Bangladesh J. Bot. **48**(3): 633-640, 2019 (September)

CALLUS INDUCTION AND REGENERATION OF *ALKANNA ORIENTALIS* VAR. *ORIENTALIS* AND *A. SIEHEANA*

CENNET YAMAN*, SERKAN URANBEY[1]*, HUSSEIN ABDULLAH AHMED[2], SEBAHATTIN ÖZCAN[1], OSMAN TUGAY[3] AND DILEK BAŞALMA[1]

Department of Field Crops, Agriculture Faculty, Bozok University, 06100 Yozgat/Turkey

Keywords: Alkanna orientalis, A. sieheana, Micropropagation, Alkannin/Shikonin

Abstract

Callus induction and proliferation of *Alkanna orientalis* var. *orientalis* and *Alkanna sieheana* containing valuable alkannin/shikonin (A/S) derivates were investigated using leaf base and stem segment explants. Stem segments and cotyledonary leaf base of both species were cultured on Murashige and Skoog medium fortified with different concentrations of BAP, Kn, NAA, IAA and IBA for callus induction and shoot regeneration. High frequency reproducible, prolific and compact calli formation was obtained from the stem segments of both species in all media tested. The frequency variations of callus induction and shoot regeneration were discussed in terms of different species, plant growth regulators and explant resources. *A. orientalis* and *A. sieheana* may be considered to be alternative plants for the A/S production *in vitro.*

Introduction

The genus *Alkanna* with 50 species is a member of the Boraginaceae family (Mahmoudi *et al.* 2012). Turkey is one of the most important gene centres for the genus represented in the flora of Turkey by 41 taxa belonging to 36 species with an 80% endemism rate (Güner *et al.* 2012). The genus has also herbaceous species and spread out in Mediterranean coast, central, eastern and western of Anatolia. Many species of the genus synthesise red pigments of the isohexenylnaphthazarins which are important plant secondary metabolites. These compounds are commonly known as alkannin/shikonin (A/S) and their derivatives are lipophilic hydroxynaphthoquinone pigments. A/S is enantiomeric hydroxynaphthoquinones and these compounds are found in at least 150 species belonging to the genera *Alkanna, Lithospermum, Cynoglossum, Echium, Anchusa* and *Onosma* of the Boraginaceae family (Kumar *et al.* 2013). Bioactive substances are mainly available in the outer surface of roots.

A/S and its derivatives are used for dyeing in areas such as textile, food, cosmetics (Pal *et al.* 2017). The compounds have also various biological activities such as a strong wound healing (Papageorgiou *et al.* 2008) antibacterial and antifungal properties (Damianakos *et al.* 2012), antiinflammatory (Mahmoudi *et al.* 2012), antioxidant (Wang *et al.* 2010), as well as anti-allergic, antineoplastic, antipyretic and antihydropic activities (Su *et al.* 2011). Recently it has been known that A/S and its derivatives exhibit antitumor activity (Damianakos *et al.* 2012) and these compounds are actively used in anticancer treatments in many countries (Yeh *et al.* 2015).

Wild forms of the genus usually contain A/S in their roots in nature. Optimum production of A/S and its derivatives in roots of plant is 7 - 9 years. Therefore, the production of these compounds with culture is difficult. Also, if the plants were collected from nature, a stable substance can not be obtained because it is collected from different locations and environments.

*Author for correspondence: <cennet.yaman@bozok.edu.tr>, <uranbey@ankara.edu.tr>. [1]Department of Field Crops, Faculty of Agriculture, Ankara University, 06100 Ankara/Turkey. [2]Department of Field Crops, Faculty of Agriculture and Natural Sciences, Uşak University, 64200 Uşak/Turkey. [3]Department of Pharmaceutical Botany, Faculty of Pharmacy, Selçuk University 42100 Konya /Turkey.

