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Effect of saccharides on secondary compounds production from stem derived callus of *Datura inoxia*

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ABSTRACT

Datura inoxia is a subshrub plant known for its toxicity which results from the presence of the tropane alkaloid scopolamine and hyoscyamine. Saccharides are one of the most important elicitors that can alter physiological and biochemical responses in plants. This study targeted to increase the production of secondary metabolites in *Datura inoxia* avoiding genetic alteration and processes using different nontoxic and biodegradable compounds, utilizing calli induced from *Datura inoxia* stems to observe the effects of mannitol and sorbitol on the production of the two major secondary metabolites, scopolamine and hyoscyamine. Methanolic extract of the whole plant and callus was used to examine the production of two secondary metabolites in *Datura inoxia* using HPLC in a qualitative and quantitative manner which revealed the increased production of scopolamine and hyoscyamine in calli. The addition of mannitol and sorbitol in the media had a negative effect on both the fresh and dry weight of the calli but production of scopolamine and hyoscyamine increased significantly. *In-vitro* anti-microbial assay of hyoscyamine against *Escherichia coli* ATCC25922 and *Candida albicans* resulted in total inhibition of both the microbes in concentrations as low as 200 μg/ml.

Abbreviations

ABA –	Abscisic acid
°C -	Degree Celsius
gm -	Gram
HPLC –	High performance liquid chromatography
h-	Hour
l –	Liter
μg –	Microgram
mg –	Milligram
ml –	Milliliter
MIC -	Minimum inhibitory concentration
MBC -	Minimum bactericidal concentration

1. Background

Datura inoxia belonging to the Solanaceae family is a medicinal plant known for its wide use as a hypnotic, sedative especially its use in colic, intestinal pain, teeth, fractures, and spinal pain [1]. The plant is also used as an antiviral, blood pressure regulator, anti-spasmogenic, anticancer, anthelmintic, anticholinergic, strong nematocidal, analgesic [2]. The plant contains atropine, which is used for enlarging the pupil. The tropane alkaloids found in *Datura inoxia* consist mainly of hyoscyamine, scopolamine and a little amount of atropine [1].

Plants produce complex organic compounds that do not have a direct function in growth, called secondary metabolites [3]. Secondary metabolites play a crucial role in the defence of plants, protecting them from pathogens [4]. Studies refer to the great importance of secondary metabolites in the manufacture of medicines and therapeutics and more than a quarter of the drugs produced in the world are derived from plant secondary metabolites [5]. Tissue culture is an important technique for obtaining economically important compounds, including compounds with pharmaceutical importance which are difficult to synthesize in the laboratory and are very costly [6]. There are many benefits associated with the production of these compounds through tissue culture techniques when compared to the process of extraction from the whole plant. These compounds can be obtained with a higher degree of purity through tissue culture techniques that are superior to those derived from whole plants, and their production is fast and not dependent on the season and does not require a large land area [7].

