INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
11–649/AWB/2012/14–4–528–532
http://www.fspublishers.org

Full Length Article

Appraisal of an Important Flavonoid, Quercetin, in Callus Cultures of C*itrullus colocynthis*

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ABSTRACT

Leaf and internode explants from *in vitro* grown seedlings of *Citrullus colocynthis* (L.) Schard. were used to establish callus cultures in MS media supplemented with different concentrations (ranging from 1 to 3 mg/L) and combinations of plant growth regulators like BAP with NAA, and Kin with 2, 4-D. BAP 3 mg/L and NAA 2 mg/L supported green to yellowish green embryogenic callus from internode explant with callus index of 300 and callus initiation commenced in 3 days. Leaf explant produced green callus under influence of 2, 4-D 2 mg/L and Kin 1 mg/L with callus index of 200 and callus initiation duration was 15 d. Leaf and internode tissues from *in vitro* grown seedlings and leaf and internode calli were analyzed for secondary metabolites through GC-MS. Nineteen compounds were detected from leaf and internode tissues. From the comparative analysis of secondary metabolites it can be concluded that regardless of a few different compounds, most of the secondary metabolites from seedlings were present in their respective calluses.

Key Words: *Citrullus colocynthis;* Callus; Quercetin; GC-MS

INTRODUCTION

Plant secondary metabolites play an important role as source of new molecules in pharmaceutical industry and need special consideration. Biotechnological approaches, specifically plant tissue culture plays a vital role in search for alternatives to production of desirable medicinal compounds from plants (Rao & Ravishankar, 2002). The capacity for plant cell, tissue and organ cultures to produce and accumulate many of the same valuable chemical compounds as the parent plant in nature, has been recognized almost since the inception of *in vitro* technology. The strong and growing demand in today's marketplace for natural, renewable products has refocused attention on *in vitro* plant materials as potential factories for secondary phytochemical products (Karuppusamy, 2009).

Polyphenols such as cinnamic acid derivatives, anthocyanins, vitamins and flavonols present in fruits, vegetables, berries and herbs are the main source of natural antioxidants (Scalbert *et al.,* 2005). Quercetin, as an antioxidant scavenges free radicals, which damage cell membranes, tamper with DNA and even cause cell death. It acts like an anti-histamine and anti-inflammatory agent (Lamson & Brignall, 2000) and may help protect cardiac diseases and cancer (Meena & Patni, 2008). The interesting biological activities of flavonoids have prompted the intensive research on physiological properties of these compounds and their effects on human health (Rusak *et al*., 2002).

Citrullus colocynthis (L.) Schard. is an Iranian medicinal plant that has traditionally been used as an abortifacient and to treat constipation, oedema, bacterial infections, cancer and diabetes (Delazar *et al.,* 2006). *C. colocynthis* is an important species of an economically important and biochemically distinct family Cucurbitaceae (Malik, 2005) producing Quercetin naturally as secondary metabolite. It is seen growing wild in the warm and arid sandy tract (Memon *et al*., 2003). The plant being annual herb becomes available seasonally; optimization of conditions for holding callus cultures of the plant remains prerequisite as an alternate and readily available source of plant material.

Present research work was carried out to optimize conditions for *in vitro* cultures and to appraise the medicinally important secondary metabolite quercetin in *in vitro* cultures of *C. colocynthis*.

MATERIALS AND METHODS

Plant material: Seeds of *C. colocynthis* were purchased from local market, tested by sowing in lawn and resultant plants were identified and confirmed.

***In vitro* seed germination:** The seeds failed to germinate in *in vitro* initial trail. Unsoaked intact seeds were unable to germinate where 5, 10 and 15 seeds were employed per petriplate on cotton pads moistened with 10, 15 and 20 mL of distilled water at room temperature. Conditions were

To cite this paper: Tanveer, H., S. Ali and M.R. Asi, 2012. Appraisal of an important flavonoid, quercetin in callus cultures of C*itrullus colocynthis. Int. J. Agric. Biol.*, 14: 528–532

optimized for the seed germination in growth room. Seeds were soaked in water for variable interims of time from 2 to 12 h, manually decoated seeds were surface sterilized by immersing in 2.5% sodium hypochlorite for variable time periods i.e. 2, 5, 10, 15 and 20 min followed by thorough rinsing in distilled water. Inoculation of seeds was carried out under aseptic conditions, in laminar air flow cabinet. The petriplates were placed in growth room. After few days, the seed germination was recorded.