Plant cell, tissue and organ cultures have been exploited for the production of secondary metabolites which could be commercially produced at an industrial scale (Piekoszewska *et al.* 2010). Many studies have reported that the secondary metabolites obtained from tissue culture techniques are higher than those obtained from field-grown plants. Callus, suspension and root culture among tissue culture techniques are known to produce secondary metabolites rapidly (Praveen and Murthy 2011). Different plant tissue culture approaches such as changing nutrient medium have been used to increase the production of A/S and its derivatives (Fujita *et al.* 1981) and genetic manipulation (Boehm *et al.* 2000). Commercial production of A/S and its derivatives were first produced by callus culture of *Lithospermum erythrorhizon* (Tabata *et al.* 1974). It is also reported that A/S and it derivatives were produced using tissue culture techniques for different species, e.g., callus culture of *Alkanna tinctoria* (Urbanek *et al.* 1996), root and suspension culture of *Lithospermum canescens* (Tatsumi *et al.* 2016), suspension culture of *Echium italicum* L. (Zare *et al.* 2010), callus culture and suspension culture of *Arnebia hispidissima* (Singh and Sharma 2014), callus culture of *Onosma bulbotrichom* (Bagheri *et al.* 2018).

There are limited studies about the production of *in vitro* secondary metabolities in *Alkanna* genus. Endemism is very high for *Alkanna* genus in Turkey and *Alkanna sieheana* Rech. Fil. and *Alkanna orientalis* var. *orientalis* species containing A/S and its derivatives are endemic and endangered species of Turkey. Propagation ratio of both species is low in nature and the species is under threat because of having slow propagation in nature, destruction of pasture areas, irregular grazing and collection. Plant tissue culture techniques are subsidiary to rapid and large-scale propagation and conservation of germplasm. However, there is no reports about callus induction and adventitious regeneration for *Alkanna orientalis* var. *orientalis* and *Alkanna sieheana* Rech. Fil. species. Therefore, aim of the present study was to develop an efficient micropropagation protocol and suitable callus production system for *in vitro* production of A/S and its derivatives in both species.

Materials and Methods

Seeds of *Alkanna orientalis* var. *orientalis* and *A. sieheana* were collected from wild flora of Konya and Yozgat provinces of Turkey at 1399 m attitude in May-June during the period of flowering.

The seeds of both the species were surface-sterilized in 20% commercial bleach for 20 min and then rinsed five times with de-ionized sterile water. The seeds were kept and inflated in sterile water for an hr, then the seed coat was peeled off. The swollen seeds containing mature zygotic embryo were germinated on MS (Murashige and Skoog 1962) basal medium supplemented with 0.25 mg/l BAP, 0.5 mg/l Kn, 1.0 mg/l IAA and 6 g/l agar in Petri dishes. Germinated seeds (healthy zygotic embryos) were subcultured at the same medium every 15 days. Healthy plantlets were used as explant source and after 30 days culture initiation. Stem segments (5 - 6 mm length) and cotyledonary leaf base (2-3 mm length, 1 - 2 mm width) were also excised from plantlets. All the explants of both the species were cultured in callus and shoot induction MS medium containing different combinations of BAP (0.25 mg/l) Kn (0.5 mg/l), NAA (0.2 0.4 and 0.7 mg/l), IAA (1.0 mg/l), IBA (2.0 mg/l). The explants were subcultured two times on the same medium at 7 - 8 weeks interval. Growth regulators (BAP, Kn, TDZ, IBA, NAA) filter-sterilized by Milipore filters (0.22 - 0.45 µm pore size) were added to the medium and pH was adjusted to 5.8 with 0.1 N NaOH before autoclaving. All cultures were incubated at 20 - 22 under a 16/8 hrs photoperiod with 35 µmol m^2/s light intensity delivered by cool white fluorescent.

The regenerated shoots were isolated and relocated to rooting medium consisting of MS basal medium containing 0.1 mg/l NAA. When the shoots reached the height of 2.2.5 cm then they were transferred to pots containing 50% soil and 50% turf and acclimatized in growth chamber with 70 - 90 per cent humidity at 18 - 20°C.

Each experiment consisted of 10 explants per Petri dishes (90 × 90 mm) and three replicates per treatment. Data given in percentage were subjected to arcsine (_X) transformation (Snedecor and Cochran 1967) before statistical analysis and all data were analysed with analysis of variance according to Düzgüneş *et al.* (1983) and the differences were compared by Duncan's multiple range test using SPSS programme at the 0.01 level of probability.

