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In-vitro Callus Development of Different Explants used for Different Medium of *Terminalia arjuna*

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ABSTRACT

An efficient protocol for *in vitro* propagation of *Terminalia arjuna* is described by callus regeneration. Four different explants were used to establish callus to develop shoot and root regeneration method. MS (Murashiege and Skoog), LS (Linsmaier and Skoog) and B5 (Gamborg) basal medium supplemented with 2,4-Dichlorophenoxyacetic acid (2,4-D, 0.1-20.0 mg L^{-1}), with combination of Naphthalene acetic acid (NAA 0.1-20 mg L^{-1}), Indole-3-acetic acid (IAA 0.1-20 mg L^{-1}) and Benzylaminopurin (0.1-20 mg L^{-1}). Ms medium was found to be the most favorable for callus induction compare with LS and B5 media. Maximum number of callus regeneration was obtained on MS medium containing 2,4-D 3.0 mg L^{-1}. The callus culture to develop the shoot and root initiation in MS basal medium substituted with 5 mg L^{-1} 2, 4-D+0.01 mg L^{-1} Kinetin and 1.0 mg L^{-1} Gibberellic acid (GA_3). The rooted shoot plantlet was transferred in to small plastic cups containing sterile vermiculate, sand and red soil in the ratio of 1:2:2 and were kept in a mist house. The regenerated plantlets were hardened in the greenhouse and successfully transferred in soil with 87% survival rate. This *in vitro* micro-propagation method with possibility of developing a new protocol was standardized the *T. arjuna* plant.

Key words: *Terminalia arjuna*, medicinal plant, combretaceae, callus regeneration, micro-propagation

INTRODUCTION

Terminalia arjuna W and A., belongs to Combretaceae, is a large evergreen tree with spreading crown, drooping branches and common in most part of India and Sri Lanka. The genus of *Terminalia* comprises more than 16 species in India and is distributed in tropical and sub-tropical regions. *T. arjuna* W. and A. (Arjuna in Sanskrit, Vellai maruthu in Tamil) is an important medicinal plant (Orwa *et al*., 2009). The bark is an excellent source of multi-protection drug providing relief in symptomatic complaint, hypertension, ulcers, cancer and dysentry. It acts against human immuno deficiency virus (HIV-1) and as hepatoprotective agent (Nishi *et al*., 1998). It has been traditionally used in the Indian indigenous systems of medicine dating back to the Vedic ages (1500-800 BC) and is an integral part of Indian culture (Mishra and Broker, 2009).

T. arjuna bark is used in various medicinal preparations. Moreover, prolonged use of this bark shows no side effects. The potential use of this drug as an adjunct in the management of hypertension, stable angina and ischaemic cardiomyopathy merit the vital importance of this tree

