Pak. J. Bot., 47(6): 2355-2359, 2015.

DEVELOPMENT OF HIGH EFFICIENCY MICROPROPAGATION PROTOCOL FOR *TAMARIX NILOTICA* EHRENB WITH VALUED MEDICINAL PROPERTIES

FAHAD AL-QURAINY, MOHAMMAD NADEEM*, SALIM KHAN, MOHAMED TARROUM AND SALEH ALANSI

Department of Botany and Microbiology, College of Science, King Saud University, Riyadh- 11451, Saudi Arabia
**Corresponding author's e-mail: mohammadnadeem911@hotmail.com; Tel.: +966-01467-5876; Fax: +966-467-8301.*

Abstract

Tamarix nilotica is an important medicinal plant grows throughout the Kingdom, except in mountains on high altitude. Propagation of *T. nilotica* through cuttings and seeds are limited. To supplement harvesting of active ingredient from plants, alternative method for the purpose has been developed. Stem node cuttings were subjected to *in vitro* culture under the influence of various cytokinin to induce shoot proliferation. Maximum shoot stimulation was found on MS medium comprising 1.0 µM TDZ followed by 2.5 µM BA, Kn and 2ip. The number of shoots declined as the concentration of cytokinin increased. A lower shoots obtained on 5.0 and 10.0 µM concentration of all the four cytokinins tested. After fair multiplication, individual shoots were subjected to different concentration of auxins IBA, NAA, 2,4-D and IAA 2.5-10.0 µM for root induction. Initial screening did not result desired rooting on MS medium. Therefore, pulse treatment of 100 µM IBA was given to the isolated shoots for 5, 10 and 15 days time. Incubation for 10 days on MS medium supplied with 100.0 µM resulted in maximum rooting when transferred on MS medium alone. Well rooted microshoots were exposed to four types of soil mixtures for acclimatization of plants. Among these soils, sand and FYM gave 100% survival under controlled green house conditions. This protocol would be helpful in regeneration and conservation of this plant species; and provide an alternative source of biomass for pharmaceutical active ingredients.

Key words: Micropropagation, *Tamarix nilotica*, Tissue culture, Medicinal plant.

Introduction

Tamaricaceae is relatively small family among four genera and 120 species (Trease and Evans, 2002); other source represents that Tamaricaceae consists of 5 genera and 78 species (Ghazanfar, 1994). The main genera of this family are *Tamarix, Reaumuria, Myricaria, Myrtama* and *Hololachna.* The plants from genera *Tamarix* and *Reaumuria* are presented in Saudi Arabia (Chaudhary 2001, http://en.wikipedia.org/wiki/Tamaricaceae 8-3, 2008). Among all, about 50 species belong to genus *Tamarix;* distributed most of the continent of the world . Only 8 species from *Tamarix* are found in Saudi Arabia, namely, *T. ramosissima* Leded., *T. aphylla* L., *T. passerinoides* Del. *T. tetragyna* Ehrenb., *T. mascatensis* Bunge. *T. aucheriana* Decne., *T. pyconocarpa* DC. and *T. nilotica* Ehrenb Bunge (Chaudhary, 2001).

Bunge (family: Tamaricaceae) commonly known as "Tarafa" is an ever-green shrub contain needle like leaves, widely distributed in eastern Najd and northern region of Saudi Arabia (Bulos, 1983). This medicinal plant species used for treatment of liver, stomach, and inflammatory problems and can be found in the book "Canon of medicine" of Avicenna (Sina, 2007; Bakr *et al.*, 2013; Abouzid & Sleem, 2011). The aqa-alcoholic extract of flowers of *T. nilotica* had showed marked hepatoprotecton against CCl_4 induced liver injury (Abouzid & Sleem, 2011). Further studies have also observed significant antioxidant (Abouzid & Sleem, 2011) and anti-inflammatory (Orfali, 2005) action of *T. nilotica* that may have resulted to its hepatoprotective activity. Clinical toxicity of *T. nilotica* is not reported in literature but few exists on the plant toxicity against some cancer cell lines of humans (Bakr *et al.*, 2013). Several phytochemicals were reported from *T. nilotica:* tannins, flavonoids syringaresinol, isoferulic acid, niloticol, methyl and ethyl esters of gallic acid, 3-hydroxy-4-methoxycinnamaldehyde, para-methoxygallic acid, quercetin 3-oglucuronides, kaempferol, 3-o-sulphated kaempferol, 7,4- dimethyl ether and free flavonols (Bakr *et al*., 2013; Abouzid & Sleem, 2011).