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Isolating secondary metabolites from the whole plant requires killing the plant which can easily be overcome by using callus cultures. Moreover, callus culture can be further optimized for single-cell suspension cultures which can then be utilized by bioreactors to produce high amounts of bioactive compounds. Callus culture for producing secondary metabolites also gives the opportunity for further manipulation to make the desired secondary metabolite release into the suspension from the cells resulting in quick and easy purification. Alteration of *in-vitro* callus culture media composition is also variable which can be improved to increase the production of many folds compared to plants [8]. Using elicitors, which can stimulate stress response in plants, to increase the production of secondary metabolites has gained popularity. Elicitation can utilize microorganisms as biotic elicitors or some abiotic substances or particular light as abiotic elicitors [9]. Supplementation of different compounds to the media has led to an increase in secondary metabolite production in many different species [10]. The addition of ABA with the concentration 3 mg/l, paclobutrazol in concentration 7 mg/l, and mannitol in concentration 5.3 mg/l to the media for callus culture of *Cinchona* increased the concentration of quinine [11]. Although the addition of sorbitol to callus culture media causes more stress than that which is induced by adding mannitol, supplementing the media for callus with either mannitol or sorbitol, alone or together led to a lack of callus growth. The addition of stimulants that cause stress (sorbitol, mannitol) leads to increased production of secondary compounds in cells [12, 13, 14]. The addition of sorbitol to the culture medium of callus derived from fenugreek cotyledons had a significant increase of choline, carpaine, trigonelline synthesis in cultured callus rather compared to those grown on sorbitol free media compared to that of extraction of the whole plant [15, 16]. The increased concentration of sorbitol resulted in an increased concentration of alkaloids in callus induced from *Catharanthus roseus* leaf and the increase was gradual with the increase of sorbitol concentration [17, 18]. The addition of mannitol, sorbitol to culture media led to increased concentration of secondary compounds cineol, thujone, camphor in callus induced from leaves of *Salvia officinalis* [19]. The concentration of saponin in plant cells of *Panax ginseng* increased significantly with the addition of 64.6 gm/l from sorbitol [20]. This study explores the hypothesis of increasing the production of scopolamine and hyoscyamine in the callus of *Datura inoxia* through the addition of sorbitol and mannitol in the callus culture media which might pave the way of producing mass amounts of these two compounds on larger industrial scales.

2. Material and methods

2.1. Callus induction

Stems of *Datura inoxia* were sterilized with 1% sodium hypochlorite for 3 min and then washed with sterile distilled water several times [21]. Stems were inoculated on Murashige and Skoog (MS) medium [22] supplemented with 6-benzylaminopurine (0.5 mg/l) and 1-naphthaleneacetic acid (1 mg/l) and were incubated in an incubator at 25±1 #x00B0;C in dark condition for 4 weeks and the same combination was used for maintenance of the induced callus for obtaining sufficient amount of callus for further experiments [21].

2.2. Treatment of callus

After obtaining the required amount of callus, callus with a weight of about 350 mg was inoculated on the same media used for callus initiation supplemented with sorbitol and mannitol at different concentrations of 0 gm/l, 5 gm/l, 10 gm/l and 15 gm/l with five replicate per treatment. After 4 weeks of culturing period, the fresh and dry weight of calli were recorded.

2.3. Extraction of secondary metabolites and quantification

Methanol was used as a solvent for the extraction of secondary metabolites. Purification and further quantification of secondary metabolites were done using High-Performance Liquid Chromatography (HPLC) technique [23], to estimate the change of secondary metabolite concentration in *Datura inoxia* callus. The results were compared with whole plant extracts. The concentration of samples with the area of standard multiplied by the concentration of standard and dilution factor by using the following law.

$$Concentration\,of\,sample = \frac{Area\,of\,sample}{Area\,of\,standard} \times Conc.\,of\,standard\,dilution\,factor$$

Results were analyzed according to the Randomize Completely Block Design (RCBD). Duncan's multiple range test was used to compare the difference between the mean at 5% probability [24].

2.4. The antibacterial and antifungal effects of hyoscyamine compound

Hyoscyamine compound solutions were prepared in various concentrations such as 5, 10, 20, 50, 100, and 200 µg/ml and diluted in normal saline. *Escherichia coli* ATCC25922 strain was cultured in blood agar (Merck, Germany) and *Candida albicans* was also cultured in Sabouraud dextrose agar medium. The bacterial solutions equal to half McFarland Standard were prepared in trypticase soy broth and exposed to hyoscyamine concentrations. Moreover, the fungal solution was prepared and exposed to hyoscyamine compound various concentrations. They were incubated for 18 h-24 h at 37 °C. The culture of each microorganism in specific media without hyoscyamine addition was considered as the control.

2.5. Data analysis

For each of the estimations, there were five biological replicates were used. Data were subjected to Statistical Package for the Social Sciences (SPSS) software version 16 for analysis of variance (ANOVA); Duncan's multiple range test (DMRT) was used to determine considerable differences among the treatments.

