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ORIGINAL ARTICLE

Study some of Physiological and Biochemical factors of *Pelargonium rostrum* under treatment Salicylic acid and Jasmonic acid in *In vitro* culture

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ABSTRACT
Plant tissue culture technique has many applications in the field of medicinal plants. The use of plant growth regulators play an important role in increasing the secondary plant compounds. Geranium is a plant of the family Geraniaceae. This plant has many medicinal properties. The aim of this study was determined the effect of different concentrations of salicylic acid, jasmonic acid on some biochemical and physiological index of secondary metabolites of the geranium. Geranium plant, greenhouses research center of Isfahan was prepared Fozveh. The plant propagation and production of callus in medium. The effects of the stimulus jasmonic acid and salicylic acid in various concentrations of 10 mM and 100 mM during the 5 days and 10 days on some physiological and biochemical indexes, as well as geranium plant secondary metabolites In vitro on cell suspension as factorial in completely randomized design with three replications. The results showed that in the samples treated with jasmonic acid phenolic compounds and flavonoids were increased. In the callus treated with salicylic acid, polyphenol oxidase and catalase were increased that polyphenol oxidase increased activity of antioxidant system. In this present study, polyphenol oxidase and peroxidase activity decreased in the treated samples was observed salicylic acid, which is due to reduced activity of this enzyme. The results of the present studies on the effect of jasmonic acid and salicylic acid on the essential oils composition and terpenes showed that a combination of high medicinal properties and compared with samples treated with salicylic acid, jasmonic acid decreased, but other compounds had increased, terpenes reacts differently to hormones is that these marks. The present study showed that, treatment with these hormones may increase or reduce some secondary metabolites especially essential oil is important alpha-pinene.
Key words: Jasmonic acid, Plant tissue culture, Salicylic acid, Secondary metabolites.

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INTRODUCTION

Plant tissues and organs are grown in vitro on artificial media, which reserve the nutrients necessary for growth. The success of plant tissue culture as a means of plant propagation is greatly induced by the nature of the culture medium used [1].

Salicylic acid and Jasmonic acid plays an important role in the defense response in many plant species to pathogen attack. These mediates the oxidative rupture that leads to cell death in the hypersensitive response, and acts as a signal for the development of the systemic acquired resistance [2http://www.plantphysiol.org/content/126/3/1024.full - ref-28]. Several studies also support a major role of Salicylic acid and Jasmonic acid in modulating the plant response to various abiotic stresses [3http://www.plantphysiol.org/content/126/3/1024.full - ref-26].

plants synthesize compounds which are able to reduce the damaging effects of several stresses. The antioxidant properties of phenolics are well-documented [4]. These compounds are present in plants as basic or can be synthesized de novo [5]. It is known that the effect of phenolics on growth is a complex process. These compounds may interfere in auxin metabolism, change membrane penetrance, influence

respiration and oxidative phosphorylation or protein synthesis [6]. The phenolic compounds such as flavonoids are active antioxidants [7] and also cause appeasement of lipid peroxidation [8]. Plants possess enzymatic systems that protect them against H_2O_2 and other harmful ROS. These include superoxide dismutase, catalase, peroxidase, polyphenol oxidase etc. [9].

The genus Pelargonium comprises of more than 250 natural species of perennial small shrub, which are limited in their geographical distribution. Pelargonium species usually grow in short grassland and sometimes with occasional shrubs and trees on stony soil varying from sand to clay-loam, shale or basalt. The plants are evergreen when cultivated, but die back during droughts and winter [10]. The Pelargonium genus are aromatic; *P. capitatum, P. graveolens* and *P. radens* are used in cultivation programs for the ennoblement of geranium oil [11].

Pelargonium rostrum is a plant for which little technical and scientific knowledge exists. It belongs to the Geraniaceae family, and its leaves are popularly used as a flavoring; as an insect repellent; in perfumery; and in aromatherapy for the treatment of gastrointestinal diseases, throat infections, and bleeding [12].

The essential oils have an important role in the pharmaceutical, food, perfume and cosmetic industries [13]. Rana et al. (2000) defined the presence of thirty compounds in the essential oil from *P. graveolens*, accounting for 99.1% of the oil. The original components identified were citronellol (33.6%), geraniol (26.8%), linalool (10.5%), citronellyl formate (9.7%), and p-menthone (6.0%) [14].

Therefore, the present study was to evaluate physiological and biochemical factors of *Pelargonium rostrum* under treatment Salicylic acid and Jasmonic acid in *In vitro* culture.