The genus *Tamarix* of the family Tamaricaceae is the largest genus in (Tamarisk). Two species of *Tamarix* found in Egypt, *Tamarix aphylla* (L.) H.Karst and *T. nilotica* (Ehrenb.) Bunge (Boulos, 1999). *T. nilotica* (Ehrenb.) in the Egyptian history Bunge was mentioned in ancient papyri in pharaonic times to treat fever, headache, inflammation and as an aphrodisiac, as well as in Egyptian Folklore medicine by locals as an antiseptic (Abouzid & Sleem, 2011). Different parts of *Tamarix* are used in Egypt, for example, cooked leaves and young branches are used in oedema of spleen; leaves mixed with ginger is useful in uterus infections; and bark boiled in water with vinegar is used as lotion against lice (Boulos, 1983). Roots have shown the presence derivatives of gallic acid, a lignan (syringaresinol) and is oferulic acid, niloticol, and 3-hydroxy-4-methoxycinnamaldehyde (Barakat *et al.*, 1987). Therefore, *T. nilotica* appeared promising as a natural source for new drugs (Barakat *et al*., 1987). The antioxidant activity is well known to be associated with phenolic compounds (Tepe *et al.*, 2006). In addition, cytotoxic drugs play a major role in cancer chemotherapy (Zunino & Capranico, 1997).

Tissue culture is being used as a potential method for multiplication and conservation of various plant species. As compared to conventional propagation, micropropagation benefits from the advantage of allowing rapid propagation within limited time and space. *T. nilotica* propagates via seeds in nature and also by vegetatively. Micropropagation would be a very useful technique for mass multiplication of *T. nilotica* to overcome slow propagation rate, low seed germination and limited cutting raising. In general, natural

habitat of a plant and genetic material conservation faces several threats, mainly due to pathological and harsh environment coupled with high cost of conservation. The development of a micropropagation system for *T. nilotica* would provide a mean for rapid plant preservation; cryopreservation for long- and medium time storage. The aim of this investigation was to reduce growth and to increase the intervals between subcultures as also reported by Engelmann, 2004.

Materials and methods

***In vitro* culture establishment:** Shoot cuttings (15 - 20 cm long) of *T. nilotica* were collected from young and apical branches of adult trees (10 year old) from the forest of Riyadh in January2014, these branches trimmed further to 1.0 cm for in vitro inoculation on media . Washing of cuttings were done for 10 min under running tap water, decontaminated in ethanol 70% (v/v) for 1 min and dipped twice (10 min each) in commercially available bleach solution (Chlorox, 3.0-4.0% available chloride) with 5-6 drops of *Teepol*. Afterward, cuttings were rinsed in a sterilized fungicide solution Carbendazim (1%, Bayer, Turkey) for 10 min followed by three washes in sterile water (5 min each). To test the influence of medium composition and growth regulators on shoot propagation, decontaminated initial explants (1 cm long cuttings with axillary bud) were placed on MS (Murashige & Skoog, 1962) supplemented with different growth regulators benzyl adenine (BA), Kinetin (Kn), TDZ (Thidiazuron) and isopentynyl (2iP). Each treatment consisted of three phytajars with 50 ml medium containing five explants each gave a total of 15 explants. Cultures were incubated in a growth chamber at 25 ±1ºC with a 16-h photoperiod and irradiance of 98 µmol m-2 s-1 supplemented by Philips L36W/21 lamps. Survival of shoot morphological characters and number of shoots/explant were noted in every culture cycle.

Rooting studies and plant acclimatization: Isolated shoots (1.5 – 2.5.0 cm long) were transferred to different rooting conditions: MS basal culture medium was chosen for rooting with different growth regulator, continuous exposure to indoleacetic acid (IAA), indolebutyric acid (IBA), α-napthaleneacetic acid (NAA) and 2-4,dichlorophenoxyacetic acid (2,4-D) at 2.5-10.0 µM concentrations. The cultures were raised under controlled condition of a growth chamber described earlier for shoot culture. A total of 25 explant cultured in five phytajars contiaing 5 explant in each jar. In another experiment, IBA at higher concentration of 100.0 µM was given to isolated shoots for 5, 10 and 15 days interval. After this pulse treatment, the shoots were transferred to MS medium alone for root induction. When roots were at least 1.9 cm or above long, the plantlets grown *in vitro,* transferred to plastic pots filled with different sterilized mixtures of sand: soil: FYM, Peat moss, Perlite and soil alone treated with a fungicide solution and transferred to a greenhouse maintained at 25±2ºC, with a 16-h photoperiod and irradiance (400 µmol m^{-2} s^{-1}) . The relative humidity was decreased perpetually for the acclimatization of the plants