3. Results and discussion

3.1. Effect of saccharide concentration on fresh and dry weight of callus

Table 1 shows the effect of mannitol at different concentrations on the fresh and dry weight of callus. Supplementation of mannitol in small amounts led to the increase in the fresh weight of the callus. However, the fresh weight of callus decreases with increased mannitol concentration. The maximum dry weight of callus was observed without any mannitol supplementation. Supplementation of mannitol with the gradual increase in concentration led to the gradual decrease of the dry weight of the callus. Table 2 shows the effects of different concentrations of sorbitol on callus fresh and dry weight. Sorbitol concentration in media negatively affects both the fresh and dry weight of callus. Maximum weight was observed without any sorbitol supplementation.

Table 1
Effect of Mannitol concentration in fresh and dry weight of callus.

Mannitol concentration (gm/l)	Fresh weight (mg)	Dry weight (mg)
0	350 b	28.73 a
5	390 a	23.11 b
10	300 c	17.85 c
15	217 d	10.03 d

* Mean values were taken from average of five replications. Mean values followed by the same letters in each column are not significantly different at $p \leq 0.05$ according to Duncan's test.

Table 2
The effect of sorbitol concentration in fresh and dry weight of callus.

Sorbitol concentration (mg/l)	Fresh weight (mg)	Dry weight (mg)
0	350	28.73
5	315	16.79
10	277	11.60
15	203	5.35

The possible reason for the decrease in the callus weight after the supplementation of mannitol and sorbitol is due to the reduction of water and nutrition availability [25]. Comparison between the effects of both the saccharides on the fresh weight and dry weight of callus has been shown in Fig. 1. Both saccharides negatively affect both the fresh and dry weight of the callus except for mannitol at 5 gm/l concentration which significantly increases the fresh weight of the callus.

3.2. Quantitative and qualitative evaluation of some secondary metabolites in callus and leaf extract of Datura inoxia by HPLC technique

Supp. Fig. 1 shows the curve of standard secondary compounds to be compared with the curves of secondary compounds extracted from callus and the leaves of the *Datura inoxia*. Fig. 2 and Supp. Fig. 2 show that the extraction from callus contained a higher concentration of secondary compounds compared to leaf extracts. The amount of scopolamine and hyoscyamine significantly increased in callus extracts reaching 202.26 µg/ml and 308.52 µg/ml respectively (Supp. Table 1). The increase of the concentration of secondary compounds in the callus is attributed to the fact that growth regulators added to culture media which stimulated and increased the production of secondary compounds in callus. Subculture also led to the emergence of somaclonal variation, leading to increased production of some secondary compounds in callus [26]. Factors such as light, humidity, explant, and plant growth stage also played a role in the difference in secondary metabolite content. These physiological and environmental differences were due to the different growth conditions of explant, in addition to the genetic factors that had been affected by physiological factors [27].

3.3. Effect of saccharides on the production of secondary compounds using HPLC technique

The results of Table 3 and Supp. Figure 3 indicate that there were significant differences between the concentration of secondary compounds due to the addition of mannitol to the media. The concentration of 15 gm/l from mannitol was the most effective in stimulating the production of hyoscyamine in callus significantly with a value of 297.58

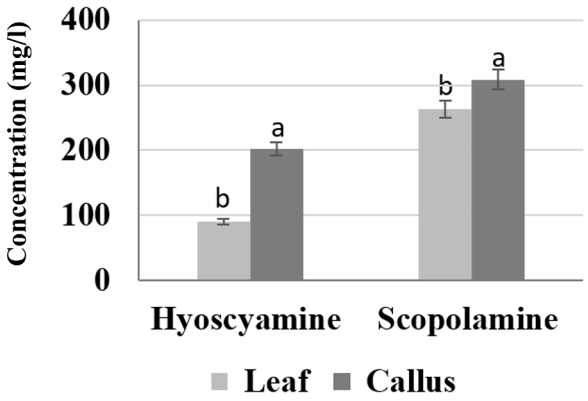

Fig. 2. Production of secondary metabolites, hyoscyamine and scopolamine in leaf and callus. Mean values were taken from average of five replications. Mean values followed by the same letters in each column are not significantly different at $p \leq 0.05$ according to Duncan's test.

