g of gallic acid with $y = 0.0005x + 0.0257$ ($R^2 = 0.9925$).

Quantifications of total flavonoid (TFC)

To measure the amount of flavonoids in the treated plants, the following steps were performed according to Kim et al. (2002). First, 0.5 mL of methanolic extract was added to 1.5 mL of 80% methanol, 100 µL of 10% aluminum chloride ($AlCl_3$), 100 µL of potassium acetate (CH_3CO_2K), and 2.8 mL of deionized water. The samples were then vortexed and kept at room temperature for 40 min. The absorbance of the samples was read at 415 nm. Quercetin determined the standard curve and the total flavonoid measurement, expressed as mg g^{-1} with $y = 0.002x - 0.0282$ ($R^2 = 0.99$).

Antioxidant potential was measurable by FRAP according to Benzie et al. (1996). Approximately 0.1 g of plant tissue was mixed well in 5 mL of distilled water. Then, it was kept at room temperature for 30 min. The sample was then filtered through Whatman filter papers and kept in dark conditions in a refrigerator until further use. The FRAP standard was prepared with tri-hydrate sodium acetate ($CH_3COONA.3H_2O$), glacial acetic acid, 10 mM 4,2,6-tripyridyl-s-triazine (TPTZ), 37% hydrochloric acid, and iron chloride ($FeCl_3$). Then, 50 µL of the obtained extract was mixed with 1.5 mL of FRAP reagent and vortexed. The sample was maintained in a 40 °C Bain-Marie bath for 4 min. Then, the samples were read at a wavelength of 593 nm by a spectrophotometer. A standard curve was plotted using 1 mM ammonium ferrous sulfate ($(NH_4)_2\ Fe(SO_4)2{\cdot}6H_2O$), and the antioxidant potential was reported as mg g^{-1} of ammonium ferrous sulfate after concluding from the following equation: $y = 0.0018x - 0.2821$ ($R^2 = 0.9864$).

Measurement of auxin and cytokinin by HPLC

To measure the amount of auxin and cytokinin, we used a modified method according to Pan et al. (2010). A Hitachi L-2450 gas chromatography (HPLC) machine measured these two traits. In distilled water, 1% formic acid with methanol and 1% formic acid in HPLC grade appeared as a mobile phase solution (40:60 ratio). Both auxin and cytokinin were measured at 220 nm. For the preparation of auxin and cytokinin standards, IAA and zeatin powder (Sigma Co.) were used. Pure ethanol assisted in dissolving the auxin hormone. First, a base solution of 25 mg L^{-1} was prepared and then the concentration was reduced in a stepwise manner to 5 mg L^{-1}.

$$Auxin\ (IAA): y = 29274517.66\ x - 47311910.50\ (R2 = 0.99)$$

$$Cytokinin\ (Zeatin): y = 522415x - 521459\ (R2 = 0.99)$$

Measurement of total protein (TP)

Protein content was evaluated according to Bradford's method (Bradford, 1976). Accordingly, 0.5 g of fresh leaf tissue was taken and mixed with 3 mL of Tris-HCL. Then, it was placed in a centrifuge at 13000 rpm at a 4 °C. After separating and creating two phases, 50 µL of the prepared extract was mixed with 2.5 mL of Bradford's solution and then placed at room temperature for 20 min. The blue color that developed was an expression of the created reaction. The absorbance of the samples was read at a

wavelength of 595 nm. Finally, the protein concentration in the fresh tissue of the plant was calculated in terms of mg g^{-1} of fresh tissue with $y = 0.0821 + 0.0891$ ($R^2 = 0.958$).

Statistical analysis

The experiment was conducted based on a completely randomized design with three replications in greenhouse conditions (2020-2021). Data analysis was done using SAS version 9.4 software. The comparison of mean values was done using the least significant difference (LSD) ($P \leq 0.01$). Figures were drawn by Microsoft Excel.

Results

Variable effects of plant extracts were observed on the morphological features, biochemical properties, and plant growth regulators of tomato plants in greenhouse cultivation.

Stem length

Based on Table 1, a significant difference was observed in the morphological and biochemical properties of tomato in all experimental treatments.

Table 1. Analysis of variance of the foliar treatments on the morphological and biochemical properties of tomato plants.