Culture medium preparation and sterilization: MS medium (Murashige & Skoog, 1962) was used as culture medium to raise in vitro cultures. The culture medium was supplemented with different growth regulators i.e., 2,4-D, NAA, Kin and BAP (ranging from 0.5 to 5 mg/L) in combinations such as 1 mg/L 2,4-D + 1 mg/L Kin (designated as DK1,1), 2 mg/L 2,4-D + 1 mg/L Kin (DK2,1), 2 mg/L2,4-D + 2 mg/L Kin (DK2,2), 0 mg/L BAP+ 5 mg/L NAA (BN 0,5), 2 mg/L BAP + 3 mg/L NAA (BN 2,3), 3 mg/L BAP + 2 mg/L NAA (BN 3,2). Culture vessels containing medium were autoclaved under 15 lbs./inch2 pressure for 20 min.

Culturing of explant and callus induction: Different parts of ten days old *in vitro* grown seedlings were excised under aseptic conditions and used as explant (cotyledon, leaf, internode & root). All the explants were transferred aseptically to the culture vessels containing sterilized medium. Culture vessels containing explants were incubated in growth room at 27˚C. To avoid the deficiency of nutrients, callus formed was transferred aseptically to fresh medium after every 10-20 d and Callus index (CI) was calculated.

Analysis of secondary metabolites by GC-MS: The essential oils were obtained from *C. colocynthis in vitro* grown plant seedlings and respective calli through Soxhlet Apparatus. GC-MS analyses of the extracted oils were carried out on Shimadzu GC-2010 series gas chromatograph interfaced with mass spectrometer (QP 2010A), Kyoto Japan equipped with DB-5MS column (30 m × 0.25 mm i.d thickness: 0.25 µm), J and W (Scientific, Folsom, CA, USA) equipped with split/splitless injector. All mass spectra were acquired in electron impact (EI) mode with ionization voltage 70 eV. Ion source and quadruple temperatures were 200˚C, transfer line temperature was 220˚C and helium at 1 mL min^{-1} constant flow. Initial column temperature was 50˚C fixed for 5 min, programmed to 230˚C at 5˚C/min; held for 5 min at 200˚C, injector temperature 200˚C and 2 µL sample was injected using SGE microliter syringe. The spectrum was scanned with acquisition mass range 50 to 600 m/z, scan rate 1.6 scans/s. Component identification was carried out using the internal standards and NIST 147 and NIST 27 libraries.

Experimental design and statistical analysis: The experiment was laid out following Randomized Complete Blocks for callus induction and secondary metabolites production with three replicates and the differences between means were assessed using Duncan's Multiple Range Test (DMRT). All differences were regarded as significant at $P < 0.05$ (Steel & Torrie, 1996).

RESULTS AND DISCUSSION

Seed germination and callus induction: Decoated seeds of *C. colocynthis* indicated highest germination in petriplates containing 10 mL of distilled water at 28ºC and the seed germination was achieved in 10 days (Fig. 1a). The presence of seed coat is also a factor influencing seed dormancy. Manual decoating of seeds supported germination as reported by Heidari *et al*. (2008). Moisture becomes critical if it is too scarce or abundant as was observed that 10 mL water provided suitable moisture to support 100% germination on cotton pads. Deviation from the prescribed amount of water decreased germination percentage on either side. The similar results were obtained by (Nerson, 2002). At high moisture germination was decreased. It seems that this decrease in germination was brought about by the thickening of the water films around the seeds, which interfered with oxygen diffusion. This assumption was supported by determinations with electrodes (Dasberg & Mendel, 1971). Temperature also plays a vital role in seed germination. Maximum seed germination was observed at temperature of 28ºC (Bansal & Sen, 1981).