MATERIALS AND METHODS

Plant materials

Stems of Pelargonium rostrum about 1.5-2 cm in size were collected, Then, the stems were rinsed with water using a mild detergent. Subsequently, they were placed in a solution of sodium hypochlorite 15% for 20 minutes. Finally, they were rinsed 3 times with double distilled water and were sterilized.

Stem explants under laminar airflow were transferred to Murashige and Skoog medium (MSO). In order to produce callus, different amounts of naphthaleneacetic acid (NAA) and benzylaminopurine (BAP) hormones were added to the MSO medium. mediums of containing hormones transferred to the culture room(16h light/8h dark) at 25 ± 2˚C. After three weeks, the produced calluses were removed from the culture and transferred to the liquid culture (M3) under 16h light/8h dark at 25 ± 2˚C for 7 days [15].

Salicylic acid and Jasmonic acid were prepared as a stock solution in ethanol and filter-sterilized through a millipore filter (0.22 mm). Sterilized Salicylic acid and Jasmonic acid were then added to the liquid media at final concentrations of 10, 100 µM. Effects of elicitation on cellular suspensions were measured after 5, 10 days. All experiments were conducted in triplicate.

Cellular growth Assay

The weight of cellular suspensions were measured at 5 and 10 days after treatment.

Total anthocyanin determination

The total anthocyanins were estimated by Solecka et al., 1999 method [16]. The anthocyanins and anthocyanidins solutions, 0.1g of leaf fresh tissue were mixed with 10 ml acidic methanol. This solution was centrifuged at dark sitution for 24 h at 25°C, then, this portion was centrifuged for 10 min at 4000g. Absorbance was measured in spectrophotometer at 550 nm.

Total phenol determination

Total phenols were determined by Folin Ciocalteu reagent [17]. 0.1 grams of plant aerial parts were extracted in 5 ml of 95% ethanol by maceration (72 h). 1 ml of this solution was mixed with 1 ml of 95% ethanol and solution value was brought with distilled water to 5 ml. Then, Folin Ciocalteu reagent (0.5 ml, 50%) was mixed with 5% Na_2CO_3 (1 ml). The mixtures were allowed to stand for 60 min and the total phenols were determined by colorimetry at 725 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L^{-1} solutions of gallic acid in ethanol : water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass), which is a common reference compound.

Total flavonoid determination

Aluminum chloride colorimetric method was used for flavonoids determination [18, 19]. 0.05 grams of plant extract in 10 ml in 60% methanol were mixed, then, 10 µl of this solution was mixed with 1 ml of 2% aluminum chloride, 6 ml of 5% potassium acetate. It remained at room temperature for 40 min; the absorbance of the reaction mixture was measured at 415 nm for flavonoid assay. The calibration curve was prepared by preparing Rutin solutions at concentrations 12.5 to 100 g ml^{-1} in methanol.

Enzyme extraction and assay

The samples, weighing about 0.1 g of freeze tissue, were homogenized with 1.5 ml of phosphate buffer pH 6.8 (0.1 M). This portion was centrifuged at 4°C for 15 min at 15,000g in a refrigerated centrifuge. The clear supernatant was taken as the enzyme source.

Peroxidase Assay

2.7 ml sodium phosphate buffer (25 Mimoles), pH 6.8, 100µl gayagol (20 Mimoles), 100µl of H_20_2 (40 Mimoles), and 100 µl of the enzyme extract. The absorbance of the reaction mixture was measured at 470 nm for peroxidase assay [20].

Polyphenoloxidase Assay

2.8 ml sodium phosphate buffer (25 Mimoles), pH 6.8, 100µl pirogalol (10 Mimoles) and 100 µl of the enzyme extract. The absorbance of the reaction mixture was measured at 420 nm for polyphenoloxidase assay [21].

Catalase Assay

The Catalase activity were assayed according to Chance and Maehly, 1955 [20]. The mixture comprised of 2.8 ml phosphate buffer pH 6.8, 200µl solution extract and 300 µl 3%H2O2. The activity of Catalase was estimated by decreased in absorbance of H_2O_2 at 530 nm using an UV-Vis spectrophotometer.

Gas Chromatography-Mass Spectametry (GC-MS) Analysis

The aerial parts of *Pelargonium rostrum* were collected. The samples were air-dried in shade at room temperature.