Statistical analysis: All the experiments were conducted in a completely randomized block design (CRD) and each treatment was repeated three times. The results are presented as a mean ± standard error (SE). Growth and phenotypic changes were regularly monitored in the cultures. Data for shoot, root and hardening of plantlets were evaluated. All the data were subjected to analysis of variance (ANOVA) followed by Duncan's range test at P = 0.05 using SPSS software version 20 (SPSS Inc., Chicago, IL).

Results and Discussion

The advantage of direct regeneration is formation of desired meristematic plant parts having rare chance of genetic variations virtually may results in formation of any organ for e.g., buds, shoots, roots, leaf or even to flower. The micropropagatin ensures genetic stability in plants as in the event of indirect organogenesis, which may result in clonal variations (Cavusoglu *et al.*, 2011). Effect of different cytokinins were evaluated on shoot induction via direct organogenesis: auxiliary buds regenerated into multiple shoots after one month of culture on MS medium supplemented with BA, TDZ, Kn, and 2ip separately. Shoot induction response to different cytokinin was variable to various PGRs tested. It has been reported that cytokinins are essential for shoot proliferation in wide range of plants (Gaspar *et al.*, 1996). Maximum 10.70 shoot per nodal explants obtained on MS medium containing 1.0 µM TDZ, however, low number of shoots were formed using the same concentration of BA, Kn and 2iP (Table 1, Fig. 1 A-C). A higher number of shoots *i.e.,* 9.8 (BA), 8.3 (Kn) and 6.5 (2ip) induced in 2.5 µM concentrations, respectively (Table 1). As the concentration of PGRs increased the number of shoots decreased: showed that higher concentrations of cytokinin have inhibitory effect on shoot induction. However, all the PGRs and concentrations were helpful to enhance multiple shoots generation.

Table 1. Effect of different cytokinins on shoot regeneration in *Tamarix nilotica* after one month of culture on MS medium. Treatment concentration (µM).

Treatments concentrations (µM)	Mean number of shoots regenerated ±SE			
	BA	KN	2iP	TDZ
1.0	4.00 ± 0.25abcd	4.30 ± 0.26abcd	2.90 ± 0.23abcd	10.70 ± 0.49a
2.5	9.80 ± 0.48a	8.30 ± 0.30a	6.50 ± 0.30a	7.00 ± 0.25b
5.0	5.40 ± 0.26ab	5.10 ± 0.31ab	5.90 ± 0.34ab	4.80 ± 0.24abc
10.0	4.40 ± 0.22abc	4.90 ± 0.27abc	5.20 ± 0.29abc	4.10 ± 0.27abcd

Values represent mean ± SE of three replicates per treatment. Means followed by same letters are not significantly different by the Duncan's range test at significance level ($p \leq 0.05$)

Our study also conforms the promotory effect of TDZ in shoot induction from nodal segments as reported (Huetteman and Preece, 1993; Shen *et al.*, 2008, 2010). TDZ has a remarkable role in micropropagation of tree species and hard to multiply with adenine-type cytokinins (Huetteman & Preece, 1993; Lu, 1993). Further it has been reported that TDZ at low concentration (less than/or 1 uM) was effective to stimulate auxiliary bud proliferation, whereas higher concentrations induced callus formation or somatic embryogenesis (Lu,1993; Mithila *et al.*, 2003). Number of shoots increased as the concentration of the PGRs reached to optimum. This has also been affirmed in *Solanum melongena* (Bhatti *et al*., 2014) and *Solanum villosum* (Iftikhar *et al*., 2015).