Callus induction was achieved in leaf and internode explants of *C. colocynthis* while cotyledon failed to respond for callus induction as reported by Savitha *et al*. (2010) that leaf and stem explants produced morphogenic callus cultures. The highest percentage of callus induction ***i.e.***, 100% was performed by leaf and internode explants. In leaf explants the callus formation initiated on the margins, where minimum time duration for callus initiation was 3 days and maximum 15 days in different combinations of PGRs (Table I). The callus duration in internode explant was altogether 15 days.

Callusing ability of MS + BN3,2 (3 mg/L BAP + 2 mg/L NAA) supplemented medium was maximum i.e., 100% with callus index of 300 as compared to other hormone combinations. The calli produced were brownish green compact embryogenic (Fig. 1c). Callus induction ability of MS medium (DK2,2) supplemented with 2 mg/L 2,4-D + 2 mg/L Kin was less and smaller calli were produced with callus index of 200 and 133 for leaf and internode explants, respectively as compared to BN3,2 medium. The calli produced were compact, yellowish green and embryogenic (Fig. 1b). Callus induction under the influence of auxin and cytokinin combinations has been described by Hegazy *et al*. (2010).

In *in vitro* cultures plant growth regulators directed the growth and growth patterns. Combination of BAP/NAA in concentrations of 2/3 (BN2,3) and 3/2 (BN3,2), respectively (Table I) supported 100% callus induction with embryogenic brownish green callus and embryogenic yellowish green callus from leaf and internode explants,

Table I: Response of *C. colocynthis* seedling explants to different combinations and concentrations of PGRs for callus induction

Medium Code (MS + PGR)	PGR	Explant	No. of explant producing callus	Callus index	Callus Initiation Duration (d)	Callus induction %age	Characteristics of callus
DK2,2	2,4-D/Kin	Leaf	2 a ± 0.5	200 a ±7	15 a ±1.5	66	Green and embryogenic
	(2+2 mg/ L)	Internode	2 a ± 0.5	133 b ±6	15 a ± 0.0	66	Green and embryogenic
BN3,2	BAP/NAA	Leaf	3 a ± 0.0	300 a ±10	3 a ± 0 .5	100	Brownish green and embryogenic
	(3+2 mg/ L)	Internode	3 a ± 0.0	300 a ±12	15 b ±0.5	100	Yellowish green and embryogenic

Means marked with the same letter in each column or row are not (P<0.05) significantly different by Duncan's Test.

Table II: Percentage of secondary metabolites from seedlings-leaf tissue and leaf callus grown in MS media supplemented with BAP 0 mg/L + NAA 5 mg/L

Names of secondary metabolites detected through GC/MS analysis	Retention time (min)/metabolites %age with SE in leaf tissue	Retention time (min)/metabolites %age with SE in leaf callus
Toluene	3.89/15.692b ±0.115	3.89/21.850b ±0.571
Nonane	4.82/12.184c ±0.248	4.82/14.578c ±0.577
Benzene 1,3-dimethyl	5.99/0.649g ±0.02	Nil
Ethylbenzene	6.10/03.237e ±0.059	6.09/3.930e ±0.207
o-Xylene	7.22/7.08ld ±0.121	7.22/3.08lef ±0.109
2-Propanoic acid, butyl ester	7.76/0.424g ±0.005	7.76/0.619g ±.017
A-sphinosterol	12.03/0.250fg ±0.063	Nil
Undecane	12.25/30.348a ±1.2	12.4/42.29a ±2.367
Quercetin	13.92/0.583g ±0.02	13.92/0.129g ±0.008
2,6octadiene,-1-01,3,7-dimethyl-acetate	14.99/0.858fg ±0.063	Nil
Tetradecane	15.36/0.173g ±0.004	15.36/0.77g ±0.034
Pentadecane	16.02/1.692g ±0.063	16.2/0.79f ±0.080
3-Cyclohexene-1-01,4-methyl-1-[1-methylethyl]	Nil	10.00/2.111g ±0.288
Hexadecanes	16.61/0.240g ±0.009	16.61/0.043g ±0.002
Octadecane	17.69/0.984fg ±0.01	17.69/0.010g ±00
Hentriactone	19.89/0.280g ±0.008	19.89/0.480g ±0.015
9,12-Octadecadienoicacid[7,7]	22.21/30.893a±0.17	22.21/9.893d ±0.0457

Means marked with the same letter in each column or row are not (P<0.05) significantly different by Duncan's Test.