Results and Discussion

Stem tips of explants of both the species were covered with light-yellowish, glossy globular calli. These organogenic calli were transferred to fresh regeneration medium after two weeks. The calli differentiated into adventitious shoot primordia and adventitious shoots started to emerge after 4 - 8 weeks culture initation for both species. The frequency of callus production and number of shoots were recorded after 8 and10 weeks, respectively, for both species. Data were collected, analysed for variance and DMRT results are given in Tables 1-2.

Table 1. Effect of different combinations of plant growth regulators on callus and shoot formation from stem explants of *A. orientalis* var. *orientalis.*

Growth regulators (mg/l)				Rate of callus induction (%)	Rate of shoot formation (%)	Number of shoots	Total number of shoots
BAP	Kn	NAA	IAA				
0.25	0.5	-	1.0	98.3 ± 4.33	40.0 ± 0.00b	1.2 ± 0.17	48.0b
0.25	0.5	0.4	-	100.0 ± 0.00	48.3 ± 1.00b	1.0 ± 0.00	48.3b
-	-	-	1.0	100.0 ± 0.00	66.6 ± 1.45a	1.3 ± 0.14	86.6a

The difference between the means indicated by the lower case letters in the same column is significantly different at $p < 0.01$.

Table 2. Effect of different combinations of plant growth regulators on callus and shoot formation from stem explants of *A. sieheana.*

Growth regulators (mg/l)				Rate of callus induction (%)	Rate of shoot formation (%)	Number of shoots per explants	Total number of shoots
BAP	Kn	NAA	IAA				
0.25	0.5	-	1.0	98.3 ± 4.33	40.0 ± 0.00b	1.2 ± 0.17	48.0b
0.25	0.5	-	1.0	100.0 ± 0.00	15.0 ± 2.60b	1.0 ± 0.00a	15.0b
0.25	0.5	0.4	-	100.0 ± 0.00	0.0 ± 0.00c	0.0b	0.0c
-	-	-	1.0	98.3 ± 4.33	43.3 ± 1.20a	1.3 ± 1.14a	56.3a

The difference between the means indicated by the lower case letters in the same column is significantly different at $p < 0.01$.

All media resulted in compact calli formation for both species. Different combinations and concentrations of BAP, Kn, IAA and NAA were statistically significant ($p < 0.01$) on shoot regeneration of stem segments of *A. orientalis* var. *orientalis.* However, no statistically significant difference was observed on the rate of callus induction and number of shoots per explant. The highest rate of shoot formation (66.6%) was determined on a MS medium containing 1.0 mg/l

IAA alone. The highest number of shoots (1.3) per explant was also observed at 1.0 mg/l IAA (Table 1).

Rate of shoot formation and number of shoots per explant were significantly ($p < 0.01$) influenced by plant growth regulators for stem segments of *A. sieheana*. The highest rate of shoot formation (43.3%) and the highest number of shoots per explant (1.3) were also found on MS medium containing 1.0 mg/l IAA alone for *A. sieheana* (Table 2).

When both the species were evaluated together, it was observed that IAA promoted regeneration from stem explants significantly, also stimulated massive and regenerative callus clusters. The morphological response of stem explants to IAA differed markedly and produced thicker and longer shoots than other growth regulators.

Cotyledonary leaf base excised from germinated plantlets swelled and produced yellow-greenish coloured compact embryogenic calli after 20 - 25 days of culture in both species. Somatic embryos differentiated into shoot primordia were visible after 4 - 5 weeks and shoot proliferation were seen after 5 - 8 weeks of culture. The frequency of shoot regeneration of both species from leaf base explants were statistically influenced ($p < 0.01$) for each medium containing different concentrations of BAP, Kn, NAA, IBA and IAA. The best callus formation (100.0%) was obtained on MS medium fortified with 1 mg/l IAA and 2 mg/l IBA alone and 0.25 mg/l BAP + 0.5 mg/l Kn + 0.7 mg/l NAA for *A. orientalis* var. *orientalis*. The highest shoot regeneration (50.0%) was observed on MS medium containing 1.0 mg/l IAA and 2.0 mg/l IBA alone. No statistically significant differences were observed in terms of the mean number of shoots and per explants (Table 3).

