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EFFECT OF SALICYLIC ACID ON INCREASING PHENOLIC COMPOUNDS OF *CATHARANTHUS VINCA* (L.) G. DON. USING SHOOT TIP *IN VITRO*

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ABSTRACT
Bioactive compounds in plants can be defined as plant secondary metabolites using in pharmacological for human healthy. Phenol compounds are produced within the *Catharanthus vinca*. Plants besides the primary biosynthetic and metabolic routes for compounds associated with plant growth and development. Several of them are found to hold various types of important functions in the treatments but this medicinal compounds are produced in little amount in plant so it's necessary to stimulate the plant to increase the amount of production. The present research was conducted in order to increase some secondary metabolites Phenolic compounds of *Catharanthus vinca*. In vitro by adding different concentrations of Salicylic acid (50,100,150 or 200) mg/l to Shoot tip media then this compounds are estimated by high performance liquid chromatography (HPLC) and compared with those in mother plant. The results showed that the concentrations (200,150 or 100) mg/l of Salicylic acid for increasing Phenolic compounds and cause highly significant differences.

KEYWORDS: *Catharanthus vinca,* Salicylic acid.

INTRODUCTION

As a traditional remedy for a wide range of ailments, *Catharanthus vinca* (L.) G. Don is an essential medicinal plant that has been used for centuries. Medicinal usage of this herb has been documented for centuries (Senbagalakshmi *et al*., 2017).

The vinca plant has a significant deal of medicinal and economic importance, as well as being aesthetically pleasing and being a beautiful blooming plant.

Since it contains a broad range of pharmaceutically valuable indole alkaloids and terpenoid compounds, *Catharanthus vinca* is a highly researched and used plant. Vincristine and vinblastine are the most valuable bisindole alkaloids because of their anti-cancer properties (Rahim *et al.*, 2018).

It is now grown all throughout the world, including in Iraq, for its medicinal and decorative qualities. Green variegation and a plethora of brightly colored blooms give it an aesthetically pleasing look (Lahuf, 2019).

The plant belongs to the perennial herbaceous group, which may be found in ponds and flowerbeds. It spreads by seeds and stems, and it blooms throughout the year in a variety of hues, including white and red (Al-Mukhtar *et al*., 2019).

Plant extracts have been used in traditional medicine to treat a wide range of ailments, including diabetes, malaria, and Hodgkin's disease, among others (Aslam *et al*., 2014).

Anticancer, antidiabetic, antihypertensive, antibacterial, antifungal and antioxidant properties are among the plant's many medical uses (Dhayanithy *et al*., 2019).

A biotechnological technique primarily tissue culture leads to the candidacy for anticancer chemical development. All kinds of bioactive substances including; alkaloids, steroids, phenols and saponins are in high demand in the commercial world. Continuous; regulated, aseptic and large-scale plant tissue culture applications are more favorable than traditional approaches in terms of their advantages. Different bioreactors are utilized for commercial-scale production of bioactive compounds. To produce shikonin, vincristine and podophyllotoxin, stirred tanks are used. Callus, suspension and hairy root cultures are used for large production of these bioactive (Patel *et al*., 2022).

Secondary metabolites derived from plant cell and organ cultures have shown promise as medicines, agrochemicals, flavorings, scents, coloring agents, bio-pesticides and food additives, among other applications. A variety of methods have been developed in recent years to measure biomass accumulation and secondary chemical production in cultures. The factors that affect the development and multiplication of cultured cells/organs and the accumulation of biomass are controlled in the initial stage of both biomass accumulation and metabolite production. The second stage controls parameters that aid in the production of metabolites (Murthy *et al*., 2014).

When certain groupings of chemicals were introduced into a culture media, the production of secondary metabolites was dramatically boosted (Maqsood & Abdul, 2017).

This compound comprises an aromatic ring with a hydroxyl group or its functional derivative, which plants produce and it was demoted to the category of "secondary metabolites" after being discovered in the soil (Hadacek *et al*., 2011).

Salicylic acid, white, crystalline organic acid, is often used as an elicitor in plants to stimulate the production of secondary metabolites. It is produced as a result of the metabolism of salicin. Salicylates are the chemical names given to the salts and esters of salicylic acid and they are derived from the word "salicylate" (Haynes, 2011).

Salicylic acid (SA) is a plant phenolic and hormone-like endogenous regulator whose involvement in defense mechanisms has been extensively documented(SA) reactivates a wide range of plant defense response genes and a wide range of physiological activities in plants; seed germination, stomatal closure, glycolysis and fruit output (Yücel & Heybet, 2016).

Referred to as elicitors because plants accumulate most secondary metabolites as a defense against pathogen infection and environmental stress, our approach makes use of that fact. Elicitors are the agents that activate plant defense mechanisms (Jian & Jian, 2013).