Table III: Percentage of quercetin in seedling-leaf tissue and respective calluses

Medium code (MS +PGRs	Seedling tissue /respective callus	PGRs used	Quercetin %age with SE
-	Leaf tissue	-	0.583±0.02
BN 0,5	Leaf callus	BAP 0 mg/L + NAA 5 mg/L	0.129±0.008
BN 2,3	--	BAP 2 mg/L + NAA 3 mg/L	0.194±0.004
BN 3,2	--	BAP 3 mg/L + NAA 2 mg/L	0.476±0.01
DK 1,1	--	2,4-D 1 mg/L + Kin 1 mg/L	0.00
DK 2,1	--	2,4-D 2 mg/L + Kin 1 mg/L	0.129±00
DK 2,2	--	2,4-D 2 mg/L + Kin 2 mg/L	0.00

Table IV: Percentage of quercetin in seedling-leaf tissue and respective calluses

Medium code (MS + PGRs	Seedling tissue/respective callus	PGRs used	Quercetin %age with SE
-	Internode tissue	-	0.537±0.02
BN 0,5	internode callus	BAP 0 mg/ L + NAA 5 mg/L	0.074±0.008
BN 2,3	--	BAP 2 mg/ L + NAA 3 mg/L	0.476±0.04
BN 3,2	--	BAP 3mg/L+ NAA 2 mg/L	0.247±0.01
DK 1,1	--	2,4-D 1 mg/ L + Kin 1 mg/L	0.031±00
DK 2,1	--	2,4-D 2 mg/ L + Kin 1 mg/L	0.00
DK 2,2	--	2,4-D 2 mg/ L + Kin 2 mg/L	0.00

respectively and both having callus index of 300. Similar results were reported by different workers (Nabi *et al*., 2002; Duangporn & Siripong, 2009), who stated that the highest percentage of callus cultures was derived from explants cultured on BAP/NAA combinations than BAP and NAA alone. The combination of NAA/BAP has also been used for the induction of multiple shoots in *Basilicum polstachyon* (Chakraborty & Roy, 2006), *Musa sapientum* (Kalimutha *et al*., 2007), *Rauwolfia serpentine* (Baksha *et al.,* 2007), *C. colocynthis* (Meena & Patni, 2007). Different combinations of 2,4-D/Kin, did not produce callus comparable to BAP/NAA (Table I). Similar results were obtained by Hegazy *et al.* (2010) who reported that callus derived from BAP/NAA combination were proved to be appropriate protocol for callus induction, while 2,4-D/Kin failed to produce the desired results.

Fig. 1: Callus induction in MS medium supplemented with BAP/NAA

a) 30-d- old seedlings of *C. colocynthis* (2×)**. b)** 18-d-old callus culture of leaf in BAP 2 mg/L + NAA 3 mg/L (2×). **c)** 18-d-old callus culture of internode in BAP 3 mg/L+ NAA 2 mg/L (2×)