Table 3. Effect of different combinations of plant growth regulators on callus and shoot formation from leaf base explants of *A. orientalis* var. *orientalis*.

Growth regulators (mg/l)					Rate of callus induction (%)	Rate of shoot formation (%)	Number of shoots
BAP	Kn	NAA	IAA	IBA			
0.25	0.5	-	1.0	-	95.0 ± 5.36	8.3 ± 1.66c	1.1 ± 0.09
0.25	0.5	0.7	-	-	100.0	40.0 ± 1.73a	1.1 ± 0.08
0.25	-	0.2	-	-	96.6 ± 5.99	21.7 ±1.00b	1.0 ± 1.0
-	-	-	1.0	-	100.0	50.0 ± 3.46a	1.2 ± 0.14
-	-	-	-	2.0	100.0	50.0 ± 3.46a	1.1 ± 0.09

The difference between the means indicated by the lower case letters in the same column is significantly different at $p < 0.01$.

Prolific callus formation (100.0%) was observed on MS medium fortified with 1 mg/l IAA and 2 mg/l IBA alone. Also, combinations of BAP, Kn and IBA produced high compact calli formation for *A. sieheana*. The best shoot regeneration (30%) was observed in MS medium containing 1.0 mg/l IAA alone. The maximum number of shoot per explant (1.2) was also found to be on the medium supplemented with 1.0 mg/l IAA, 2.0 mg/l. The data revealed that treatments of stem and cotyledonary leaf base of both species with 1.0 mg/l IAA induced shoots more than control and other cytokines types and also stimulated compact callus formation (Table 4).

The regenerated shoots of both the species were isolated and transferred to rooting medium containing 0.5 mg/l IAA. Rooting occurred in the medium but there was a slight enhancement of rooting quality in MS medium containing IAA. Rooted shoots were removed and washed and transferred to pots and 74% of regenerated shoots formed healthy leafy structures after acclimatization period. Both species responded better to IAA comparatively induced higher

compact callus production when two explant types were used. Treatments of two types of explants of both the species with 1.0 mg/l IAA promoted significantly more regenerative calli and shoots than did other cytokines and auxins. Higher concentrations of cytokines may also not be recommended for shoot proliferation for both species. BAP and Kn were the least effective for shoot multiplication and resulted in shoots to turn yellowish with some vitrification. Cotyledonary leaf base and stem explants were responsive to spontaneous formation of morphogenetic calli for

Table 4. Effect of different combinations of plant growth regulators on callus and shoot formation from leaf base explants of *A. sieheana.*

Growth regulators (mg/l)					Rate of callus induction (%)	Rate of shoot formation (%)	Number of shoots
BAP	Kn	NAA	IAA	IBA			
0.25	0.5	-	1.0	-	100.0a	15.0 ± 2.60b	1.0 ± 0.0a
0.25	0.5	0.7	-	-	100.0a	20.0 ± 0.0ab	1.2 ± 0.12a
0.25	-	0.2	-	-	86.6 ± 1.66b	21.7 ± 1.00ab	1.0 ± 0.0a
-	-	-	1.0	-	100.0a	30.0 ± 1.73a	1.2 ± 0.09a
-	-	-	-	2.0	100.0a	0.0c	0.0b

The difference between the means indicated by the lower case letters in the same column is significantly different at $p < 0.01$.

