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EFFECT OF SUCROSE CONCENTRATIONS ON INCREASING OF ACTIVE COMPOUNDS IN CALLUS OF DURENTA ERECTA L. IN VITRO

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ABSTRACT
The present research was conducted in order to increase some secondary metabolites compounds of Durenta erecta L. in vitro by adding different concentrations of sucrose (30, 45, 60 or 75) g/l to callus media. Quantitative and qualitative analysis of secondary metabolite were estimated by using high performance liquid chromatography (HPLC). The results revealed that the concentrations 75 g/l sucrose caused highly significant production in most of the secondary metabolites from callus of Durenta erecta L.

KEYWORDS: callus induction, in vitro, Durenta erecta L., secondary metabolites, sucrose.

INTRODUCTION

Medicinal plants have been used by mankind since ancient times, and many bioactive plant secondary metabolites are applied nowadays both directly as drugs, and as raw materials for semi-synthetic modifications. However, the structural complexity often thwarts cost-efficient chemical synthesis, and the usually low content in the native plant necessitates the processing of large amounts of field-cultivated raw material. The biotechnological manufacturing of such compounds offers a number of advantages like predictable, stable, and year-round sustainable production, scalability, and easier extraction and purification. Plant cell and tissue culture represents one possible alternative to the extraction of phytochemicals from plant material. Although a broad commercialization of such processes has not yet occurred, ongoing research indicates that plant in vitro systems such as cell suspension cultures, organ cultures, and transgenic hairy roots hold a promising potential as sources for bioactive compounds. Progress in the areas of biosynthetic pathway elucidation and genetic manipulation has expanded the possibilities to utilize plant metabolic engineering and heterologous production in microorganisms. This review aims to summarize recent advances in the in vitro production of high-value plant secondary metabolites of medicinal importance.[1] Plant tissue culture techniques are essential to many types of academic inquiry, as well as to many applied aspects of plant science. In the past, plant tissue culture techniques have been used in academic investigations of totipotency and the roles of hormones in cytodifferentiation and organogenesis.[2]

Elicitors are the compounds obtained from living (biotic) and non-living sources (abiotic) which can induce plant responses to stress leading to aggravated biosynthesis of secondary plant metabolites and the process is termed as an ‘elicitation’ Based on originated sources, elicitors are classified into two types viz. Abiotic elicitors and Biotic elicitors. Abiotic elicitors include physical, chemical and hormonal factors i.e. UV radiations, osmotic stress, drought, salinity and temperature stress (low and high); heavy metals and intracellular signaling molecules (jasmonic acid, methyl jasmonate, salicylic acid, brassino steroids, polyamines, abscisic acid and gibberellic acid and gaseous molecules such as ethylene and nitric oxide) In contrast, biotic elicitors are substances of biological origin that include polysaccharides and pathogens (yeast, bacterial and fungal extracts).[3]

Duranta erecta L. belongs to the Verbenaceae family and it comprises 35 species. It is a native plant of Asia, Africa, and South and Central America. Duranta is, commonly known as “golden dew drop,” is an upright scrambling shrub with a height of 1–3 m[4], and different parts of the plant are used to treat a variety of diseases. The fruit and leaves are used in traditional folk medicine for the treatment of malaria, intestinal worms, and abscess and, in some cases, serve as vermifuge or diuretic. D. erecta is said to possess antitumor activity and significant antibacterial activity. It has numerous natural uses including antifungal and insecticidal properties. The ethyl acetate extract of leaves reportedly exhibited significant anti plasmodial activity against the chloroquine-sensitive and chloroquine-resistant strains of

Plasmodium falciparum. Methanolic extracts of different parts such as leaves, stem, and roots of D. erecta exhibited antifungal properties against Aspergillus flavus, Alternaria sp., Penicillium sp., Rhizopus sp., and Trichoderma sp.[5,6]

MATERIALS AND METHODS

Plant material and sterilization: The explants of Duranta erecta from new branches were collected from gardens in Baghdad Iraq on 1/10/2023. leaves of Duranta erecta were rinsed with running water for 30 minutes then transfer to laminar air flow cabinet where submerged in 70% ethanol for 1 minutes then washed with sterilized D.W for 5min then rinsed with sodium hypochlorite at concentration 2% then washed with sterilized D.W for 5min three times and culture on universal tubes which contain MS medium.[7]

Callus induction: Explants (leaves) of Duranta erecta were dissected and cultured on universal tubes contain MS medium with different concentrations of the auxin 2, 4-D (0, 0.5, 1.5, 2.5 or 3.5) mg/l and then we choose the concentration 2.5 mg/l of 2,4-D and different concentrations of sucrose, table (1), then distributed into 10 replicates for each concentrations which incubated in dark conditions at a temperature 25±2°C , the results recorded after 21 day.[8]

Table (1): MS medium supplemented with different concentrations of 2, 4-D and different concentrations of sucrose.