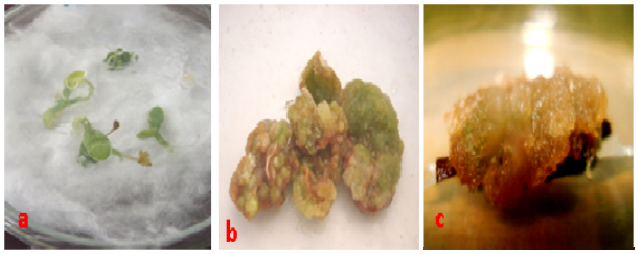
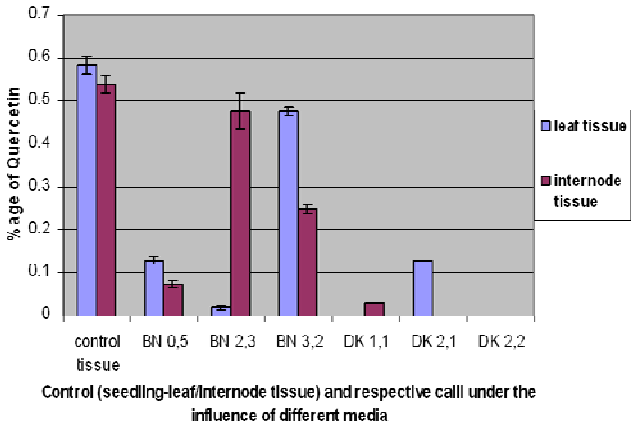


Fig. 2: Percentage of quercetin from control tissues/ respective calluses



Secondary metabolites production in callus cultures and analysis: Callus cultures of *C. colocynthis* produced were processed through GC-MS analyses for percentage composition of secondary metabolites to compare with seedling tissues for the same. Callus cultures can be employed and exploited for secondary metabolites production. In tissue culture procedures, this approach is relatively frequently used for production of flavonoids (Jedinák *et al*., 2004). Many different types of secondary metabolites were found in seedling tissues of *C. colocynthis* and respective calluses but 9 compounds were common in seedling tissues as well as in callus cultures including quercetin. Common secondary metabolites present in leaf tissue and leaf calluses were toluene, α-sphinosterol, quercetin, tetradecane, pentadecane, hexadecane, octadecane, hentriactone and 9,12-octadecadienoicacid[7,7] (Table II). The quantity of these compounds was variable in seedling tissues and respective callus cultures. Some compounds detected in seedling tissues were absent in callus cultures such as Benzene 1,3-dimethyl, α-sphinosterol, 2,6octadiene,-1-01,3,7-dimethyl-acetate, and vice versa such as 3-cyclohexene-1-01,4-methyl-1-[1-methylethyl] (Table II). But the desired secondary metabolite i.e., quercetin was present in *in vivo* as well as *in vitro* grown tissues. The production of secondary metabolites in callus cultures can vary greatly. Usually, the accumulation is lower or higher than concentrations found *in vivo* or fail to accumulate. Biotechnology offers an opportunity to exploit cells, tissues, organs or entire organisms by growing them *in vitro* to get desired compounds (Rao & Ravishankar, 2002).

The GC-MS analyses of seedling leaf and internode tissues and respective calluses revealed the presence of saponins, steroids, alkaloids and flavonoids. Presence of flavonoids has been reported from other plant species like *Lycium barbarum* (Harsh *et al*., 1983), *Passia palmer* (Ulubelen *et al*., 1984), *Cassia angustifolia* (Goswami & Reddi, 2004) and *Jatropha curcus* L. (Saxena *et al*., 2005). Flavonoids can be produced by using different biotechnological approaches, such as callus cultures, cell suspension cultures and/or organ cultures (Rusak *et al*., 2002).

The composition of Quercetin from leaf and internode calluses developed in MS medium supplemented with different PGRs showed that combination as well as concentration of PGRs in a combination, affects the synthesis of secondary metabolites and optimization of medium can enhance the production of secondary metabolites (Karuppusamy, 2009). Among different media used, medium code BN3, 2 (3 mg L^{-1} BAP + 2 mg L^{-1} NAA) proved the best to produce callus cultures containing comparable percentage of Quercetin (0.476±0.01) as in seedling tissues (Table III & IV). Medium DK1,1 (2,4-D 1 mg/L + Kin 1 mg/L) and DK2,2 (2,4-D 2 mg/L + Kin 2 mg/L) were unable to induce the synthesis of Quercetin in callus cultures of leaf tissue whereas Quercetin production (0.129±0.0%) was detected in the callus cultures supported by the same combination of PGRs with different concentrations such as DK2,1 (2,4-D 2 mg/L + Kin 1 mg/L) as reported by Hegazy *et al*. (2010).