plant (Dwivedi and Gupta, 2002). Stem bark contain Arjunolic acid, tomentosic acid, β-sitosterol, ellagic acid, (+) - leucodelphinidin (Rastogi and Mehrotra, 1993a), arjunic acid (Row *et al*., 1970a) arjunetin (Row *et al*., 1970b), arjungenin, arjunglucoside I and II (Rastogi and Mehrotra, 1993b), tannins containing catechin, gallocatechin, epicatechin, epigallocatechin (Rastogi and Mehrotra, 1993c) arjunolone, baicalein (Sharma *et al*., 1982; Sharma, 1996), arjunglucoside III (Rastogi and Mehrotra, 1993c), terminoic acid (Ahmad *et al*., 1983), arjunolitin (Tripathi *et al*., 1992), arjunglucoside IV, V (Wang *et al*., 2010a) arjunasides A-E (Wang *et al*., 2010b), 2α, 3β-dihydroxy urs-12, 18 dien-28-oic acid 28-O-β-D-glucopyranosyl ester (Wang *et al*., 2010c), casuarianin (Kuo *et al*., 2005), arjunophthanoloside (Ali *et al*., 2003a), terminoside-A (Ali *et al*., 2003b), arjunin (Kandil and Nassar, 1998), terminarjunoside I, II (Alam *et al*., 2008). Fruits contain Arjunone, cerasidin, β-sitisterol, friedelin, methyl oleanolate, gallic acid, ellagic acid, arjunic acid, hentriacontane, myristyl oleate, arachidic stearate (Rastogi and Mehrotra, 1993c), terminolitin (Singh *et al*., 1995). Root bark contain Arjunoside I, II, 8-hydroxyl hexadecanoic, oleanolic, arjunic acids, arjunolic acid, β-sitosterol (Anjaneyulu and Rama-Prasad, 1982a), terminic acid (Anjaneyulu and Rama-Prasad, 1983), arjunoside III, IV, arjunoside I, arjunetin, ellagic acid, gallic acid, leucocyanidin (Anjaneyulu and Rama-Prasad, 1982b), arjunetoside (Upadhyay *et al*., 2001), 16, 17-dihydroneridienone 3-O-β-D-glucopyranosyl-(1>6)-O-β-D-galactopyranoside (Yadav and Rathore, 2001). Seeds contain 14, 16-dianhydrogitoxigenin 3-β-D-xylopyranosyl-(1>2)-O-β-D-galactopyranoside (Yadava and Rathore, 2000). It is used in hypertension and heart diseases. The plant might be considered a boon to patients suffering from blood pressure. The pharmacological studies have shown that bark of *T. arjuna* has antiviral (Kusumoto *et al*., 1995) anti mutagenic (Kaur *et al*., 2001) antiplague (Shaila *et al*., 1997), anticancer (Nagpal *et al*., 2000) and hypotensive (Takahashi *et al*., 1997) properties. *T. arjuna* has been used traditionally as a cardiotonic and current users include treatment for angina, hypertension, arrhythmias and congestive heart failure. Animal studies have also indicated that crataegus extracts may also have potential use as anti ischemic and lipid-lowering agents. The bark of the *T. arjuna* has a long history of use as a cardiotonic as well and has been indicated in the treatment of coronary artery disease, heart failure, hypercholesterolemia and for relief of anginal pain. Additionally, it has been found to have antibacterial and anti-mutagenic properties. Micropropagation of *Terminalia arjuna* by Ramesh *et al*. (2001). Nishi *et al*. (1998) reported for somatic embryogenesis of some selected genotypes, effect of antioxidant and absorbent in *Terminalia arjuna*.

T. arjuna seeds life span is very short and viability also very poor. The present study was conducted to standardized a simple and efficient protocol for *in vitro* micropropagation through explants such as leaf, cotyledon, hypocotyls and epicotyls of *T. arjuna* which would highly useful for the conservation of this vulnerable tree species.

MATERIALS AND METHODS

Collection of plants: *Terminalia arjuna* explants and seeds were collected from the Botany Field Research Laboratory in 2011, Maduravoyal, University of Madras, Tamil Nadu.

Explant sterilization: Different explants viz., cotyledon, hypocotyls, epicotyl and leaves were collected from 10-12 days old seedling. Explants were washed thoroughly in running tap water for 30 min then in Teepol (1% v/v solution mild detergent used as cleaning agent) for 2-3 min and washed several times with distilled water. Explants immersed in 70% (v/v) ethanol for 1 min followed by surface sterilized with 0.1% $HgCl_2$ solution for 1-2 min and again washed well in

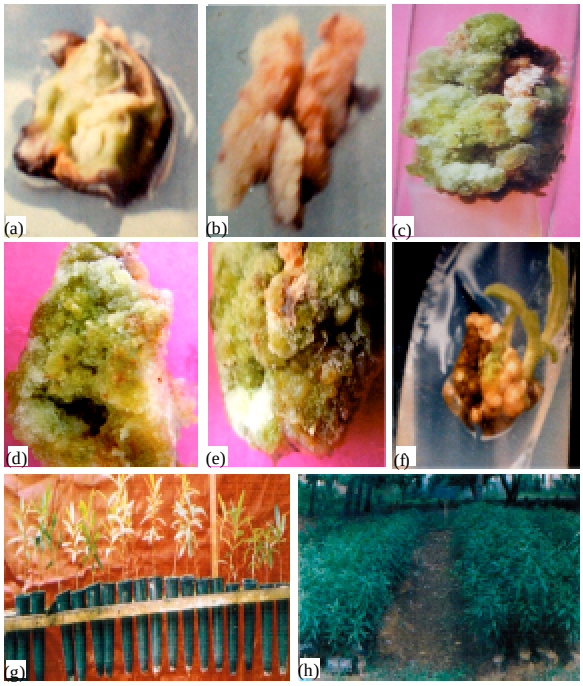

Fig. 1 (a-h): (a) MS medium callus, (b) LS medium callus, (c) Green friable callus, (d) Yellowish green friable callus, (e) Cream compact callus, (f) Callus Shoots initiation, (g) Rooted plantlet transfer to the plastic cups and maintained for one month and (h) Hardening process of plantlet transfer to polythene bags

distilled water 3-4 times to remove the traces of $HgCl_2$ (Mercuric chloride). The leaf and young stem, shoot part were cut in to (0.5-1.0 cm) small pieces and were inoculated in the different medium.