S.O.V	df	Stem Length	Branch number	Internode distance	Total phenol	Total Flavonoid	Anti-Oxidant Activity	Protein
Treatments	18	7.3**	2.4**	0.16**	15.2**	1.1**	20.8**	1576.2**
Error	38	1.11	0.162	0.720	0.102	0.050	0.997	71.374
CV		2.78	4.36	6.95	2.23	4.57	3.66	4.42

** indicate significance ($P \leq 0.01$).

Comparison of mean values regarding stem length (Figure 2A) shows that most treatments had a bell-shaped effect, indicating the impact of increasing concentration. For instance, in the *Allium jesdianum* treatment, stem length increased in response to concentrations of up to 5000 mg L^{-1} (Fig. 2A). As shown in Figure 2A, increasing the *Conocarpus erectus* extract concentration led to a noteworthy increase in plant height. A significant difference ($P \leq 0.01$) was observed in plant height between the applied concentrations of 1000 and 5000 mg L^{-1}. Conversely, no significant difference occurred between the 1000 and 8000 mg L^{-1} treatments. Minimal height was observed in the control treatment (33.96 ± 1.77) and the highest length was noted in the *Dorema aucheri* 8000 mg L^{-1} treatment (40.47 ± 1.77) (Fig. 2A). Overall, spraying medicinal plant extracts increased plant growth. However, it is necessary to note that differences occurred in the effects of the various extracts.

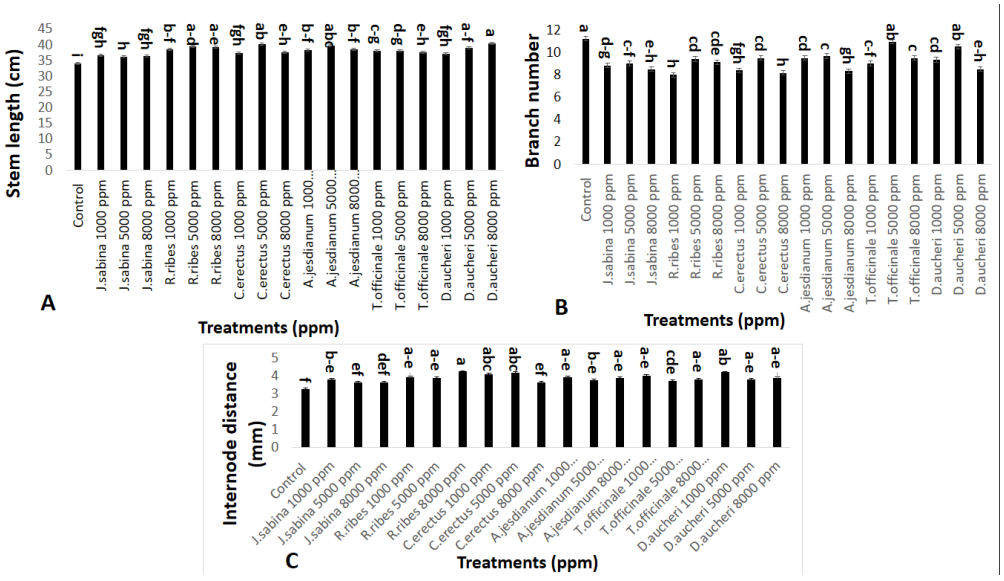

Fig. 2. (A) SL variation. (B) BN variation. (C) ID variation under effect of medicinal plant extract foliar spraying.

Branch number, internode distance, auxin, and cytokinin content

The mean value of branch number (BN) (Fig. 2B) showed that the number of branches decreased while the plant height increased. The control plant had the highest number of branches (11.21 ± 0.094), which indicates that the treatments significantly affected this parameter. Furthermore, the internode distance increased, with the lowest value observed in the control (3.27 ± 0.32) (Fig. 2C). Figure 3C shows that the auxin/cytokinin ratio increased in response to treatments leading to higher growth rates. The results in Figures 3A, B, and C demonstrated that the control plants had the shortest height, shortest internode distance, and the maximum number of branches.

According to Figure 3A and Table 2, foliar extract spraying changed the internal auxin levels compared to the control. For instance, in the control plant, the internal auxin was 179 ± 0.00 µg g-1 FW, while some treatments showed an increase or decrease in the amount of auxin. An increase in auxin was observed in plants that were treated using *Allium jesdianum* 5000 mg L^{-1} (186 ± 0/00 µg g^{-1} FW), but a decrease was noted in response to the Taraxacum officinale treatment (177-178 ± 0.00 µg g^{-1} FW) (Fig. 3A).