No.	Component	Concentration (mg/l)
1	MS	4400
2	sucrose	0 , 30000 , 45000, 60000 , 75000
3	agar	8000
4	2,4-D	0 , 0.5 , 1.5 , 2.5 , 3.5
5	BA	0.2
6	Glycine	100
7	Asparagine	150

Measuring fresh and dry weight of callus: The fresh weight of callus was measured by using the sensitive balance then the callus was dried using oven at 70°C until the dry weight is stable then measured by sensitive balance.[9]

Extraction and analysis of secondary metabolite from callus of Duranta erecta: 500 mg of callus were dissolved in 20 ml hexane to remove fat, after decarded fat layer, the residue subjected, 1oo ml of 80:20 (methanol: water) the extract was subjected to the ultra-sonication (Branson sonifier, 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, and then 20 ul of the sample injected into HPLC system according the optimum condition.[10]

Estimation the increase in secondary metabolites compound by device High performance liquid chromatography (HPLC): Analysis of phenols and flavonoids in Duranta callus extract.

The main compound were separated on FLC (Fast Liquid Chromatographic) column under the optimum condition Column: phenomenex C-18, 3µm particle size (50 x 2.0 mm I.D) column, Mobile phase: linear gradient of, solvent A 0.1% phosphoric acid: solvent B was (6:3:1, v/v/v) of acetonitrile: methanol: 0.1% phosphoric acid, gradient program from 0%B to 100%B for 15 minutes. flow rate 1.2 ml/min. Detection: UV 280 nm.

Seq	Subjects	Retention time minute	Areaµ volt	Concentration 50 µg/ml
1	Acteoside	2.498	267677	
2	Iso-acteoside	3.187	312986	
3	Durantoside	4.127	334497	
4	Quercetin	5.525	3344593	
5	Methyl apigenin -7-O-β pyranuronate	6.345	278571	

Calculation

Concentration rea of sample of sample ug/ml = ----------------- x conc. of standard x dilution Factor Area of standard 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.

Statistical Analysis: Completely randomized design (CRD) was put to use in the design of the experiments for studying various interactions and changes. Least significant difference (LSD) procedure was adopted to compare the difference in various experiments and their results, with a 1% probability.[11]

RESULT AND DISCUSSION

Callus morphology: Callus morphology serves as a parameter for assessing the regeneration capacity of the callus.[12] Visual observations of callus morphology, including color and texture, were conducted on the 15th and 30th days after planting. The color of the callus formed varied across treatments, with the majority exhibiting a brown hue (Fig. 1). Differences in callus color indicate variations in the quality, growth, and developmental stages of each callus. Callus color can range from light yellow, dark yellow, and brownish white, to light brown, dark brown.[13]

Fig. (1): Callus of Durenta erecta L. at different concentrations of sucrose.

Illustrates that the majority of obtained calli exhibited various shades of brown. The browning of callus is attributed to the accumulation and oxidation of phenolic compounds in plant tissues and culture media. Phenolic compounds are produced or released in significant quantities as a defense mechanism, particularly in response to injury or exposure to stressful conditions.[14]

Browning reactions in plant tissue culture are closely associated with the activity of phenylalanine ammonialyase (PAL) [15], an enzyme that plays an important role in the biosynthesis of phenolic compounds.[16] PAL activity can be enhanced by exogenously administered sucrose in the media. Browning can also serve as an indication of the aging process (senescence) or physiological decline in callus cells. Prolonged culture incubation (30 days after planting) resulted in the formation of darker or dark brown calli. The dark brown color of the callus may also indicate the initiation of secondary metabolite synthesis by the cells.[17]

The effect of different concentrations of sucrose on fresh and dry weight in callus

The result in (table 2) showed the concentration (60 and 75) g/l sucrose had the highest callus dry weight (27.65 and 26.45 mg) that had high significant than the other treatments while the lowest callus dry weight (14.25 mg) at concentration 30 g/l of sucrose, the results at same table showed that no significant differences in all concentration of sucrose in fresh weight.

Table (2): Effect of Concentrations of sucrose (g/l) on means of fresh and dry weight of callus (mg).