Gas Chromatography-Mass Spectametry (GC-MS) Analysis. The chemical composition of the aerial parts essential oil was analyzed using GC and GC-MS. The GC/MS analysis was carried out with an 20 Agilent 5975 GC-MSD system in research laboratory of Islamic Azad University, Khorasgan Branch, Isfahan, Iran. HP-5MS column (30m × 0.25mm. 0.25mm film thickness) 20 was used with helium as carrier gas (1.2mL/min). GC oven temperature was kept 20 at 50 C_2 B0C for 3 min and programmed to 280 C_2 B0C at a rate of 5 C_2 B0C/min, and kept 20 constant at 290 C_2 B0C for 3 min, at spilitless mode. The injector temperature was at 20 280 C_2 B0C. Transfer 20 line temperature 280 C_2 B0C. MS were taken at 70 20 eV. Mass ranger was from m/z 35 to 450. Head space GC-MS was used in this study. This method can use plant dry matter for chemical analysis.

Statistical analysis

The presented data included means of three separate experiments ± SD. In order to analyze the data, SPSS 20 software and ANOVA test were used. Thus, the statistical significance between phytochemical activities values of the extracts was evaluated with a LSD test. P values less than 0.05 were considered to be statistically significant.

RESULTS

Analysis of data on cell suspension weight showed that salicylic acid caused significant increase in cellular suspension weight at concentrations of 10, 100 µM during the 5, 10 days, compared to control treatment ($P<0.05$), so that when salicylic acid concentration was increased (100 µM), the amount of cell suspension weight was increased (Figure 1). When jasmonic acid concentration was increased in 5 days treatment, the cellular suspension weight were significantly increased compared to 10 µM treatment, but this increase compared to 10 µM treatment was not significant. While, when jasmonic acid concentration was increased in 10 day treatment, the amount of cellular suspension weight was increased and this increase compared to 10 µM treatment was significant (Figure 1).

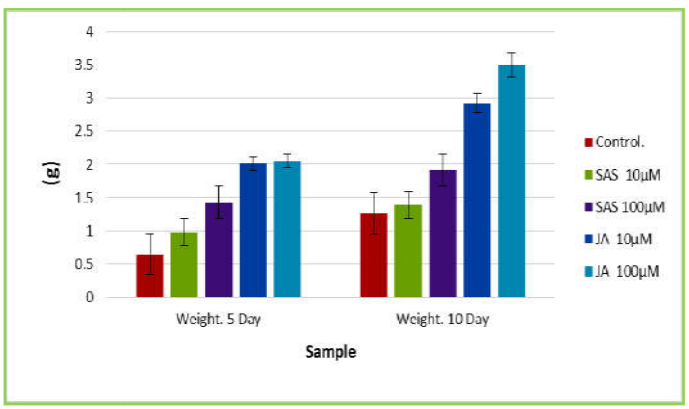

Figure 1. Significant decrease and increase of cellular suspension weight of under treatment groups with jasmonic acid in compared with salicylic acid.

Table 1 show the weight of cellular suspensions. The weight varied from 0.976 ± 0.098 to 3.506 ± 0.22 g in the cellular suspensions. Salicylic acid treatment of 10 µM and 5 days with weight of 3.506 ± 0.22 g had the highest amount among the cellular suspension in this study.

Table 1. Weight in the studied cellular suspensions

Groups	[1]Mean Total phenol ±SD (g)
JA-10µM. 5 days group	2.011±0.098
JA-100µM. 5 days group	2.053±0.230
JA-10µM. 10days group	2.929±0.119
JA-100µM. 10days group	3.506±0.22
SA-10µM. 5days group	0.976±0.098
SA-100µM. 5days group	1.436±0.530
SA-10µM. 10days group	1.386±0.119
SA-100µM. 10days group	1.922±0.223

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

Analysis of data on anthocyanin showed that salicylic acid caused significant decrease in anthocyanin at concentrations of 10, 100 µM during the 5 days, compared to control treatment ($P<0.05$), so that treatment with salicylic acid during the 10 days showed that salicylic acid caused significant increase in anthocyanin at concentration of 100 µM compared to 10 µM treatment ($P<0.05$) (Figure 2). When jasmonic acid concentration was increased in 5 days treatment, the anthocyanin were significantly increased compared to 10 µM treatment ($P<0.05$). So that when jasmonic acid concentration was increased in 10 days treatment, the amount of anthocyanin was declined, and this reduction compared to control and 10 µM treatment was significant (Figure 2).