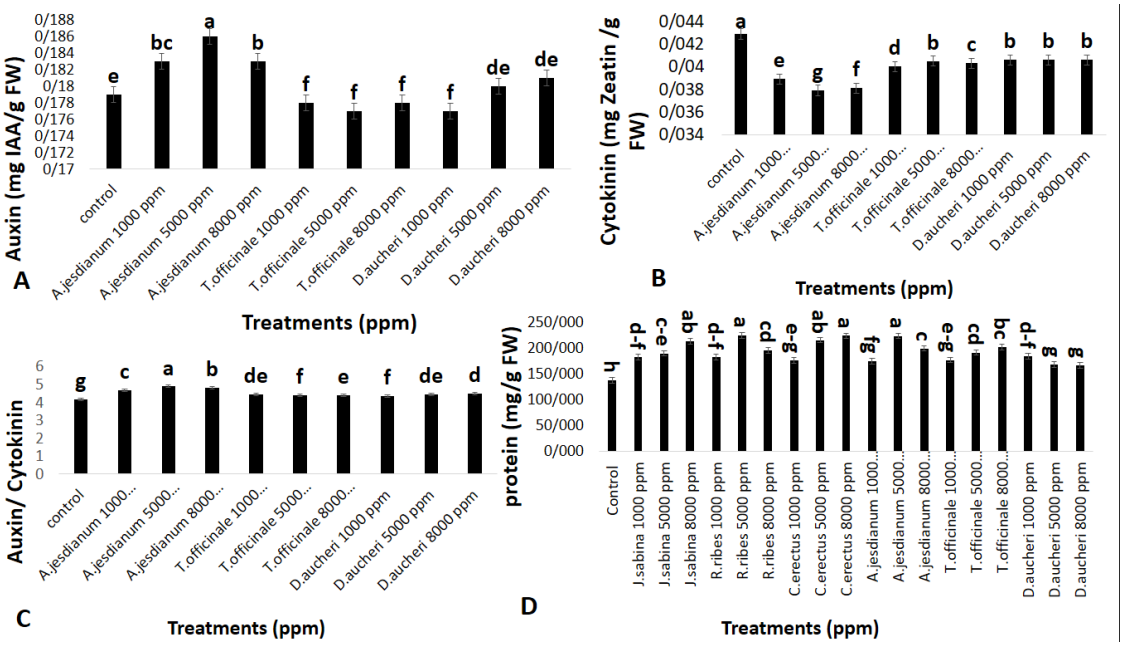

Fig. 3. (A) AC variation. (B) CC variation. (C) auxin/cytokinin content variation. (D) TP variation in response to medicinal plant extract foliar spraying.

Table 2. Analysis of variance of the foliar treatments on PGR traits.

S.O.V	df	Auxin	Cytokinin	Auxin/ Cytokinin
Treatments	9	0.0002488**	0.000062**	0.1454**
Error	29	0.0000059	0.0000001	0.00040
CV		0.42	0.25	0.44

** indicate significance (P≤0.01).

Total protein (TP)

According to Figure 3, the foliar application of plant extracts increased the protein content while increasing the plant height (Fig. 3D). The findings indicated that the control plant had the lowest protein content (136 mg g^{-1} ± 4.878). Furthermore, Figure 3D revealed that the total protein increased as the applied concentration increased from 1000 to 8000 mg L^{-1} in *Juniperus sabina, Taraxacum officinale,* and *Conocarpus erectus* treatments. From the results in Figure 3B, cytokinin decreased in response to spraying. The highest level of cytokinin, approximately 43 ± 0.00 µg g^{-1}, was observed in the control group, whereas a decreasing trend was noted in response to the remaining treatments (Table 3).