Concentrations of sucrose(g/l)	Mean of fresh weight (mg)	Mean of dry weight (mg)
Cont.	170.00	16.95 B
30	227.00	14.25 B
45	167.00	15.90 B
60	257.33	27.65 A
75	180.33	26.45 A
LSD (0.01)	N.S.	6.690

Previous research by[18] demonstrated that the supplementation of elicitors at biomass production in plant culture serves as an indication of optimal nutrient absorption from the culture media, which acts as a source for cellular metabolic activity.[19] Sucrose plays a dual role as an external energy source and in maintaining osmotic pressure in the environment, thereby promoting growth in various types of plants in vitro.[20] Increased osmotic pressure in the tissue facilitates water absorption, solute uptake, and the assimilation of growth regulators from the growth medium, leading to a high frequency of shoot regeneration.[21] Therefore, osmotic pressure is crucial for cell growth and proliferation in plant tissue culture propagation. Importantly, it has been reported that high sucrose concentrations result in a significant increase in osmotic pressure.[22]

The effect of different concentrations of sucrose on medical compounds in callus using HPLC technique: The result in (table 3) showed different concentrations of medical compounds were increase depending on the increasing concentration of sucrose compared with mother plant. The Acteoside gave high significant difference (2029.98µg/ml) at concentration 75 g/l sucrose while the lowest average at Control that measured (1278.74 µg/ml). The Isoacteoside gave high significant difference (1262.63, 1092.20, 1032.36, 847.27 µg/ml) at concentration (75, 30, 60, 45 g/l) of sucrose while the lowest average at control which gave (353.33 µg/ml) at cont. treatment. The Durantoside gave high significant difference at concentration 75 g/l sucrose that measured (1223.87 µg/ml) which no significant difference than treats. (45, 60 g/l) sucrose , while the lowest average at control and 30 g/l treatment (727.38, 464.04 µg/ml). The Quercetin gave high significant difference at concentration (75 and 60 g/l) sucrose that measured (93.77 and 88.42 µg/ml)with no significant difference between them , while the lowest average at control treatment (61.91 µg/ml). Finally, the methyl apigenin-7-O-B-pyrnuronate measured the high significant difference (1177.20 µg/ml) at concentration (75 g/l) sucrose where the control treatment measured (759.81µg/ml) the lowest average of Methyl apigenin-7-O-B-pyrnuronate compound.

Table (3): Effect of Concentrations of sucrose on increasing medicinal compounds in callus of Duranta erecta.

Compounds	Concentrations of sucrose (g/l)					LSD (0.01)
	Cont.	30	45	60	75	
Acteoside	1278.74 D	1500.01 C	1550.15 C	1925.95 B	2029.98 A	64.54
Isoacteoside	353.33 B	1092.20 A	847.27 A	1032.36 A	1262.63 A	347.12
Durantoside	727.38 B	464.04 B	937.19 AB	1092.23AB	1223.87 A	437.85
Quercetin	61.91 C	73.34 B	76.68 B	88.42 A	93.77 A	9.32
Methyl apigenin-7-O-B-pyrnuronate	759.81 D	916.289 C	1040.21BC	1139.47AB	1177.20 A	129.85

These findings suggest that the efficacy of the elicitor is influenced by its concentration at different harvest times. Previous research has indicated that elicitor concentration is a limiting factor in secondary metabolite synthesis.[23] The elicitor acts as an effector and the optimal concentration depends on the presence of receptors, such as receptor like kinases and wall-associated kinases, in the plant cell membrane. The interaction between the elicitor and the receptor occurs when the secondary metabolite reaches its optimal level.[24]

In this study, sucrose served as an elicitor to induce secondary metabolite in Duranta erecta callus. Each elicitor has an optimal concentration[25], and excessively high concentrations can trigger a hypersensitive response that leads to cell death.[26] Sucrose acts as an osmotic agent, creating osmotic pressure that stimulates the generation of reactive oxygen species, such as hydrogen peroxide (H_2O_2), hydroxyl radicals, superoxide radicals, and alkoxyl radicals. The production of H_2O_2 in the root suspension culture of Hypericum perforatum L. indicates oxidative stress, which serves as a second messenger in plant defense reactions.[27]

Thus, the increase in secondary metabolite biosynthesis is a result of the plant cells' defense response.[28] Sucrose activates various enzymes involved in flavonoid biosynthesis through the shikimate pathway, including PAL, chalcone synthase, chalcone isomerase, and isoflavone synthase.[29]

CONCLUSION

Adding different concentration of sucrose for callus media cause high significant increase in all study medical compound of Duranta erecta. L.

RECOMMENDATION

Utilize other elicitors to increase secondary metabolites that used as medicinal plant.

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