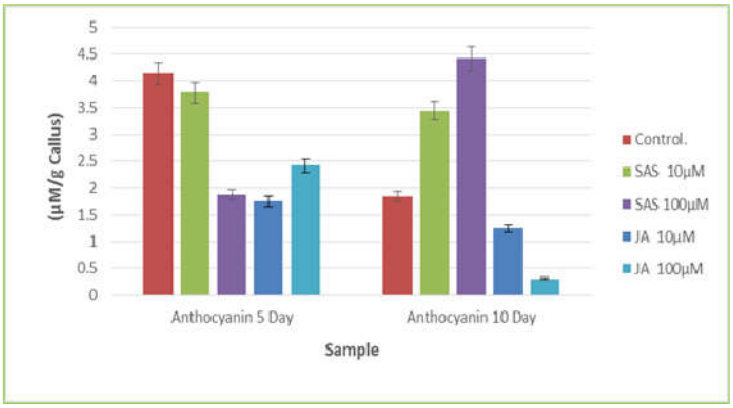

Figure 2. Significant decrease and increase of anthocyanin of under treatment groups with jasmonic acid in compared with salicylic acid.

Table 2 show the content of anthocyanin that were measured by Solecka et al., 1999 method. The anthocyanin varied from 0.404 ± 0.030 to 4.42 ± 0.138 µM g-1 fw in the cellular suspension. Salicylic acid treatment of 10 µM and 5 days with total anthocyanin of 4.42 ± 0.138 µM g-1 fw had the highest amount among the cellular suspension in this study.

Table 2. Anthocyanin in the studied cellular suspensions

Groups	[1]Mean Total phenol ±SD ($^{\mu}$M g-1 fw)
JA-10µM. 5 days group	1.75±0.125
JA-100µM. 5 days group	2.42±0.058
JA-10µM. 10days group	1.24±0.243
JA-100µM. 10days group	0.3±0.038
SA-10µM. 5days group	3.78±0.125
SA-100µM. 5days group	0.404±0.03
SA-10µM. 10days group	3.45±0.243
SA-100µM. 10days group	4.42±0.138

[1]Each value in the table was obtained by calculating the average ofthree experiments ± standard deviation.

Analysis of data on total phenol showed that salicylic acid caused significant reduction in polyphenol content at concentrations of 100 µM during the 5 days, compared to other treatment($P<0.05$), so that when salicylic acid concentration was decreased (10 µM), the amount of polyphenol content was increased (Figure 3). When jasmonic acid concentration was increased in 5 days treatment, the phenol content were significantly decreased compared to 10 µM treatment ($P<0.05$). So that when jasmonic acid concentration was increased in 10 days treatment, the amount of polyphenol content was declined, but this reduction was not significant (Figure 3).

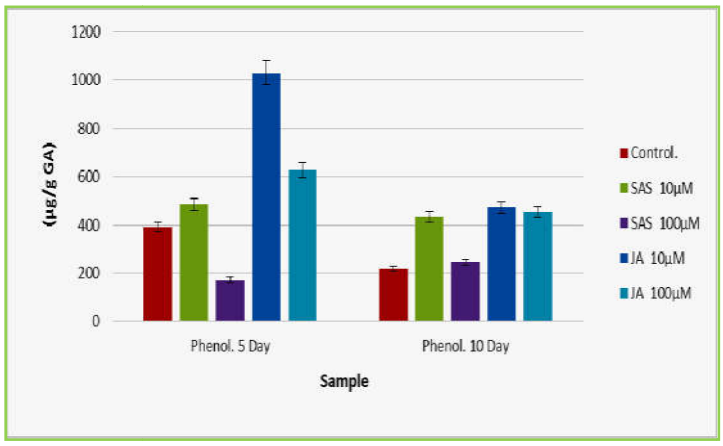

Figure 3. Significant decrease and increase of total phenol content of under treatment groups with jasmonic acid in compared with salicylic acid.

Table 3 show the content of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: $y=29.85x+0.043$, $r_2= 0.990$). The total phenol varied from 172.52 ± 15.3 to 1030.15 ± 16.4 µg g-1 GA in the cellular suspension. Jasmonic acid treatment of 10 µM and 5 days with total phenol content of 1030.15±16.4 µg g-1 GA had the highest amount among the cellular suspension in this study.

Table 3. Phenol content in the studied cellular suspensions

Groups	[1]Mean Total phenol ±SD (mg g-1 GA)
JA-10µM. 5 days group	1030.15±16.4
JA-100µM. 5 days group	628.14±15.3
JA-10µM. 10days group	472.36±21.2
JA-100µM. 10days group	453.93±36.3
SA-10µM. 5days group	172.52±15.3
SA-100µM. 5days group	485±16.3
SA-10µM. 10days group	432.16±0.029
SA-100µM. 10days group	246.23±0.086

[1]Each value in the table was obtained by calculating the average ofthree experiments ± standard deviation.