As presented in Figure 3C, it is apparent that the auxin/cytokinin ratio, a critical factor in growth

and development, improved in response to the treatments. Specifically, the control group had the lowest ratio (4.193), whereas the rest of the treatment groups showed higher ratios despite some occasional fluctuations. Additionally, Figure 2A confirmed that the control group had the shortest stem length. Upon analyzing the data from the *Allium jesdianum* plant extract application, an obvious increase in the auxin/cytokinin ratio was observed in comparison with the control group. Figure 3C revealed that the ratio in the control group was 4.193 units, but increased in response to the *Allium jesdianum* plant extract. However, upon further examination (Fig. 2A and Table 3), it was discovered that as the concentration of the extract increased from 5000 to 8000 mg L-1, the auxin/cytokinin ratio decreased, followed by a decrease in plant height (Fig. 4). Furthermore, Figure 4 displays the effects of *Conocarpus erectus* extract on the growth of tomato plants, providing a helpful explanation.

The *Conocarpus erectus* extract increased vegetative growth, compared to the control group (Fig. 4D). This increase in growth may be attributed to the diverse presence of specialized compounds like phenols, flavonoids, etc., in the biochemical profile of compounds within the plant extracts (Table 4).

Table 3. Mean value of PGR variation in response to foliar spraying medicinal plant extracts.

Treatments	Auxin (mg g^{-1} FW)		Cytokinin (mg g^{-1} FW)		Auxin / Cytokinin	
Control	0.179	e	0.0429	a	4.193	g
***A.jesdianum* 1000 mg L^{-1}**	0.183	bc	0.0389	e	4.700	c
***A.jesdianum* 5000 mg L^{-1}**	0.186	a	0.0379	g	4.903	a
***A.jesdianum* 8000 mg L^{-1}**	0.183	b	0.0381	f	4.818	b
***T.officinale* 1000 mg L^{-1}**	0.178	f	0.0400	d	4.453	de
***T.officinale* 5000 mg L^{-1}**	0.177	f	0.0405	b	4.384	f
***T.officinale* 8000 mg L^{-1}**	0.178	f	0.0403	c	4.422	e
***D.aucheri* 1000 mg L^{-1}**	0.177	f	0.0406	b	4.374	f
***D.aucheri* 5000 mg L^{-1}**	0.180	de	0.0406	b	4.448	de
***D.aucheri* 8000 mg L^{-1}**	0.181	de	0.0406	b	4.476	d
LSD	0.01		0.01		0.01	

Numbers with the same letters are not significant.

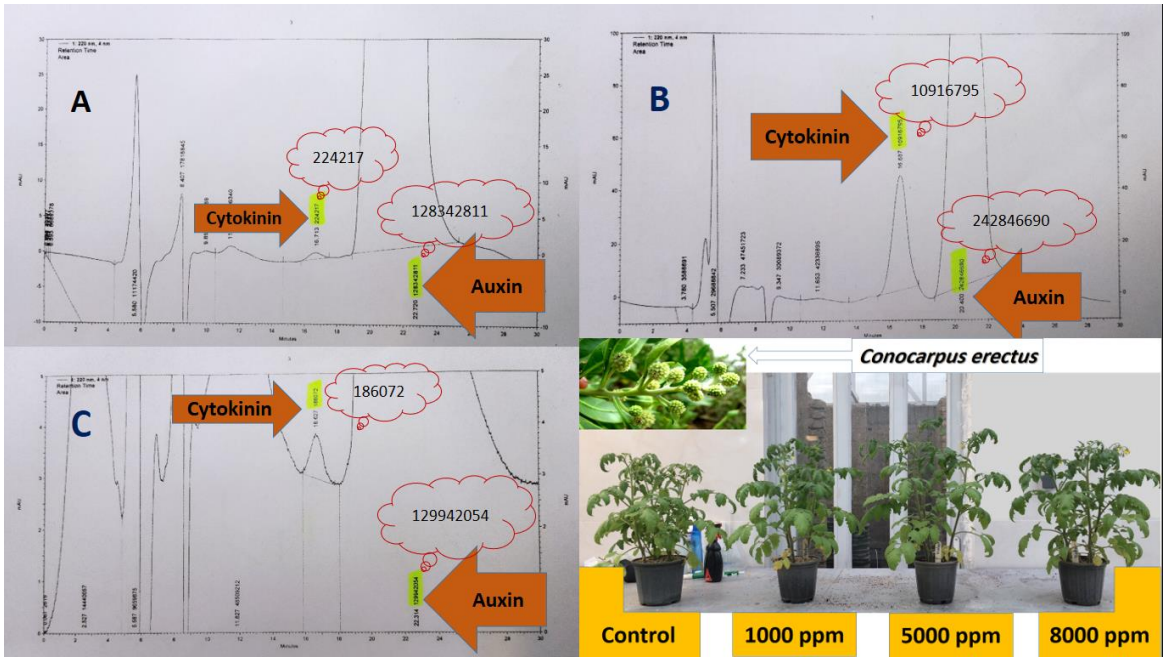

Fig. 4. Graphs of (A) control treatment. (B) PGR addition. (C) Conocarpus treatment 8000 mg L^{-1}. (D) morphological tomato changes under the effect of Conocarpus erectus extract.