Development of a successful micropropagation protocol requires an efficient system of rooting. Therefore, individual shoots were subjected to different auxins *i.e.,* IBA, NAA, 2,4-D and IAA at various concentrations for a month. A very low level 13-30% rooting was observed across all the PGRs tested in different concentration (Table 2). This may have happened due to carry over effect of cytokinins inhibiting root induction. Improved Induction of roots was achieved when shoots were cultured on MS medium supplemented with high 100 µM concentration of IBA for short time 5, 10 & 15 days and then transferred to MS medium without any PGRs. About 50-86 % (Table 3, Fig. 1D) rooting obtained after one month culture on MS medium devoid of any PGR. Maximum 86% rooting was observed at 10 days pulse treatment of IBA. This is in agreement with results obtained in shoots of *O. arabicus* by Al-Qurainy *et al.*, (2013). Maximum (90%) acclimatization of rooted plants was achieved in sterilized Sand + Soil + Farmyard (1:1:1) (Table 4, Fig. 1E). Other experimented medium fail to give higher rate of survival of the plants. In the present investigation the observations regarding potting medium is also reported in other plants (Baskaran & Jayabalan, 2005; Ghauri *et al.*, 2013).

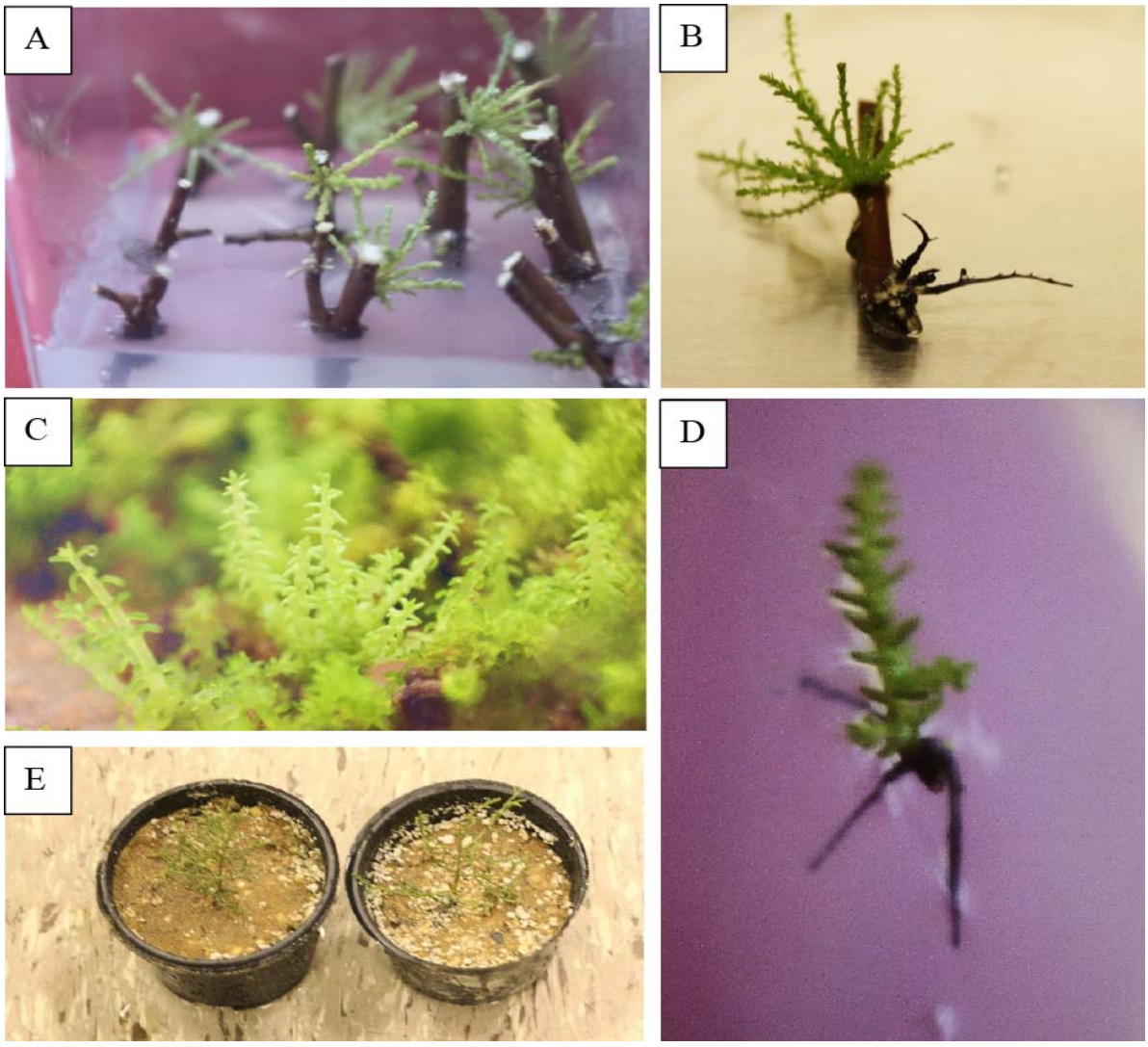

Fig. 1. Micropropagation of *Tamarix nilotica* through axillay shoot bud induction
A: Induction of axillary bud on MS medium containing 1.0 µM TDZ., B & C: Shoot proliferation and growth., D: Rooting in individual shoot on MS medium after pulse treatment of IBA for 10 days., E: Acclimatized plants ready for transplant.

Table 2. Evaluation of different auxins in root induction of *Tamarix nilotica* on MS medium supplemented with various concentration.

Treatments concentrations (µM)	Mean rooting % ± SE			
	IAA	NAA	24D	IBA
2.5	26.66 ± 3.3a	23.33 ± 3.33a	30.00 ±5.77a	20.00 ± 5.77a
5.0	20.00 ± 5.7ab	20.00 ± 5.77ab	20.00 ± 5.77ab	16.66 ± 3.33ab
7.5	16.66 ± 3.3abc	20.00 ± 0.00ab	16.66 ± 3.33abc	13.33 ±3.33 abc
10.0	13.33 ± 3.3abcd	13.33 ± 3.33abc	16.66 ± 3.33abc	13.33 ±3.33 abc

Values represent mean ± SE of three replicates per treatment. Means followed by same letters are not significantly different by the Duncan's range test at significance level ($p \leq 0.05$)