Analysis of data on total flavonoid showed that salicylic acid caused significant increase in polyphenol content at concentrations of 10 µM during the 5 days, compared to other treatment($P<0.05$), so that when salicylic acid concentration was increased (100 µM), the amount of polyphenol content was decreased (Figure 4). When jasmonic acid concentration was increased in 5 days treatment, the phenol content were significantly decreased compared to 10 µM treatment ($P<0.05$). So that when jasmonic acid concentration was increased in 10 days treatment, the amount of polyphenol content was declined, but this reduction compared to 10 µM treatment was not significant (Figure 4).

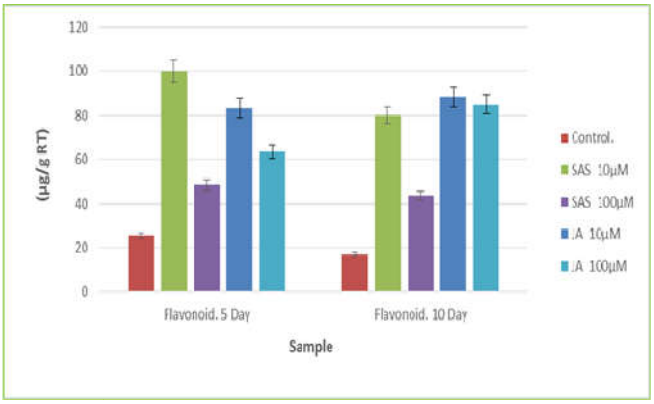

Figure 4. Significant decrease and increase of total flavonoid content of under treatment groups with jasmonic acid in compared with salicylic acid.

The flavonoid content of the cellular suspension in terms of rutin equivalent (the standard curve equation: $y = 0.0603x + 0.0007$, $r_2 = 0.985$) were between 43.61 ± 8.1 µg g-1 RTA and 88.39 ± 4.2 µg g-1 RTA (Table 2). The flavonoid content in the cellular suspension of under treatment with SA 100 µM during the 10 days (43.61 ± 8.1 µg g-1 RTA) and under treatment with SA 10 µM during the 5 days (48.59 ± 5.9 mg g-1 RTA) were lower than that in the cellular suspension of treatment with JA 10, 100 µM during the 5, 10 days (Figure 4). Table 4 also show the content of total flavonoid that were measured in terms of rutin.

Table 4. Flavonoid content in the studied cellular suspensions

Groups	[1]Mean Total phenol ±SD (mg g-1 RTA)
JA-10µM. 5 days group	83.41±8.1
JA-100µM. 5 days group	63.51±3.9
JA-10µM. 10days group	88.39±4.2
JA-100µM. 10days group	85.07±6.5
SA-10µM. 5days group	48.59±5.9
SA-100µM. 5days group	100±10.1
SA-10µM. 10days group	80.09±7.2
SA-100µM. 10days group	43.61±8.1

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

Increasing concentrations of salicylic acid, significantly decreased peroxidase enzyme activity at concentration of 10µM during the 5,10 days, compared to control treatment ($P<0.05$), so that treatment with salicylic acid at concentration of 100µM showed that salicylic acid caused significant decrease in peroxidase enzyme activity at 10 days compared to 5 days treatment ($P<0.05$) (Figure 5). When jasmonic acid concentration was increased in 10 day treatment, the peroxidase enzyme activity were significantly decreased compared to 10 µM treatment ($P<0.05$) (Figure 5).

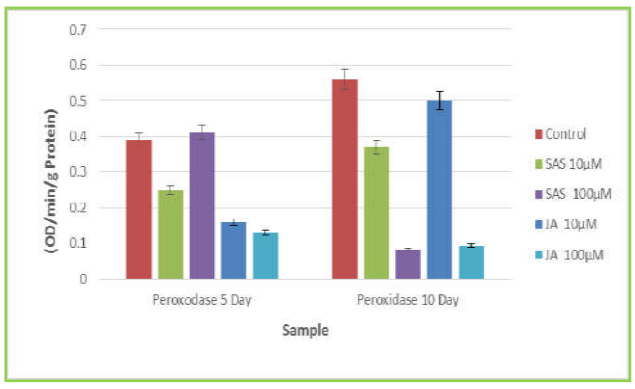

Figure 5. Significant decrease and increase of peroxidase enzyme activity of under treatment groups with jasmonic acid in compared with salicylic acid.