Table 4. Biochemical profile and amount of auxin in the medicinal plant extracts.

Medicinal plant	Total phenol (mg g^{-1})	Flavonoid (mg g^{-1})	Antioxidant potential (%)	Auxin content (mg g^{-1})
J. sabina	31.187	15.893	74.285	0.3838
A. jesdianum	21.087	15.898	12.780	0.3260
T. officinale	22.595	14.757	80.483	0.2832
C. erectus	62.545	18.56	67.568	0.2553
D. aucheri	31.663	17.043	58.46	0.3567
R. ribes	40.269	15.5	80.805	0.1483

Total phenol content (TPC)

Plant extracts affected the amount of total phenol in the tomato plants (Fig. 5A). The results revealed that the 8000 mg L^{-1} *Allium jesdianum* treatment displayed the highest amount of total phenol (18.086 ± 0.184 mg g^{-1}), and the control group showed the lowest amount (10.033 ± 0.184 mg g^{-1}) (Fig. 5A). An overall upward trend was observed in the amount of total phenol with the increase in extract concentration. Significant differences among the treatment groups reflected the diversity of plant compounds and metabolites in the extracts. Based on data in Figure 5, an overall increase in phenol content was observed upon foliar application of plant extracts when compared to the control treatment. In a relevant study, plant growth correlated with an increase in antioxidant compounds and secondary metabolites, both enzymatic and non-enzymatic (Desoky et al., 2020).

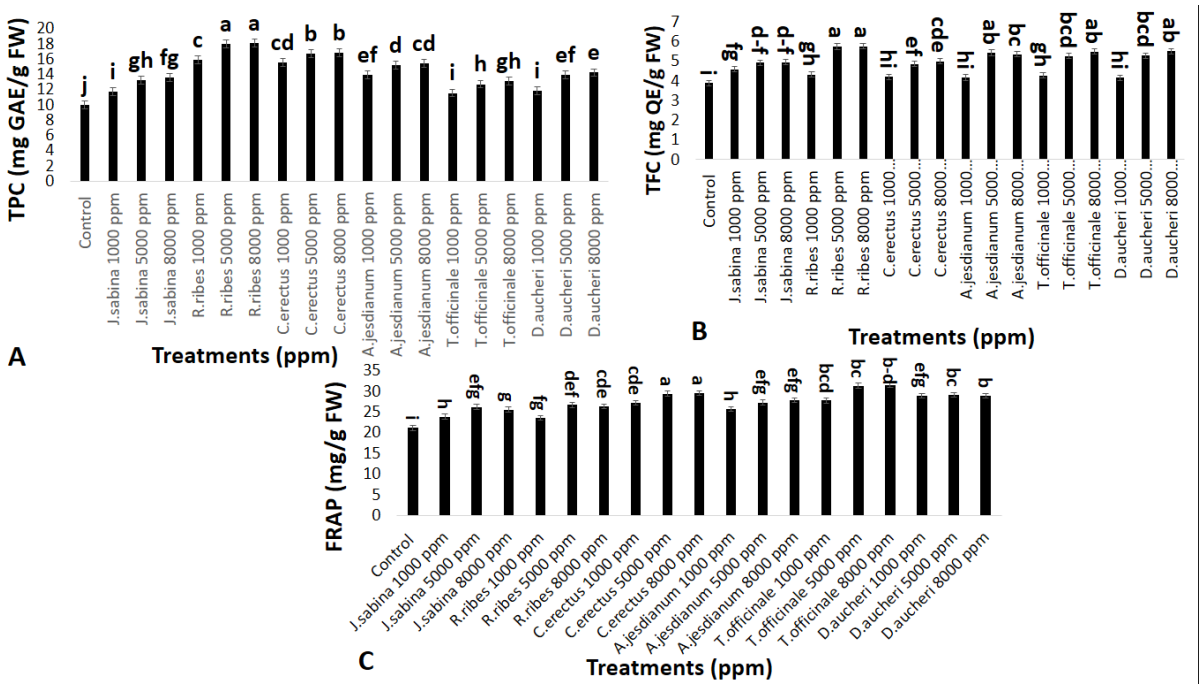

Fig. 5. (A) Changes in total phenol content (TPC), (B) total flavonoid content (TFC), and (C) antioxidant potential (FRAP) caused by foliar spraying medicinal plant extracts.