Culture media: All the different explants (0.5-1.0 cm) were implanted on the MS (Murashige and Skoog, 1962), LS (Linsmaier and Skoog, 1965) and B5 (Gamborg *et al*., 1968) medium. These media were supplemented with different hormone concentration viz., 2,4-D, NAA, IAA and BAP (High media, India) were used. The pH of the media was adjusted to 5.8 with 1N HCl or 1N NaOH, before addition of agar. The tubes and conical flasks were closed with cotton plug and contained of medium. Media were autoclaved at 1.05 kPa in 121°C for 20 min.

Culture conditions: The cultures were incubated under the conditions of white fluorescent light at 16 h, photo-period, 60 μ EM^{-2} light intensity, in 25±2°C.

Hardening: Rooted plantlets were removed from the culture flasks. After washing away the agar with water, they were transferred in to small plastic cups containing sterile vermiculate, sand and red soil in the ratio of 1:2:2 and were kept in a mist for one month (Fig. 1g). The hardening process of regenerated plantlets were successfully transferred in to ground soil contain polythene bags. The survival rate of the tissue culture plant 87% (Fig. 1h).

Table 1: Effect of different explants and media used for 2,4-dichlorophenoxyactic acid (2,4-D) on callus induction (%)

	MS				LS				B5			
2,4-D (mg L^{-1})	L	C	H	E	L	C	H	E	L	C	H	E
0.1	40	35	52	29	45	45	15	23	20	26	20	14
0.5	54	46	38	40	45	48	25	36	32	36	28	29
1.0	70	44	63	47	55	46	48	35	48	48	45	35
1.5	79	62	65	75	52	56	49	42	58	45	44	35
2.0	91	65	76	84	84	60	75	42	60	64	48	48
3.0	95	82	88	91	88	65	64	48	54	58	56	46
4.0	78	67	60	79	75	26	44	48	64	40	62	48
5.0	65	50	45	75	72	29	28	65	52	32	68	32
10.0	59	29	32	42	63	20	-	20	44	-	-	-
20.0	-	-	-	-	-	-	-	-	-	-	-	-
Type of callus	YG	G	CN	CC	YG	G	CN	CC	YG	G	CC	CC

C: Cotyledon, H : Hypocotyl, E : Epicotyl, L: Leaf -:No response, YG: Yellowish green friable callus, G: Green friable callus, CN: Cream nodular callus, CC: Cream Compact callus, MS: Murashiege and Skoog, LS: Linsmaier and Skoog, BS: Gamborg

RESULTS AND DISCUSSION

Effect of 2,4-D callus induction (%) in different explants of *T. arjuna*: Callus initiation was observed from the MS, LS, B5 medium supplemented with 2, 4-D (0.1-10 mg L^{-1}) (Fig. 1a, b). The response shown by different explants varied widely depending on the concentration (Table 1). The overall callus induction frequency on MS medium varied from 40-95% with leaf explants, while in hypocotyls, epicotyls and cotyledon the frequency range was 38-88, 29-91 and 29-82%, respectively. The optimum frequency of callus induction was elicited with the supplementation of 1.5-3.0 mg L^{-1} of 2, 4-D more or less uniformly for all the explants of the four explants. Among the 4 explants maximum callus induction was observed with leaf explant followed by the epicotyls, hypocotyl and cotyledon (L>E>H >C) cotyledonary and leaf explants yielded (Fig. 1d) yellowish green friable callus whereas hypocotyl gave light green friable callus (Fig. 1c) in scant amount while creamy friable callus in scant quantity was obtained with epicotyl (Fig. 1e). The callusing frequency in LS medium ranged from 45-88% leaf explants. Mostly cream-colored modular callus was observed abundantly. The various concentrations of 2,4-D added to MS, LS and B5 media showed maximum induction of calli in MS and LS medium at the concentration of 3 mg L^{-1}. Maximum amount of callus growth was obtained on MS medium followed by LS, B5, media (MS>LS>B5). (Kumar *et al*., 2010) the upper hypocotyl with shoot apices was observed to be the best explant for callus formation.