The comparative analysis of leaf and internode seedling tissues and respective callus cultures showed the occurrence of Quercetin (Table III & IV) in comparable amounts in callus cultures supported by medium containing combination of BAP and NAA rather than other combinations like 2,4-D and Kin or NAA alone. Yield of Quercetin in *in vitro* culture can be increased by manipulating hormones concentration of culture medium (Ashrafi *et al*., 2010). BAP 2 mg/L + NAA 3 mg/L and vice versa proved best concentrations of BAP and NAA to produce equal amounts of Quercetin in internode and leaf explant generated callus cultures respectively (Fig. 2).

CONCLUSION

BAP and NAA in concentrations of 2 to 3 mg/L can trigger Quercetin production in *in vitro* cultures of *C. colocynthis* and these cultures can be used in place of green herb for Quercetin as medicine and for extraction of the important flavonoid. Quercetin from callus cultures can prove to be an important metabolite of *C. colocynthis* as it

has been isolated from other plant species like *Adina cordifolia* (Meneni *et al.,* 2002) and *Acacia catechu* (Jain *et al.,* 2007).

REFERNCES

Ashrafi, S., M.R. Mofid, M. Otroshi, M. Ebrahimi and M. Khosroshahli, 2010. Effects of plant growth regulators on the callogenesis and Taxol production in cell suspension of *Taxus baccata* L. *Trakia J. Sci.*, 8: 36–43

Baksha, R., M.A.A. Jahan, R. Khatun and J.L. Munshi, 2007. *In vitro* rapid clonal propagation of *Rauvolfia serpentina* (Linn.) benth. *Bangladesh J. Sci. Indus. Res.*, 42: 37–44

Bansal, R.P. and D.N. Sen, 1981. Differential germination behaviour in seeds of Indian arid zone. *Folia Geobot. Phytotax.,* 16: 317–330

Chakraborty, S. and S.C. Roy, 2006. Micro propagation of Cyphomandra betacea (Cav.) Sendt a potential horticultural and medicinal plant, by axillary bud multiplication. *Phytomorphology,* 56: 29–33

Dasberg, S. and K. Mendel, 1971. The Effect of Soil Water and Aeration on Seed Germination. *J. Exp. Bot.,* 22: 992–998

Delazar, A., S. Gibbons, A.R. Kosari, H. Nazemiyeh, M. Modarresi, L. Nahar and S.D. Sarker, 2006. Flavone c-glycosides and cucurbitacin glycosides from Citrullus colocynthis. *DARU*, 14: 109–114

Duangporn, P. and P. Siripong, 2009. Effect of auxin and cytokinin on phyllanthusol A production by callus cultures of Phyllanthus acidus skeels. *American-Eurasian J. Agric. Environ. Sci.,* 5: 258–263

Goswami, A. and A. Reddi, 2004. Antimicrobial activity of flavonoids of medicinally important plant *Cassia angustifolia in vivo* and *in vitro*. *J. Phytol. Res.,* 17: 179–181

Harsh, M.L., T.N. Nag and S. Jain, 1983. Arid zone plants of Rajasthan a source of antimicrobials. *Commun. Phys. Ecol*., 8: 129–131

Hegazy, K., A. Mohamed, A. Amal, Ali, I. Sami and M. Mahmoud, 2010. Enhancement of callus induction and cucurbitacin content in *Citrullus colocynthis* L. (Schrad) using plant growth regulators. *J. Alabama Acad. Sci.,* 81: 4694–4701

Heidari, M., M. Rahemi and M.H. Daneshvar, 2008. Effects of mechanical, chemical scarification and stratification on seed germination of Prunus scoparia (Spach.) and Prunus webbii (Spach.) Vierh. *American-Eurasian J. Agric. Environ. Sci*., 3: 114–117