As antitumor agents, these chemical substances are used into anticancer treatment plans. They prevent the development of the mitotic spindle during cell division by inhibiting tubulin polymerization. DNS repair mechanisms and RNS polymerase are also inhibited in cancer cells, resulting in an inhibition of RNS polymerase. These chemicals are most often used to treat leukemia and lymphoma in anticancer treatment (Mayer *et al*., 2020).

Almost all plants contain phenolic chemicals, particularly flavonoids. Among the many effects flavonoids have in the body are those related to their ability to fight cancer and diabetes as well as those related to their ability to fight bacteria, viruses and fungi. They also have effects on the liver hormones and immune system as well as effects on inflammation, anxiolysis, antiulcer, antiosteoporotic, antibacterial, antifungal and antiviral functions (Al-Snafi, 2020).

MATERIALS AND METHODS

Plant materials and sterilization

The explant of *Chatharanthus vinca* , newly branches were collected from gardens in Baghdad Iraq on 20/4/2021, shoot tip of *C. vinca* were rinsed with running water for 30 minutes then transfer to laminar air flow cabinet where submerged in 95% ethanol for 1 minutes next, washed with sterilization D.W. for 5 min after that rinsed with sodium hypochlorite at 3% and washed with sterilization D.W. for 5 min three times and culture in universal tubes which contain MS medium (Tanaka *et al*., 2001; *Shaw et al*., 2014).

Growth medium

Explants (shoot tip) of *C. vinca* was dissected culture on universal tubes containing MS medium with different concentrations of the BA (0, 1, 2, 3 or 4) then distributed in to 10 replicated for each concentration which incubated at 16/8 hrs. light photoperiod at illumination intensity was 1000lux and incubated at temperature 25±2 °C, the results recorded after 21 day (Nair & Chung, 2014).

Fresh and dry weight measurements of shoot tips growth

The fresh weight of Plantlets was measured by using the sensitive balance then the Plantlets was dried using oven at 70°C until the dry weight is stable then measured by sensitive balanced (Castiglione *et al.*, 2011).

Table 1: MS medium supplement with different concentration of Salicylic acid.

No.	Component	Concentrations (mg/l)
1.	MS	4400
2.	Sugar	30000
3.	2,4-D	0.5
4.	Thimine	10
5.	BA	2
6.	Agar-Agar	8000
7.	Salicylic acid	5, 10, 15 or 20

Extraction and analysis of secondary metabolite from shoot tip of *C. vinca*.

The main phenolic compounds were separated on FLC (Fast Liquid Chromatographic) column under the optimum condition Column: phenomenex C-18, 3µm particle size (50 x 2.0 m m I.D) column, Mobile phase: linear gradient of, solvent A 0.1% phosphoric acid: solvent B was of acetonitrile added 0.1% phosphoric acid, linear gradient program from 0% B to 100%B for 15 minutes.
flow rate 1.0 ml/min.
detection: UV 280 nm

Calculation the concentration of samples (Phenolic compounds) was measured by using the following formula:

$$\text{comcentration of sample}\left(\frac{\mu g}{ml}\right) = \frac{\text{Area of sample}}{(\text{Area of standard})} \text{X con} \cdot \text{of standard X dilution Factor}$$

Equipment

The separation occurred on liquid chromatography Shimadzu 10AV LC equipped with binary delivery pump model LC-10A shimadzu the eluted peaks were monitored by UV-Vis 10 A- SPD spectrophotometer.

Extraction of secondary metabolite from shoot tip of C*. vinca* (L) G.Don.

0.5 gm of leaves powder and 100 mg of shoot tip were dissolved in 20 ml hexane to remove fat layer, then the organic layer dissolved 100 ml of 80:20 (methanol: water), The extract was subjected to ultra- sonication (Branson sonicater, (USA) at 60 % duty cycles for 25 min at 25°C followed by centrifugation at 7,500 rpm for 15 min. The clear supernatant of each sample was subjected to charcoal treatment to remove pigments prior to evaporation under vacuum (Buchi Rotavapor Re Type). Dried samples were re suspended in 1.0 ml HPLC grade methanol by overtaxing, the mixture were passed through 2.5 um disposable filter, and stored at 4°C for further analysis, then20 ul of the sample injected into HPLC system according the optimum condition (Bandar *et al*., 2013).

Statistical analysis and experimental design

Experiments are designed according to the completely randomize design (CRD) to study the effect of various transactions in the studied traits and compared the differences between the treatments according to least significant differences (LSD) probability of 5% (Salkind and Ramsey, 2007).

RESULT AND DISCUSSION

Table 2 showed that the concentrations (1, 2) mg/l BA gave the highest number of branch (7.33, 4.33) respectively that had high significant differences than the other treatments while the lowest number of branch in control treatment was recorded (1.33). The results in the same table showed that highest significant differences in length of shoot tip (19. 7, 18.0) cm in concentrations (1, 2) mg/l BA and the lowest average (5.7) cm in control treatment. The same table showed there was no significant differences in the number of leaves.