Total flavonoid content (TFC)

Plant extract applications on tomato plants led to an increase in total flavonoids. The control group exhibited the lowest amount of flavonoids (3.886 ± 0.130 mg g^{-1}). In all treatment groups, the foliar application of extracts increased the TFC compared to the control. Rheum ribes extract (8000 mg L^{-1}) caused the highest increase in tomato flavonoid content (Fig. 5B).

Antioxidant potential

As expected, the control treatment had the lowest

amount of antioxidant potential (21.114 ± 0.576 mg g^{-1}), whereas the 5000 and 8000 mg L^{-1} *Conocarpus erectus* treatments caused the highest increase in tomato antioxidant potential (31.327 and 31.512 ± 0.576 mg g^{-1}, respectively) (Fig. 5C).

Discussion

Stem length, branch number, internode distance, auxin, and cytokinin

Most treatments exhibited a bell-shaped response curve, indicating the normal distribution of the impact of extract concentration on plant growth (Fig. 2A). These outcomes imply that moderate concentrations of the *Conocarpus erectus* extract cause greater stimulatory effects on plant growth. However, above a certain concentration level (i.e., 8000 mg L^{-1}), the extract may inhibit plant growth. *Juniperus sabina* and *Taraxacum officinale* extracts increased the plant height compared to the control. In line with the current results, foliar spraying of two medicinal plant extracts, fennel (*Foeniculum vulgare* L.) and toothpick (*Ammi visnaga* L.) at 2000 mg L^{-1} reportedly increased the growth and yield of beans under salt stress. In another study, holy basil leaf extract (*Ocimum sanctum* L.), *Astragalus tribuloides* L., and *Calotropis procera* L. affected mung bean plants (Gupta, 2016). Limited studies have considered the effect of plant extracts on plant growth, especially the effect of medicinal plant extracts on other plants (De Saeger et al., 2020). Although changes in plant height were observed at different concentrations, the differences were not statistically significant. This suggests that increasing the concentration of these extracts does not negatively affect the growth process or that the concentrations in this study may not be significant enough to make a difference. Figure 2 demonstrates that as the plant height increased, the number of branches decreased, and the internode distance increased. This observation may be attributed to the high levels of antioxidant substances in the compounds that counter free radicals and cell destruction. According to Figure 3C, treatments that increased plant height exhibited an increase in the auxin/cytokinin ratio, consistent with previous studies that suggested how high amounts of auxin can increase the internode distance. Research on *Arabidopsis thaliana* revealed that seaweed aqueous extract increased the number of leaves and plant height due to the high amount of internal auxin in seaweed (Rayorath et al., 2008). Similarly, under the influence of auxin and cytokinin, the expression of the cdc2a At gene increased (Taiz et al., 2015). In previous studies, areas with high cell division also had high expressions of the cdc2 At gene (Masoumi-Asl, 2017). Figure 3A and Table 2 showed that foliar extract spraying can alter the internal levels of auxin compared to the control, confirming previous studies on the role of plant growth regulators in growth. The balance of plant growth regulators controls cell growth and division, and deviation from this balance can alter plant growth. However, the plant detects and adjusts the levels of internal regulators to promote growth (Taiz et al., 2015). Scientists have found that healthy and proper cell division requires the presence of auxin and cytokinin. However, the optimal amount of these growth regulators for effective results depends on plant conditions and environmental situations (Tazi et al., 2015). However, it is important to note that applying these regulators does not always have a positive effect, and the effects can vary depending on the concentration and the type of plant. Some studies have found that increasing the concentration of the spray solution can have opposite effects (Jacqmard et al., 2019; Purohit and Pandya, 2013). Furthermore, some treatments showed a stimulating effect after extract application at lower concentrations and turned into an inhibitory effect at higher concentrations. This finding is significant because it shows that plants contain compounds that can affect the biosynthesis of plant growth regulators, which may explain some of the results observed in our experiment. The treatment with *Allium jesdianum* resulted in an increase in vegetative growth compared to the control plant. Mechanisms and growth processes in plants are complex. Discoveries in measurement, materials, and methods have increased our knowledge accordingly. Scientific findings showed that plant growth involves two processes, i.e., cell division and development, with gibberellin and cytokinin playing a role in cell division and auxin stimulating cell enlargement (Taiz et al., 2015). Growth regulators such as auxin, cytokinin, gibberellin, ethylene, abscisic acid, jasmonic acid, brassinosteroids, and salicylic acid are involved in this process. Among these regulators, auxin and cytokinin play crucial roles in cell growth and division, making them indicators for physiological studies. Tryptophan is the precursor of auxin produced from the chorismate pathway, a product of the shikimic acid pathway. The latter is a well-known pathway for the production of phenolic compounds. Conversely, cytokinin starts from the biochemical pathway of mevalonate when combining acetyl co-enzyme A with one of the sub-units of the citric acid cycle (Taiz et al., 2015). As we know, plant extracts contain a wide range