Table 3
Effect of mannitol concentration in the production of secondary compounds from callus.

Secondary products	Mannitol concentration (gm/l)			
	0	5	10	15
Hyoscyamine (µg / ml)	202.26 b	228.24 b	159.78 c	297.58 a
Scopolamine (µg / ml)	308.52 c	358.23 b	353.82 b	443.55 a

* Mean values were taken from average of five replications. Mean values followed by the same letters in each column are not significantly different at $p \leq 0.05$ according to Duncan's test.

µg/ml, while the least effective concentration was 10 gm/l where the concentration of hyoscyamine was 259.78 µg/ml, which also differ significantly from control treatment of 5 gm/l. The addition of mannitol concentration had a significant effect on the increased concentration of scopolamine significantly compared to control treatment. The highest concentration of scopolamine was 443.55 µg/ml when 15 gm/l mannitol was added to culture media. The concentration of scopolamine didn't show a significant difference when media was supplemented with 5, 10 gm/l mannitol, and the lowest concentration was 308.52 µg/ml at control. The increased concentration of secondary compounds may be due to the addition of stimulants which increase the production of secondary metabolites [12], and addition of stimulants also increases the accumulation of secondary metabolite [27] (Fig. 3a).

Table 4 and Supp. Fig. 4 shows the effects of sorbitol on hyoscyamine

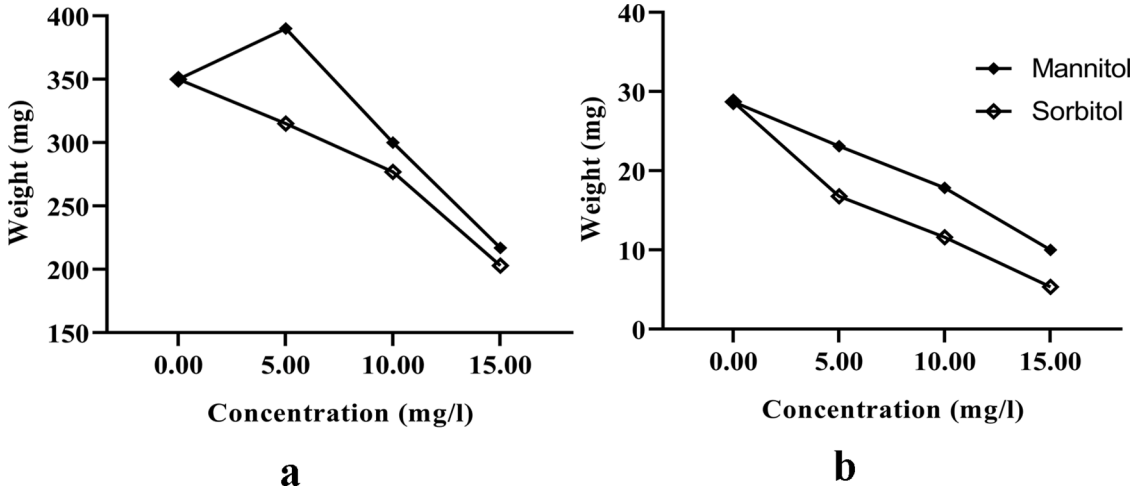

Fig. 1. Comparison of the effects of mannitol and sorbitol on (a) fresh weight of callus; (b) dry weight of callus.