generating direct shoot initiation and plant regeneration of both the species. Both the species containing A/S derivates also showed calligenic potentials for *in vitro* secondary metabolite production. *Boraginaceae* did not also produce sufficient shikonin raw material for commercial production (Pal and Chaudhury 2010). Plant cell culture techniques help in producing of *in vitro* secondary metabolites and the plant cell cultures including direct plant regeneration may be an interesting alternative for production of secondary metabolites (Piekoszewska *et al.* 2010). Alkannin pigmets was also produced by callus and cell cultures of *A. tinctoria* (Mita *et al.* 1994). The type of plant growth regulators and explant may be fundamental factors for production of A/S derivates (Urbanek *et al.* 1996) which optimized suspension cultures of *A. tinctoria* for efficient production of alkannin. B5 medium supplemented with 2 mg/l 2-4, D, 0.5 mg/l BAP and 7 g/l agar was reported to be suitable for callus formation of *A. tinctoria* using root. Plant cell and tissue culture methods using different explant types and medium have been successfully utilized on the other species containing A/S derivates such as *Lithospermum erythrorhizon* (Fujita *et al.* 1983), *Arnebiaeu chroma* (Manjkhola *et al.* 2005) and *Arnebia hispidissima* (Phulwaria and Shekhawat 2013). MS medium supplemented with 0.25 mg/l BAP, 0.1 mg/l IAA 0.5 mg/l Kn and 100 mg/l casein hydrolysate was the best regeneration medium for *A. hispidissima* using shoot tip explants (Pal and Chaudhury 2010). Also, efficient regeneration of *Oenothera erythrosepala* was using shoot tips on MS medium supplemented with 1.0 mg/l BAP, 0.1 mg/l NAA, or 0.1 mg/l IAA (Suzuki *et al.* 1990). Shoots were regenerated from callus derived from immature inflorescence explants for *A. hispidissima* on MS medium containing 4.52 µM 2,4-D and 3.33 µM BAP was effective for the proliferation of callus (Phulwaria and Shekhawat 2013). The data in the present study for generation of calli and shoot regeneration of *Alkanna orientalis* var. *orientalis* and *A. sieheana* are reasonably comparable as to the other plant species containing A/S derivates stated previously. In conclusion, it may be said that for the first time, a reproducible protocol related to callus induction and regeneration of endangered species *Alkanna orientalis* var. *orientalis* and *Alkanna sieheana* containing valuable metabolites like A/S derivates were developed. The regeneration procedure can reduce the propagation cycle of both species, contribute to the mass propagation and *ex situ* conservation of these species. Development of a suitable callus formation

for the production of secondary metabolites can be very useful in meeting the supply of raw products for a large scale *in vitro* production of A/S (Fig. 1).