Jain, R., V. Patni and D.K. Arora, 2007. Isolation and identification of flavonoid "quercetin" from Acacia catechu (L.f.) Willd- A Katha yielding plant. *J. Phytol. Res*., 20: 43–45

Jedinák, A., J. Faragó, I. Pšenáková and T. Maliar, 2004. Approaches to flavonoid production in plant tissue cultures. *Rev. Biol. Bratislia,* 59: 697–710

Kalimutha, K., M. Saravanakumar and R. Senthilkumar, 2007. *In vitro* micropropagation of *Musa sapientum* L. (Cavendish Dwarf). *African J. Biotechnol*., 6: 1106–1109

Karuppusamy, S., 2009. A review on trends in production of secondary metabolites from higher plants by *in vitro* tissue, organ and cell culture. *J. Med. Plant Res.*, 3: 1222–1239

Lamson, D.W. and M.S. Brignall, 2000. Antioxidants and cancer III: quercetin. *Alt. Med. Rev*., 5: 196–208

Malik, T.A., 2005. *Principals of Botany,* pp: 281–283. The Caravan Press

Memon, U., A.H. Brohi, S.W. Ahmed, I. Azhar and H. Bano, 2003. Antibacterial screening of *Citrullus colocynthis. Pakistan J. Pharm. Sci.,* 16: 1–6

Meena, M.C. and V. Patni, 2007. *In vitro* clonal propagation of *Citrullus colocynthis* (Linn.) Schrad: A threatened medicinal plant. *Plant Cell Biotechnol. Mol. Biol*., 8: 147–152

Meena, M.C. and V. Patni, 2008. Isolation and Identification of Flavonoid "Quercetin" from *Citrullus colocynthis* (Linn.) Schrad. *Asian J. Exp. Sci.,* 22: 137–142

Meneni, S. Rao, H. Duddeck and R. Dembinski, 2002. Isolation and structural elucidation of 3,4′,5,7-tetraacetyl quercetin from *Adina cordifolia* (Karam ki Gaach). *Fitoterapia*, 73: 353–355

Murashige, T. and F. Skoog, 1962. Arevised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*, 15: 437–496

Nabi, S.A., M.M. Rashid, M.A. Amin and M.G. Rasul, 2002. Organogenesis in teasle gourd (Momordica dioica Roxb.). *Plant Tissue Cult*., 12: 173–180

Nerson, H., 2002. Effects of seed maturity, extraction practises and storage duration on germinability in watermelon. *Sci. Hortic.,* 93: 245–256

Rao, S.M. and G.A. Ravishankar, 2002. Plant cell cultures: chemical factories of secondary metabolites. *Biotechnol. Adv*., 20: 101–153

Rusak, G., H.O. Gutzeit and J. Ludwig-Muller, 2002. Effects of structurally related flavonoids on hsp gene expression in human promyeloid leukaemia cells. *Food Technol. Biotechnol.,* 40: 267–273

Savitha, R., T. Shasthree and B. Mallaiah, 2010. High frequency of plantlet regeneration and multiple shoot induction from leaf and stem explant of *Citrullus colosynthis* (L.) schrad, an endangered medicinal cucurbit. *Int. J. Pharm. Biol. Sci.,* 2: 1–8

Saxena, S., R. Sharma, A. Batra and S. Rajore, 2005. Isolation and identification of flavonoid "Vitexin" from *Jatropha curcas* L. *J. Plant Sci. Res.,* 21: 116–117

Scalbert, A., T. Ian Johnson and M. Saltmarsh, 2005. Polyphenols: antioxidants and beyond dietary polyphenols and health: Proceedings of the 1st International Conference on polyphenols and health. *American J. Clin. Nutr.,* 81: 215S–217S

Steel, R.G.D. and V.J.H. Torrie, 1996. *Principles and Procedures of Statistics: A Biometrical Approach.* McGraw-Hill, New York, USA

Ulubelen, A., J.J. Mabry, G. Dellamonicas and J. Chopin, 1984. Flavonoids of *Passiflora plamer*. *J. Nat. Prod.,* 47: 384–385

(Received 16 November 2011; Accepted 28 March 2012)