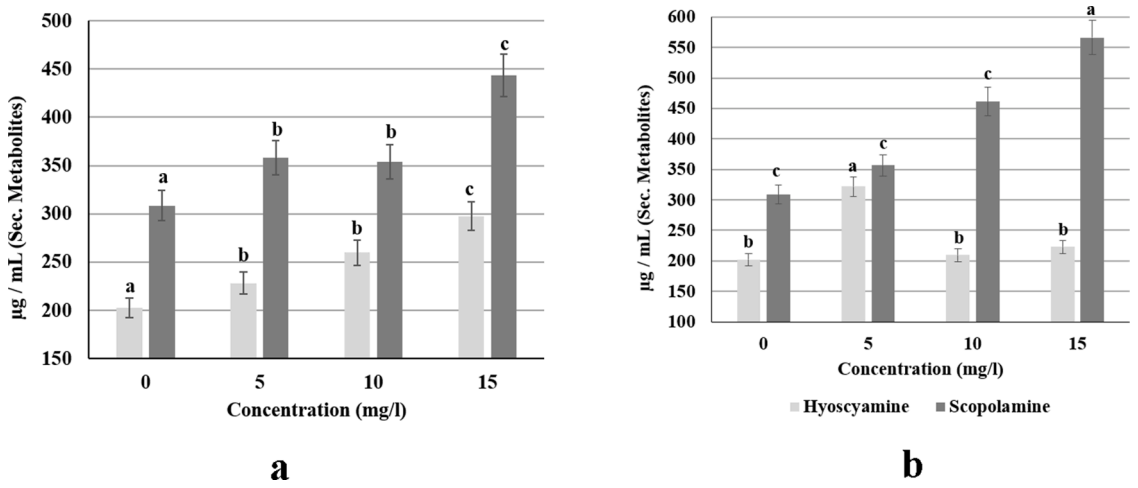

Fig. 3. Comparison of the effects of mannitol and sorbitol on the production of (a) hyoscyamine and (b) scopolamine. Mean values were taken from average of five replications. Mean values followed by the same letters in each column are not significantly different at $p \leq 0.05$ according to Duncan's test.

and scopolamine production. The concentration of hyoscyamine reached the maximum concentration of 321.65 µg/ml when the media was supplemented with sorbitol at a concentration of 5 gm/l. Concentration higher than this doesn't show any significant difference in hyoscyamine production than the control. But the production of scopolamine gradually increased with the increase in sorbitol concentration reaching a maximum concentration of 566.31 µg/ml when the media was supplemented with 15 gm/l sorbitol. The low concentration of secondary compounds may be due to the increased concentration of sugar for treatment (Fig. 3b). This leads to increased stress and therefore leads to a decrease in the production of primary metabolites, and consequently to the concentration of some secondary metabolites [19]. A comparison of the effects of mannitol and sorbitol on the production of secondary metabolites (hyoscyamine and scopolamine) in callus is shown in Fig. 3 (a, b). Production of hyoscyamine and scopolamine increased significantly in the presence of sorbitol in high concentrations compared to that of mannitol.

3.4. The antimicrobial effect of hyoscyamine compound

The effects of hyoscyamine concentrations *E. coli* ATCC25922 exhibited that 100 and 200 µg/ml of this compound inhibited the bacterial growth completely. Therefore, 100 µg/ml was considered as the minimum inhibitory concentration (MIC) and 200 µg/ml was defined as minimum bactericidal concentration (MBC) (Fig. 4). Moreover, the concentration of 200 µg/ml could inhibit the growth of *C. albicans* being the MIC level. Table 5

4. Conclusion

The addition of sorbitol at 5 gm/l to callus culture media led to an increase of the concentration of hyoscyamine significantly, while supplementation at 15 gm/l had a significant effect on the increase of the compound scopolamine in callus compared to the control. The addition of 15 gm/l mannitol to callus culture media had a significant effect on the concentration of scopolamine and hyoscyamine which increased compared to control treatment. Moreover, the current study revealed that secondary metabolites like scopolamine and hyoscyamine have great anti-microbial potential.