of phenols, flavonoids, alkaloids, and terpenes, as shown by the biochemical profile of the compounds in plant extracts in this study (Table 4). For example, one of the important and basic compounds found in the *Taraxacum officinale* plant extract is coumaric acid, convertible to salicylic acid through its biochemical pathway (Taiz et al., 2015). It is worth noting that at low concentrations, salicylic acid can stimulate growth, whereas at high concentrations, it can act as a growth inhibitor (Horváth et al., 2007). The increase in the concentration of *Taraxacum officinale* plant extract may be due to changes in the precursors of other essential plant compounds. The same may apply to other extracts because each of these extracts is rich in various compounds that deserve closer observations. Accordingly, plant compounds in the extracts can act as biological stimulants or growth inhibitors, depending on the nature of the plant. Also, other compounds found in plants can have different effects on plants. For example, benzoic acid derivatives can reduce chlorophyll, prevent photosynthesis, and affect growth (Azad et al., 2023). Since this is the first step of such research, further molecular and biochemical assessments can inspire more research in the future to give stronger reasons for the observations.

Total protein (TP)

Hussain et al. (2002) indicated a positive correlation between plant growth and total protein content. This correlation is because plant growth and development rely heavily on protein synthesis, which is necessary for photosynthesis, cell division, and nutrient uptake. Several factors can influence total protein content. These factors include environmental conditions, such as light, temperature, and soil nutrients, as well as genetic factors, such as plant species and cultivars. For example, some studies have shown that increasing nitrogen availability in the soil can increase plant growth and total protein content. However, other studies have found that drought stress or high salt concentrations can decrease plant growth and protein levels. Researchers may use the protein content to assess plant growth and development, particularly in studies where direct biomass measurement or plant size may be difficult or time-consuming. However, it is essential to note that changes in total protein content can change in response to factors other than growth, such as changes in gene expression or protein turnover rates. Overall, the relationship between plant growth and total protein content is complex and can depend on many factors.

Nonetheless, total protein content is an essential indicator of plant health and can usefully inform plant research (Hussain et al., 2002). Plants contain diverse compounds, each of which plays crucial biological roles (Sun and Shahrajabian, 2023). As our treatments involve using whole plant extracts rather than single compounds, identifying effective and principal compounds can be a subject of future research programs. However, the present study showed that these plant extracts can influence the amount of plant growth regulators, including essential growth indicators, i.e., auxin and cytokinin (Taiz et al., 2015). This fact demonstrates the potential role of these extracts in promoting plant growth and development. Upon closer examination, it is apparent that progress in plant growth correlates with plant protein content (Fig. 2A and 3B). Notably, the lowest total protein and plant length occurred in the control.