Table 5 show the peroxidase enzyme activity that were measured by Chance and Maehly, 1955 method. The peroxidase enzyme activity varied from 0.083± 0.032 to 0.5 ± 0.027 OD/min/g protein in the cellular suspension. Jasmonic acid treatment of 10 µM and 10 days with peroxidase activity of 0.5 ± 0.027 OD/min/g protein had the highest amount among the cellular suspensions in this study.

Table 5. Peroxidase enzyme activity in the studied cell suspensions

Groups	[1]Mean Total phenol ±SD (OD/min/g Protein)
JA-10µM. 5 days group	0.16±0.026
JA-100µM. 5 days group	0.13±0.023
JA-10µM. 10days group	0.5±0.027
JA-100µM. 10days group	0.093±0.032
SA-10µM. 5days group	0.41±0.023
SA-100µM. 5days group	0.25±0.016
SA-10µM. 10days group	0.37±0.027
SA-100µM. 10days group	0.083±0.032

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

Analysis of data on polyphenol oxidase activity showed that salicylic acid caused significant increase in polyphenol oxidase activity at concentrations of 10, 100 µM during the 5 days, compared to control treatment ($P<0.05$), so that when salicylic acid concentration was increased (100 µM), the amount of polyphenol oxidase activity was increased (Figure 6). So that treatment with salicylic acid at concentration of 100µM during the 10 days showed that salicylic acid caused significant decrease in polyphenol oxidase activity at 100µM treatment compared to 10µM treatment ($P<0.05$) (Figure 6).When jasmonic acid concentration was increased in 5 days treatment, the polyphenol oxidase enzyme activity were significantly decreased compared to 10 µM treatment ($P<0.05$). So that when jasmonic acid concentration was increased in 10 days treatment, the amount of polyphenol oxidase activity was increased and this increase compared to 10 µM treatment was significant (Figure 6).

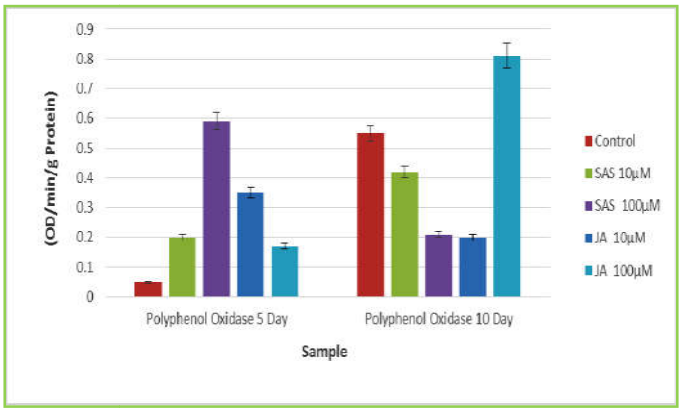

Figure 6. Significant decrease and increase of polyphenol oxidase enzyme activity of under treatment groups with jasmonic acid in compared with salicylic acid.

The polyphenol oxidase activity of the cellular suspensions were between 0.17 ± 0.016 OD/min/g protein and 0.81 ± 0.015 OD/min/g protein (Table 6). The polyphenol oxidase activity in the cellular suspensions of under treatment with JA 100 µM during the 5 days (0.17 ± 0.016 OD/min/g protein) was the highest than that in the other cellular suspensions.

Table 6. Polyphenol oxidase enzyme activity in the studied cell suspensions

Groups	[1]Mean Total phenol ±SD (OD/min/g Protein)
JA-10µM. 5 days group	0.35±0.012
JA-100µM. 5 days group	0.17±0.016
JA-10µM. 10days group	0.2±0.045
JA-100µM. 10days group	0.81±0.015
SA-10µM. 5days group	0.59±0.016
SA-100µM. 5days group	0.2±0.002
SA-10µM. 10days group	0.42±0.045
SA-100µM. 10days group	0.21±0.005

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

Analysis of data on catalase activity showed that salicylic acid caused significant increase in catalase activity at concentrations of 10, 100 µM during the 5 days, compared to control treatment ($P<0.05$), so that when salicylic acid concentration was increased (100 µM), the amount of catalase activity was increased (Figure 7). So that treatment with salicylic acid at concentration of 100µM during the 10 days showed that salicylic acid caused significant decrease in catalase activity at 100µM treatment compared to 10µM treatment ($P<0.05$) (Figure 7).When jasmonic acid concentration was increased in 5, 10 days treatment, the catalase activity were significantly decreased compared to 10 µM treatment ($P<0.05$) (Figure 7).