Table 3. Effect of pulse treatment of IBA on rooting of *T. nilotica* on MS medium.

Treatment Time period (days)	Mean rooting % ±SE	Mean root length (cm) ±SE	Mean shoot length (cm) ±SE
5	60.00 ± 5.77ab	2.20 ± 0.20ab	2.74 ± 0.07abc
10	86.66 ± 3.33a	3.00 ± 0.21a	3.31 ± 0.10a
15	50.00 ± 5.77abc	1.90 ± 0.17abc	2.89 ± 0.09ab

Values represent mean ± SE of three replicates. Means followed by same letters are not significantly different by the Duncan's range test at significance level ($p \leq 0.05$)

Table 4. Effect of potting mix on survival of micro-shoots after acclimatization under controlled conditions of greenhouse.

S. No.	Potting mixture	Survival %
1	Sand: soil: FYM	90.0
2	Perlite	50.0
3	Peat moss	70.0
4	soil	60.0

Values are means of three replicates 10 plants each

Conclusion

Tamarix nilotica was successfully propagated through stem node cutting by using TDZ. This study has opened gateways for producing cell, tissue and suspension cultures of *T. nilotica*. The medicinal potential of this plant requires further studies on callus, cell and suspension culture to obtain active constituents from the alternative source to conserve the plant in natural habitat. This will ensure a continuous and reliable source for stable production.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the study through the research group project #RGP-VPP-014.

References

Abouzid, S. and A. Sleem. 2011. Hepatoprotective and antioxidant activities of *Tamarix nilotica* flowers. *Pharm Biol.*, 49(4): 392-395.

Al Qurainy, F., M. Nadeem, S. Khan, S. Alansi and M. Tarroum. 2013. Efficient regeneration of a potentialmedicinal plant *Ochradenus baccatus* Delile from cotyledon and shoot axis. *Pak. J. Bot.*, 45(2): 501-505.

Bakr, R.O., M.A. E-A. El, Raey and R.S. Ashour. 2013. Phenolic content, radical scavenging activity and cytotoxicity of *Tamarix nilotica* (Ehrenb.) bunge growing in Egypt, *J. Pharmacognosy Phytotherapy*, 5(3): 47-52.

Barakat, H.H., M.A.M. Nawwar, J. Buddrus and M. Linsheid. 1987. Niloticol, a phenolic glyceride and two phenolic aldehydes from the roots of *Tamarix nilotica*. *Phytochemistry,* 26(6): 1837-1838.

Baskaran, P. and N. Jayabalan. 2005. An efficient micropropagation system for *Eclipta alba* – a valuable medicinal herb. *In vitro cell. Dev. Biol. Plant.*, 41(4): 532-539.