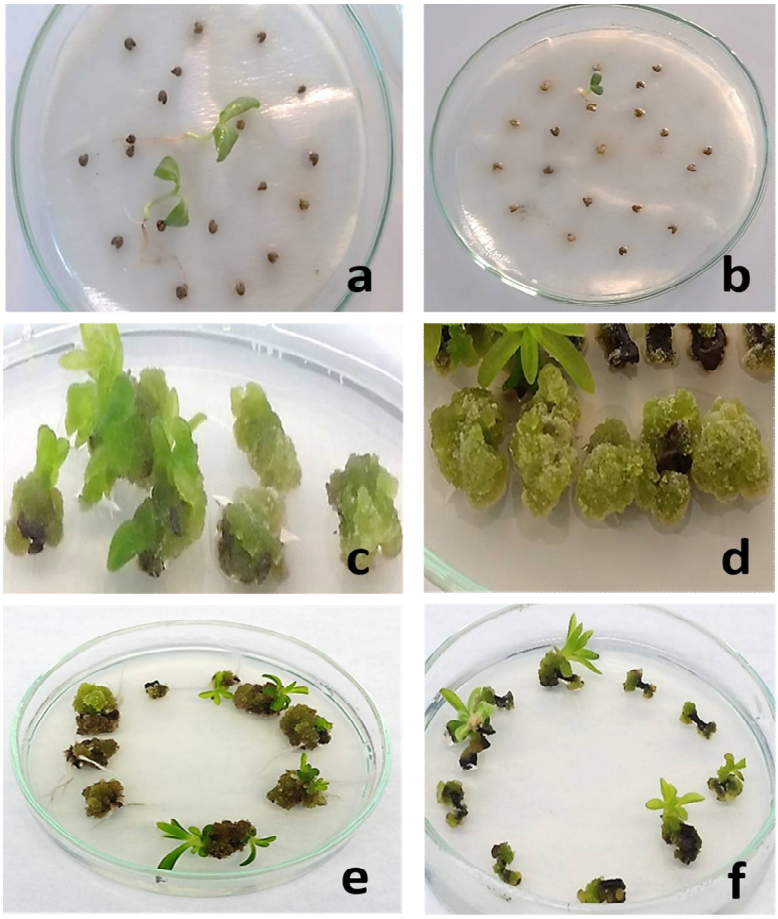

Fig. 1. Callus and adventitious shoot regeneration in *A. orientalis* var. *orientalis* and *A. sieheana* (a, b). *In vitro g*ermination of seeds of both species after 2 weeks of culture (c). *In vitro* callus induction and shoot primordia development from stem explants of *A. orientalis* var. *orientalis*, (d) Compact calli regeneration from stem explants of *A. sieheana* after 5 - 6 weeks of culture (e, f). Callus induction and plant regeneration from leaf base explant of both species on MS medium containing 1.0 mg/l IAA after 4 - 5 weeks of culture.

References

Bagheri F, Tahvilian R, Karimi N, Chalabi M and Azam M 2018. Shikonin production by callus culture of *Onosma bulbotrichom* as active pharmaceutical ingredient. Iran J. Pharm. Res. **17**(2): 495-504.

Boehm R, Sommer S, Li SM and Heide L 2000. Genetic engineering on shikonin biosynthesis: expression of the bacterial *ubiA* gene in *Lithospermum erythrorhizon*. Plant Cell Physiol. **41**: 911-919.

Chung BY, Lee YB, Baek MH, Kim JH, Wi SG and Kim JS 2006. Effects of low-dose gamma-irradiation on production of shikonin derivatives in callus cultures of *Lithospermum erythrorhizon* S. Radiation Physics and Chemistry **75**(9): 1018-1023.

Damianakos H, Kretschmer N, Sykłowska-Baranek K, Pietrosiuk A, Bauer R and Chinou I 2012. Antimicrobial and cytotoxic isohexenylnaphthazarins from *Arnebia euchroma* (Royle) Jonst. (Boraginaceae) callus and cell suspension culture. Molecules **17**(12): 14310-14322.

Düzgüneş O, Kesici T and Gürbüz F 1983. İstatistikMetotları 1. A.Ü.Zir.Fak. Yay. No: 862, Ankara.

Fujita Y, Hara Y, Ogino T and Suga C 1981. Production of shikonin derivatives by cell suspension cultures of *Lithospermum erythrorhizon*: I. Effects of nitrogen sources on the production of shikonin derivatives. Plant Cell Rep. **1**(2): 59-60.

Fujita Y, Maeda Y, Suga C and Morimoto T 1983. Production of shikonin derivatives by cell suspension cultures of *Lithospermum erythrorhizon*. III. Comparison of shikonin derivatives of cultured cells and Ko-shikon. Plant Cell Rep. **2**(4): 192-193.

Güner A, Aslan S, Ekim T, Vural M and Babaç T 2012. Türkiye Bitkileri Listesi. NGBB and Flora Araştırmaları Derneği Yayını, İstanbul.

Kumar N, Kumar R and Kishore K 2013. *Onosma* L.: A review of phytochemistry and ethnopharmacology. Pharmacogn. Rev. **7**: 140-151.

Mahmoudi SZ, Seyedabadi M, Esfahani HRM, Amanzadeh Y and Ostad SN 2012. Anti-inflammatory and analgesic activity of *Alkanna bracteosa* and *Alkanna tricophila*. Natural Product Research **26**(6): 564-569.

Manjkhola S, Dhar U and Joshi M 2005.Organogenesis, embryo genesis and synthetic seed production in *Arnebia euchroma* - A critically endangered medicinal plant of the Himalaya. In vitro Cell. Dev. Biol-Plant **41**(3): 244-248.

Mita G, Gerardi C, Miceli A., Bollini R and De Leo P 1994. Pigment production from *in vitro* cultures of *Alkanna tinctoria* Tausch, Plant Cell Report **13**(7): 406-410.

Murashige T and Skoog EA 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum **15**: 473-497.