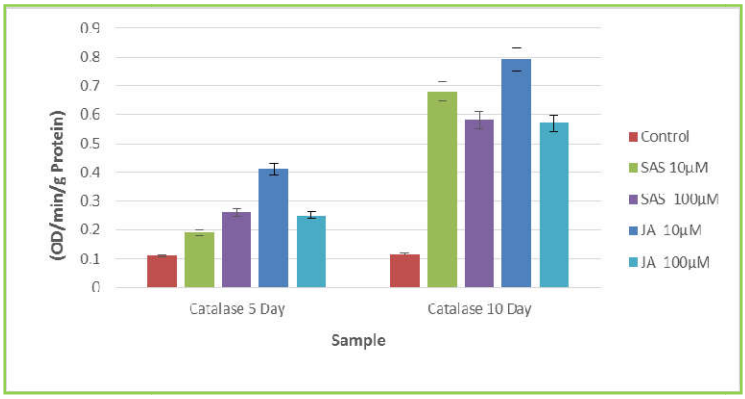

Figure 7. Significant decrease and increase of catalase enzyme activity of under treatment groups with jasmonic acid in compared with salicylic acid.

The catalase activity of the cellular suspensions were between 0.19 ± 0.012 OD/min/g protein and 0.79 ± 0.022 OD/min/g protein (Table 7). The catalase activity in the cellular suspensions of under treatment with JA 10 µM during the 10 days (0.79 ± 0.022 OD/min/g protein) was the highest amount in compared to the other cellular suspensions.

Table 7. Catalase enzyme activity in the studied cell suspensions

Groups	[1]Mean Total phenol ±SD (OD/min/g Protein)
JA-10µM. 5 days group	0.41±0.012
JA-100µM. 5 days group	0.25±0.051
JA-10µM. 10days group	0.7+±0.022
JA-100µM. 10days group	0.57±0.038
SA-10µM. 5days group	0.19±0.012
SA-100µM. 5days group	0.26±0.014
SA-10µM. 10days group	0.68±0.002
SA-100µM. 10days group	0.58±0.021

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

The results chemical composition of the cellular suspensions essential oil of under treatment with salicylic acid during the 10 days, showed that 18, 54 main compounds identified in cellular suspensions from 10 µM, 100 µM treaetments. Thus, The results chemical composition of the cellular suspensions essential oil of under treatment with jasmonic acid during the 10 days, showed that 15, 20 main compounds identified in cellular suspensions from 10 µM, 100 µM treaetments.
Major compounds of cellular suspensions of under treatment with salicylic acid at 10 µM were: α-pinene (74.153%), Cyclotetrasiloxane (5.140%), Cyclohexasiloxane (5.029%) and major compounds of cellular suspensions of under treatment with salicylic acid at 100 µM were: α-pinene (49.330%), Cyclotetrasiloxane (8.973%), Cyclohexasiloxane (4.601%) (Table 8).
Major compounds of cellular suspensions of under treatment with jasmonic acid at 10 µM were: α-pinene (79.012%), Cyclotetrasiloxane (4.563%), Cyclohexasiloxane (3.423%) and major compounds of cellular suspensions of under treatment with jasmonic acid at 100 µM were: α-pinene (47.705%), Cyclotetrasiloxane (3.290%), Cyclohexasiloxane (2.463%) (Table 8).

Table 8. Chemical composition of cellular suspensions essential oil of under treatment with salicylic acid and jasmonic acid

*Compound	Rt[a] (min)	Control (%)	SA 10µM (%)	SA 100µM (%)	JA 10µM (%)	JA 100µM (%)
α-Pinene	7.231	92.605	74.153	49.330	79.012	47.705
Cyclotetrasiloxane	8.444	1.132	5.140	8.973	4.563	3.290
Cyclohexasiloxane	13.448	0.49	5.029	4.601	3.423	2.463
Cyclopentasiloxane	10.970	0	2.081	3.736	2.032	1.388
Pentasiloxane	15.6723	0	2.048	3.420	1.22	0.89

[a] Rt(Retention time)