Bhatti, K.H., M.D. Jamil and M. Tufail. 2014. Direct organogenesis (shoot and root) of egg plant (*Solanum melongena* L.) through tissue culture. *World Appl. Sci. J.*, 30(3): 317-321.

Boulos. L. 1999. *Flora of Egypt*. Al Hadara Publishing, Cairo, Egypt. Vol. 2, pp. 124.

Bulos, L. 1983. *Medicinal Plant of North Africa*, Michigan. pp. 286.

Cavusoglu, A., Z. Ipekci-Altas, K. Bajrovic, N. Gozukirmizi and A. Zehir. 2011. Direct and indirect plant regeneration from various explants of eastern cottonwood clones (*Populus deltoides* Bartram ex Marsh.) with tissue culture. *Afr. J. Biotechnol*., 10(16): 3216-3221.

Chaudhary, S.A. 2001. *Flora of the Kingdom of Saudi Arabia*, Ministry of Agricultureand Water, National Herbarium, National Agriculture and Water Research Center,Riyadh, KSA, vol.1, pp.405-408.

Engelmann, F. 2004. Plant cryopreservation: progress and prospects. *In Vitro Cell. Dev. Biol. Plant.*, 40: 427-433.

Gaspar, T., C. Kevers, C. Penel, H. Greppin, D.M. Reid, and T.A. Thorpe. 1996. Plant hormones and plant growth regulators in plant tissue culture. *In Vitro Cell. Dev. Biol. Plant*., 32: 272.

Ghauri, E.G., M.S. Afridi, G.A. Marwat, Inayatur-Rahman and M. Akram. 2013. Micro-propagation of *Stevia rebaudiana* bertoni through root explants. *Pak. J. Bot*., 45(4): 1411-1416.

Ghazanfar, S.A. 1994. *Handbook of Arabian Medicinal Plants*, CRC. Press, Boca Roton, Ann Arbor, London, Tokyo, pp. 203.

Huetteman, C.A. and J.E. Preece. 1993. TDZ: A potent cytokinin for woody plant tissue culture. *Plant Cell Tissue Organ Cult.,* 33: 105-119.

Iftikhar, A., R. Qureshi, M. Munir, G. Shabbir, M. Hussain and M.A. Khan. 2015. *In vitro* micropropagation of Solanum Villosum─A potential alternative food plant. *Pak. J. Bot.,* 47(4): 1495-1500.

Lu, C.Y. 1993. The use of TDZ in tissue culture. *In Vitro Cell. Dev. Biol. Plant.,* 29: 92-96.

Mithila, J., J.C. Hall, J.M.R. Victor and P.K. Saxena. 2003. Thidiazuron induces shoot organogenesis at low concentrations and somatic embryogenesis at high concentrations on leaf and petiole explants of African violet (*Saintpaulia ionantha* Wendl.). *Plant Cell Rep.,* 21: 408-414.

Murashige, T. and F. Skoog. 1962. A revised medium for rapidgrowth and bioassay with tobacco tissue culture. *Physiol. Plant*., 15: 473-497.

Orfali, R.S. 2005. Phytochemical and Biological Study of *Tamarix nilotica* growing in Saudi Arabia, King Saud University.

Shen, X., M.E. Kane and J. Chen. 2008. Effects of genotypes, explant sources, and plant growth regulators on indirect shoot organogenesis in *Dieffenbachia* cultivars. *In Vitro Cell. Dev. Biol. Plant*, 44: 282-288.

Shen, X., W.S. Castle and F.G. Jr. Gmitter. 2010. *In vitro* shoot proliferation and root induction of shoot tip explants from mature male plants of *Casuarina cunninghamiana* Miq. *Hortscience,* 45(5): 797-800.

Sina, I. 2007. *Al Qanoon Fil Tib*, vol. 522, Mataba Munshi Naval Kishore, Lucknow, India.

Tepe, B., M. Sokmen, H.A. Akpulat and A. Sokmen. 2006. Screening of the antioxidant potentials of six *Salvia* species from Turkey. *Food Chem*., 95: 200-204.

Trease, G.E. and W.C. Evans. 2002. Pharmacognosy, WB Saunders Company Ltd, London, 15th ed., pp.30.

Zunino, F. and G. Capranico. 1997. In: *Cancer Therapeutics: Experimental and Clinical Agents*. (Ed.): B.A. Teicher. Cancer Drug Discovery and Development, Humana Press, Totowa, NJ. pp. 195-214.

(Received for publication 13 February 2015)