Table 4
Effect of sorbitol concentration in the production of secondary compounds from callus.

Secondary products	Sorbitol concentration (mg/l)			
	0	5	10	15
Hyoscyamine (µg / ml)	202.26 b	321.65 a	209.17 b	222.75 b
Scopolamine (µg / ml)	308.52 c	356.52 c	461.42 b	566.31 a

* Mean values were taken from average of five replications. Mean values followed by the same letters in each column are not significantly different at $p \leq 0.05$ according to Duncan's test.

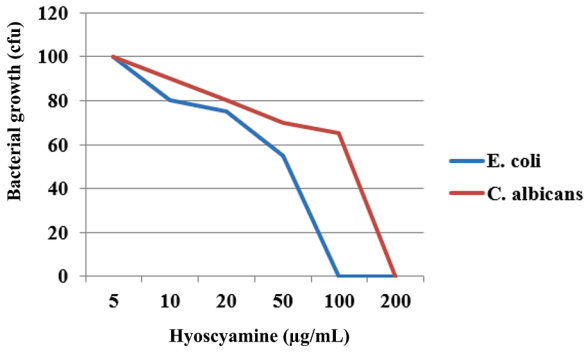

Fig. 4. The effects of various concentrations of hyoscyamine against *E. coli* and *C. albicans*.

Table 5
Effect of sorbitol concentration in the production of secondary compounds from callus.

Callus secondary metabolites (µg / ml)	Concentration of sorbitol (mg/l)			
	0	5	10	15
Hyoscyamine	202.26 b	321.65 a	209.17 b	222.75 b
Scopolamine	308.52 c	356.52 c	461.42 b	566.31 a

* Mean values were taken from average of five replications. Mean values followed by the same letters in each column are not significantly different at $p \leq 0.05$ according to Duncan's test.

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Authors' contributions

B.M.T.conceptualized the experiment. B.M.T. and AJT performed the experiments, analyzed data. B.M.T. and MNH wrote the manuscript. MNH and FHB edited and reviewed the manuscript.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Declaration of Competing Interest

The Authors declare that there is no conflict of interest. All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.btre.2022.e00701.