The table 2 showed that the concentrations (1, 2) mg/l BA gave the highest shoot tip fresh weight (1114, 1273) mg that had high significant differences than the other treatment while the lowest fresh weight showed in concentration (4) mg /l which was reached (377) mg. The same table showed that the treatment (1) mg/l BA gave a highest dry weight(175.0)mg with a significant difference than other treatments except (2) mg/l BA which is reached to (169.3)mg while the lowest dry weight showed in treatment (4)mg/l BA which gave (58.0) mg.

Table 2: Effect of different concentrations of BA on Number of branch, the length, Number of Leaves, fresh and dry weight of Plantlets of *C. vinca* (L) G. Don.

BA Concentration mg/l	Number of branch	The length Cm	Number of Leaves	Fresh weight (mg)	Dry weight (mg)
Control	1.33b	5.7b	7.17	589b	85.0b
1	7.33a	19.7a	7.83	1114a	175.0a
2	4.33ab	18.0a	7.83	1273a	169.3a
3	2.67b	9.7b	6.67	573b	88.3b
4	2.00b	9.3b	6.50	377b	58.0b
LSD(0.05)	3.13	8.52	N.S.	655.3	64.01

The result in table 3 showed that adding Salicylic acid led to increasing the concentration of Salicylic acid. The treatments 150 and 200 mg/l giving a high concentration reached to (737,879) µg/ml which are significantly different than other treatments but had no difference between them. The lowest concentration of Salicylic acid reported with control treatment which reached to359 µg/ml.

while the Gallic acid was recorded the high significant increasing in concentration (200) mg/l Salicylic acid that measured (783.0) µg/ml and significantly different than other treatments, the lowest values of Gallic acid recorded with in mother plant which reached to (152.9) µg/ml. Vanillic acid shows high significant differences recorded with (100, 150 or 200) mg/l Salicylic acid reached to (618, 466 or 602) µg/ml respectively. While lowest concentration observed with treatment 50 mg/l Salicylic acid which gave 143 µg/ml that had no differences from the treats (Mother plant and cont.).

Feruolic acid Showed high significant difference treats (100,150 or200) mg/l Salicylic acid that measured (819, 746 and 823) µg/ml respectively while lowest concentration recorded in mother plant (223) µg/ml.
Rutin compound have lowest concentration in mother plant (521) µg/ml and high concentration with significant difference recorded in treatment (200) mg/l Salicylic acid were recorded (6797) µg/ml.

Caffeic acid highly significant value recorded with treats(150,200)mg/l Salicylic acid reached to (455,475) µg/ml, while the lowest concentration observed in treatment 50 mg /l which gives (193) µg/ml that had no difference than treats(cont. and100) mg/l Salicylic acid.

Quercetin Showed high significant difference in mother plant that recorded (739) µg/ml while was lowest concentration in control treatment (190) µg/ml.

Kaempferol compound showed high significant differences with treatments (100, 150 or 200) mg/l Salicylic acid where reported (5258, 5385 and 5634) µg/ml respectively and had no significant differences between them, while treatment (50) mg/l recorded (3705) µg/ml and treatment (count) mg/l recorded (1881) µg/ml. In mother plant showed the lowest concentration reached to (631) µg/ml.

Adding different concentrations of Salicylic acid for shoot tip media cause high significant increase in all study secondary metabolites (phenolic compounds) of *C. vinca* (L.) G. Don. especially with high concentrations of Salicylic acid. This results agree with (Al-oubaidi & Ameen, 2014).When he used different concentrations of Salicylic acid with *Calendula officinalis* L.

Table 3: phenolic compounds of shoot tip *C. vinca* (L) G. Don which cultured on MS medium supplemented with different concentrations of Salicylic acid.

Compounds	Mother Plant	Cont.	50	100	150	200	LSD 0.05
Salicylic acid	513cd	359d	360d	587bc	737ab	879a	208.70
Gallic acid	152.9f	318.2d	259.2e	502.8c	684.6b	738.0a	47.98
Vanillic acid	264b	235b	143b	618a	466a	602a	181.60
Feruolic acid	223c	454b	508b	819a	746a	823a	151.30
Rutin	521f	2711e	3796d	4611c	5341b	6797a	362.00
Caffeic acid	329bc	199c	193c	260c	455ab	475a	138.90
Querectin	739a	190c	192c	335bc	471b	469b	246.40
Kaempferol	631d	1881c	3705b	5258a	5385a	5634a	1151.40

Effect of different concentrations of BA on multiplication of C. vinca.

Effect of different concentrations of Salicylic acid on phenolic compounds of C. vinca.

Recommendation

Utilize other Chemical compounds to increase secondary metabolites that used as medicinal compounds.

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