Nasim SA, Dhir B, Kapoor R, Fatima S and Mahmooduzzafar MA 2010. Alliin production in various tissues and organs of *Allium sativum* grown under normal and sulphur-supplemented *in vitro* conditions. Plant Cell Tissue Organ Cult. **101**(1): 59-63.

Pal M and Chaudhury A 2010. High frequency direct plant regeneration, micropropagation and shikonin induction in *Arnebia hispidissima*. J. Crop Sci. Biotech. **13**(1): 13-20.

Pal M, Kumar V, Yadav R, Gulati D and Yadav RC 2017. Potential and prospects of shikonin production enhancement in medicinal plants. Proc. Natl. Acad. Sci., India, Sect. B Biol. Scipp 1-10.

Papageorgiou VP, AssimopoulouAN and Ballis AC 2008. Alkannins and shikonins: A new class of wound healing agents. Curr. Med. Chem. **15**(30): 3248-3267

Phulwaria M and Shekhawat NS 2013. An efficient *in vitro* shoot regeneration from immature inflorescence and *ex vitro* rooting of *Arnebia hispidissima* (Lehm). DC. - A red dye (Alkannin) yielding plant. Physiol Mol. Biol. Plants **19**(3): 435-441.

Piekoszewska A, Ekiert H and Zubek S 2010. Arbutin production in *Rutagraveolens* L. and *Hypericumperforatum* L. *in vitro* cultures. ActaPhysiol Plant **32**: 223-229.

Praveen N and Murthy HN 2011. Effects of macroelements and nitrogen source on biomass accumulation and withanolide - A production from cell suspension cultures of *Withania somnifera* (L.) Dunal. Plant Cell Tissue Organ Cult. **104**(1): 119-124.

Shekhawat MS and Shekhawat NS 2011. Micropropagation of *Arnebia hispidissima* (Lehm). DC. and production of alkannin from callus and cell suspension culture. Acta Physiol. Plant **33**(4): 1445-1450.

Singh B and Sharma RA 2014. Antioxidant and antimicrobial activities of *Arnebia hispidissima*. American Journal of Advanced Drug Delivery **2**(2): 224-237.

Snedecor GW and Cochran WG 1967. Statistical Methods. The Iowa State University.

Tabata M, Mizukami H, Hiraoka N and Konoshima M 1974. Pigment formation in callus cultures of *Lithospermum erythrorhizon*.. Phytochemistry **13**: 927-932.

Tatsumi K, Yano M, Kaminade K and et al. 2016. Characterization of shikonin derivative secretion in *Lithospermum erythrorhizon* hairy roots as a model of lipid-soluble metabolite secretion from plants. Front Plant Sci. **7**:1066.

Touno K, Tamaoka J, Ohashi Y and Shimomura K 2005. Ethylene induced shikonin biosynthesis in shoot culture of *Lithospermum erythrorhizon.* Plant Physiology Biochemistry **43**(2): 101-105.

Urbanek H, Katarzyna Bergier, K, Marian Saniewski M and Patykowski J 1996. Effect of jasmonates and exogenous polysaccharides on production of alkannin pigments in suspension cultures of *Alkanna tinctoria*. Plant Cell Reports **15**(8): 637-641.

Wang Z, Liu T, Gan L, Wang T, Yuan X, Zhang B, Chen H and Zheng Q 2010. Shikonin protects mouse brain against cerebral ischemia/reperfusion injury through its antioxidant activity. European Journal of Pharmacology **643**: 211-217.

Yeh YC, Liu TJ and Lai HC 2015. Shikonin induces apoptosis, necrosis, and premature senescence of human A549 lung cancer cells through upregulation of p53 expression, evidence-based complementary and alternative Medicine 1-13.

Zare KH, Nazemiyeh H, Movafeghi A, Khosrowshahli M, Motallebi-Azar A, Dadpour M and Omidi Y 2010. Bioprocess engineering of *Echium italicum* L.: Induction of shikonin and alkannin derivatives by two-liquid-phase suspension cultures. Plant Cell Tiss Organ Cult. **100** (2): 157-164.

(Manuscript received on 20 November, 2018; revised on 13 February, 2019)