References

[1] D. Hérouart, et al., Variations in the leaf alkaloid content of androgenic diploid plants of Datura innoxia, Planta Med. 54 (01) (1988) 14–17.
[2] P. Sharma, Classical uses of medicinal plants, chaukhambha vishvabharati, Oriental Publisher and Distributors, Varanasi, India 66 (1996) 67.
[3] K. Ramawat, Plant Biotechnology. Reprint of the Second Edition, S. Chand and Company. Ltd. New Delhi, India, 2004, p. 456.
[4] L. Taiz, E. Zeiger, Plant Physiology, 3rd ed, Sinauer Associates Inc Publishers, Sunderland, MA, 2002, p. 690, p.
[5] M. Vanisree, et al., Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures, Bot. Bull. Acad. Sin 45 (1) (2004) 1–22.
[6] S. Purohit, Agriculture Biotechnology, Published by Updesh Purohit for Agrobios (India), 1999, p. 833.
[7] F. DiCosmo, M. Misawa, Plant cell and tissue culture: alternatives for metabolite production, Biotechnol. Adv. 13 (3) (1995) 425–453.
[8] T. Efferth, Biotechnology applications of plant callus cultures, Engineering 5 (1) (2019) 50–59.
[9] Z. Beygi, et al., Enhanced accumulation of trigonelline by elicitation and osmotic stresses in fenugreek callus culture, Plant Cell, Tissue and Organ Culture (PCTOC) 147 (1) (2021) 169–174.
[10] B.M. Twaij, Z.H. Jazar, M.N. Hasan, The effects of elicitors and precursor on in-vitro cultures of Trifolium resupinatum for sustainable metabolite accumulation and antioxidant activity, Biocatal. Agric. Biotechnol. 22 (2019), 101337.
[11] D. Pratiwi, P. Sari, D. Ratnadewi, Cinchona cells performance in in vitro culture: quinine alkaloid production with application of different elicitors. IOP Conference Series: Earth and Environmental Science, IOP Publishing, 2018.
[12] C. Huang, Z.-.G. Qian, J.-.J. Zhong, Enhancement of ginsenoside biosynthesis in cell cultures of Panax ginseng by N, N′-dicyclohexylcarbodiimide elicitation, J. Biotechnol. 165 (1) (2013) 30–36.
[13] G. Panwar, S. Guru, Stimulation of reserpine production in the whole plant culture of Rauwolfia serpentina L. by elicitors and precursor feeding, J. Plant Biochem. Biotechnol. 24 (1) (2015) 49–55.
[14] R. Razavizadeh, et al., Comparative study of osmotic stress effects on the defense mechanisms and secondary metabolites in Carum copticum seedling and callus, J. Plant Process and Function. 5 (18) (2017) 24–33.
[15] S.A. Hassan, E.H. Jassim, Effect of NaCl and sorbitol on the production of some alkaloids of fenugreek cotyledons derived callus, Al-Nahrain J. Sci. 21 (1) (2018) 90–97.
[16] E.A. Hussein, E.M. Aqlan, Effect of mannitol and sodium chloride on some total secondary metabolites of fenugreek calli cultured in vitro, Plant Tissue Culture and Biotechnol. 21 (1) (2011) 35–43.
[17] A.J. Taha, B.M.A. Twaij, Effect of some chemical and physical elicitors on some secondary compound induction of Ricinus communis through callus induction, Al-Mustansiriyah J. Sci. 28 (3) (2017).
[18] B. Singh, P.M. Sahu, R.A. Sharma, Effect of elicitors on the production of pyrroloquinazoline alkaloids by stimulating anthranilate synthase activity in Adhatoda vasica Nees cell cultures, Planta 246 (6) (2017) 1125–1137.
[19] S.K. Ameen, H.I. Mohamed, Influence of succharides on secondary metabolites production from leaves induced callus of Salvia officinalis in vitro, Anbar J. Agricultural Sci. 8 (4) (2010).
[20] J. Wu, et al., Enhancement of saponin production in Panax ginseng cell culture by osmotic stress and nutrient feeding, Enzyme Microb. Technol. 36 (1) (2005) 133–138.
[21] B. Twaij, A. Taha, M. Hasan, In vitro callus induction and regeneration of medicinal plant Datura innoxia, Am. J. Biochem. Biotechnol. 32 (57900553) (2016) 758–769.
[22] T. Murashige, F. Skoog, A revised medium for rapid growth and bio assays with tobacco tissue cultures, Physiol. Plant 15 (3) (1962) 473–497.
[23] K.-.H. Plank, K.G. Wagner, Determination of hyoscyamine and scopolamine in Datura innoxia plants by high performance liquid chromatography, Zeitschrift für Naturforschung C 41 (4) (1986) 391–395.
[24] A. Glaser, C. Biggs, An Introduction to Statistical Methods in GenStat, VSN. International. Hamell. Hempstead. UK, 2010.
[25] M. Xu, et al., Effect of factors on callus biomass and synthetic mass of hypericin in Hypericum perforatum, Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China J. Chinese Materia Medica 28 (10) (2003) 921–923.
[26] F. Bourgaud, et al., Production of plant secondary metabolites: a historical perspective, Plant Sci. 161 (5) (2001) 839–851.
[27] H. Taha, M. El-Bahr, M. Seif-El-Nasr, In vitro studies on Egyptian Catharanthus roseus (L.) G. Don. IV: manipulation of some amino acids as precursors for enhanced of indole alkaloids production in suspension cultures, Aust. J. Basic Appl. Sci. 3 (4) (2009) 3137–3144.