Total phenol, flavonoid, and antioxidant potential

Systemic acquired resistance (SAR) receives stimuli from external plant extracts (Hassanzadeh, 2014). Usually, the biological response of plants to chemicals varies, which is called the tolerance threshold (Gulzar et al., 2017; Ishak and Sahid, 2016). These responses can be stimulatory or suppressive in different concentrations (Qasem and Foy, 2001). In line with our research, foliar spraying of two medicinal plant extracts, fennel (*Foeniculum vulgare*) and toothpick (*Ammi visnaga*) (2000 mg L^{-1}), increased the growth and yield of beans under salt stress. This research indicated that foliar spraying of these extracts activated the enzymatic and non-enzymatic defense systems. Plants exhibited various biological responses to chemicals, often referred to as the tolerance threshold (Gulzar et al., 2017; Ishak and Sahid, 2016). These responses can be stimulatory or inhibitory at different concentrations (Qasem and Foy, 2001). In line with our study, foliar spraying of extracts from two medicinal plants, fennel (*Foeniculum vulgare*) and toothpick (*Ammi visnaga*), at a concentration of 2000 mg L^{-1}, increased the growth and yield of beans under salt stress. This research suggested that foliar spraying of these extracts activated the enzymatic and non-enzymatic defense systems. The foliar application of medicinal plant extracts significantly affected phenols, flavonoids, and antioxidant potential ($P \leq 0.01$) compared to the control (Table 1 and Fig. 5). This finding is consistent with previous studies that plant

extracts can alter specialized metabolites (SM) in plants (Desoky et al., 2020; Salvi et al., 2019). It is worth noting that toothpick had a more auxiliary effect than fennel, highlighting the difference in specialized metabolites. The results of this study suggested that the biological compounds and specialized metabolites (phenols, flavonoids, terpenes, etc.) found in these plants can enter the biosynthesis pathway of plant regulators, leading to changes in their amounts (Fig. 3 and 4 and Table 2). A higher tomato growth rate resulted from the auxin content of the extracts, and the difference in growth between different treatments may be due to the difference in auxin levels. Plants produce free radicals with adverse effects on growth. However, phenolic and antioxidant compounds in these selected plants may promote growth in non-stress conditions. The foliar application of plant extracts increased protein levels and plant height (Fig. 3D). Since growth can change in response to multiple factors, it is essential to note that the protein index alone cannot be a criterion for judging the changes in growth rate.

Plant extracts can stimulate the defense system of plants (Hassanzadeh, 2014). In a study by Desoky et al. (2020), fennel and toothpick plant extracts were applied to mung bean plants, thereby increasing the activity of enzymatic and non-enzymatic antioxidants. The current research showed a similar stimulatory effect of the medicinal plant extracts on the biochemical profile of tomatoes. Generally, the total phenol content increased in response to higher extract concentrations up to a specific point. Furthermore, significant differences occurred among the treatment groups due to variations in the extract compounds and metabolites.

Similar to the trend observed in total phenol, an increase in the concentration of foliar spray extract also led to a rise in flavonoid content (Fig. 5A), indicating the stimulation of the defense and biochemical system under the influence of medicinal plant extracts. This finding is consistent with another study that reported the foliar application of seaweed extract and its enhancing effect on anthocyanin content (hydrocinnamic acid), flavonols, and color in grapevine (Salvi et al., 2019). The increase in flavonoid compounds in both studies can be due to a stimulated defense system under the influence of plant extracts. Specific plant compounds may increase in amount in response to stimuli from particular compounds, depending on the biochemical profile of those compounds.

The effect of plant extracts on the amount of antioxidant potential showed that an increase in the concentration of foliar sprayed extracts led to a rise in the amount of antioxidant potential, which is consistent with previous findings, demonstrating the ability of plant extracts to stimulate the defense system and increase the activity of antioxidant systems (Hassanzadeh, 2014).

Conclusion

Our study highlighted the potential use of herbal compounds in medicinal plants as a practical adjunctive treatment in various stages of crop cultivation, planning, and harvesting. Thus, these plant extracts are a promising source for organic production. Our findings suggested that the extracts of the medicinal plants in this study, particularly *Allium jesdianum*, can be effective compounds (5000 mg L^{-1}) for tomato cultivation. The positive impact of plant extracts on plant growth and biochemical profile highlighted their potential for enhancing sustainable and organic agricultural practices. More research can increase the current knowledge on plant extract usage for various agricultural settings and crop types, thus expanding opportunities to explore optimal concentrations and application methods. Overall, our study provided valuable insights into the potential of plant extracts as a natural and sustainable alternative to chemical fertilizers. This research is a first step toward future research and can reveal multiple aspects of plant relationships evaluated herein.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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