* Compounds listed in order of elution

DISCUSSION

Secondary metabolite production by medicinal plants and its utilization are one of the plant research areas which are being managed by using various elicitors, such salicylic acid and jasmonic acid [22].
Elicitors are determined as molecules that motivate defense or stress-induced responses in plants [23]. The exogenous use of elicitors to *In vitro* cultures is useful for studying plant responses to potential microbe/insect attack as well as for raised biotechnological output of value-added secondary metabolites in fermentation systems. Elicitors including JA and its derivatives are known to motive production of secondary metabolites in plants [24, 25]. Salicylic acid is an significant, plant-signaling compound that activates defense-related genes, and when used as an elicitor, SA is useful for studying the accumulation of pathogenesis related compounds [26]. Fungal elicitors, mainly derived from the cell walls of fungal pathogens, are known to induce the de novo synthesis of antimicrobial phytoalexins [27].
Jasmonic acid and its derivatives have an perfect role in the cascade of events that occur in the elicitation process, causing either directly or indirectly the activation of the genes of secondary metabolism. There is one news [28] of the induction of secondary plant product accumulation (anthocyanin) in response to treatment of germinating soybean seedlings with methyl jasmonate, indicating that this defense mechanism is operative not only in plant cell suspension cultures but also in differentiated plants.
The fact that exogenously provided methyl jasmonate activates, in different plant systems, a multitude of"jasmonate induced proteins" [29] can now be explained in a satisfactory manner by the fact that, in each plant, a multitude of species-specific genes complicated in the formation of high [30], and low molecular weight combinations are expressed in response to these signal-transducer molecules. Without knowledge of its action in plants, jasmonic acid has been compared to the prostaglandins, chemically similar mammalian hormones. This supposition may now prove to be correct in light of the induction of proteinase inhibitors [30], storage proteins [28] and the results presented here.
According to the results under different treatments of salicylic acid and jasmonic acid in the study, weight, total phenol and total flavonoid sighnificantly increased as compared to the control ($P<0.05$). Our results are further supported by the results of other studies using SA to raise the production of certain secondary metabolites. The increase in secondary metabolites in the suspension culture of *Saussurea medusa* treated with 0.02 mM SA was reported by Yu et al. 2006 [31].
The our results showed that the samples treated with jasmonic acid sighnificantly weight, phenolic compounds and flavonoids were increased and sighnificantly anthocyanins were decreased. Therefore, the hormone as a biological inducer efficient biosynthesis of secondary metabolites that is improving in

many medicinal plants used.
On the other hand, the samples treated with jasmonic acid sighnificantly polyphenoloxidase enzyme activity were increased. This results were not similar with results of Yoshimurak *et al.* (2000) [32], they reported that high concentrations of heavy metals can decrease activity of antioxidant enzymes.
The callus treated with salicylic acid, catalase was increased that catalase increased activity of antioxidant system and reduce damage to the plasma membrane lipids.
In this present study, polyphenol oxidase and peroxidase activity decreased in the treated samples was observed salicylic acid, which is due to reduced activity of this enzyme, salicylic acid directly involved in removing free radicals and clean up this species increased activity of enzymes is prevented.
The results of the present studies on the effect of jasmonic acid and salicylic acid on the essential oils composition and terpenes alpha-pinene showed that a combination of high medicinal properties and compared with samples treated with salicylic acid, jasmonic acid decreased, but other terpene compounds had increased, terpenes reacts differently to hormones is that these marks. The present research study phytochemical reaction geranium plant hormones jasmonic acid and salicylic acid was performed.
Given the results of this study, treatment with these hormones may increase or reduce some secondary metabolites especially essential oil is important terpenes.
It has been accepted that flavonoids show antioxidant effect and their effects on human nutrition and health are remarkable. The mechanisms of action of flavonoids are through scavenging or chelating process [33, 34]. Phenolic compounds are a class of antioxidant agents which act as free radical terminators [35]. Arora *et al*. (2000) show that phenolics (especially flavonoids) are able to change peroxidation kinetics by modifying the lipid packing order. They confirm membranes by decreasing membrane fluidity and prevent the diffusion of free radicals and restrict peroxidative response [36, 37].
On the other hand, *in vitro* studies have shown that flavonoids can directly scavenge molecular species of active oxygen: superoxide, hydrogen peroxide, hydroxyl radical, singlet oxygen or peroxyl radical. Their antioxidant action resides generally in their ability to donate electrons or hydrogen atoms [38]. Polyphenols posess perfection structural chemistry for this effect and have been shown to be more effective *in vitro* than vitamins E and C on molar basis [7].
According to our study, the high contents of these phytochemicals in the samples treated with jasmonic acid can explain its high radical scavenging activity. Free radicals are involved in multitude disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases.

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