Effect of NAA callus induction (%) in different explants of *T. arjuna*: Callus initiation occurred from leaf, cotyledon, hypocotyl and epicotyl explants on all the media supplemented with different concentration of NAA (0.1-10.0 mg L^{-1}) (Table 2). After 3 weeks of induction, The callusing response on MS medium varied from 0-92% and the maximum was at 5 and 10 mg L^{-1} NAA with leaf explants in MS medium. However, the lower concentration of NAA (0.1-0.5 mg L^{-1}) did not induce any callusing. A high green friable callus in moderate amounts was obtained with leaf explant with high frequency 0-92% at 5-10 mg L^{-1} concentration. In hypocotyls and epicotyls callusing potential ranged from 0-84% the lower concentration of NAA (0.1-0.5 mg L^{-1}) has not induced any callusing. In LS medium the callusing frequency ranged from 0-78%. The lower concentrations (0.1-1.0 mg L^{-1}) callus did not induce all other explants. In cotyledonary explant the

Table 2: Effect of different explants and media used for naphthalene acetic acid (NAA) on callus induction (%)

	MS				LS				B5			
NAA (mg L^{-1})	L	C	H	E	L	C	H	E	L	C	H	E
0.1	-	-	-	-	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-	-	-	-	-
1.0	-	8	38	47	-	-	-	-	-	-	8	-
1.5	35	14	52	58	14	4	16	15	11	8	9	7
2.0	48	20	59	66	55	18	14	24	14	15	7	8
3.0	65	27	66	70	35	32	24	33	15	14	12	14
4.0	78	48	75	78	45	45	25	36	18	18	16	16
5.0	90	42	84	84	78	54	35	35	27	25	18	19
10.0	92	54	74	82	78	22	36	42	18	28	20	18
20.0	35	28	48	36	27	20	18	12	15	13	14	12
Type of callus	YG	G	CN	CC	YG	G	CN	CC	YG	G	CC	CC

C: Cotyledon, H : Hypocotyl, E : Epicotyl, L : Leaf -:No response, YG :Yellowish green friable callus, G: Green friable callus, CN: Cream nodular callus, CC: Cream compact callus. MS: Murashiege and Skoog, LS: Linsmaier and Skoog, BS: Gamborg

callusing frequency ranged from 0-54% mainly the light green friable callus in moderate quantities was noticed. In hypocotyl it ranged from 0-36%, a white cream friable callus in scant quantity was obtained. The epicotyl range 0-42% of cream compact callus was produced. Callusing frequency varied from 0-27% in B5 medium when supplemented with different concentrations of NAA. The lower range of supplementation (0.1-1.0 mg L^{-1}) did not induce callusing. Leaf, cotyledon and hypocotyl explants yielded a yellowish green, light green friable and white creamy friable callus.

Effect of IAA callus induction (%) in different explants of *T. arjuna*: All the explants, inoculated on three media (MS, LS and B5.) prepared with various concentrations of IAA (0.1-10 mg L^{-1}) showed differential response (Table 3). The epicotyl explants only showed a good response for callus induction. But leaf hypocotyls and cotyledon did not respond for callus induction in the presence of IAA. The Epicotyl explants responded for callus induction, the frequency of callus in MS medium varied from 0-66% and maximum was observed at 5 mg L^{-1} concentration of IAA mainly cream colored modular callus was observed (Table 4).

Effect of BAP callus induction (%) in different explants of *T. arjuna*: The callus initiation was observed with all explants in 10-12 days of culturing on three media (MS, LS and B5) amend with various concentrations of BAP (0.1-20 mg L^{-1}) the efficiency of callusing of different explants varied between media and supplemented BAP at different concentrations (Table 4). In MS medium, the frequency of callusing varied from 0-88% for leaf explants. Maximum callus induction was observed at BAP 4 mg L^{-1} concentration. Among the three media, MS media was found to be better than LS and B5 media (Rathore *et al.*, 2008). MS medium containing 1.5 mg L^{-1} BAP was the best for callus initiation of *T. bellerica*.

Potential callus developing shoot and root: Among the four different calli observed, the green friable calli produced from leaf explants developed plantlets (Fig. 1f) upon incubation for shoot and root initiation in MS basal medium substituted with 5 mg L^{-1} 2,4-D+0.01 mg L^{-1} kinetin and 1.0 mg L^{-1} GA_3. All the other calli finally resulted in browning, shriveled and did not develop into plantlets (Kumar *et al.*, 2010). MS media supplemented with Kn, 2,4-D and GA_3 (1-5 mg L^{-1}) shoot

Table 3: Effect of different explants and media used for Indole-3-acetic acid (IAA) on callus induction (%)

	MS				LS				B5			
IAA (mg L^{-1})	L	C	H	E	L	C	H	E	L	C	H	E
0.1	-	-	-	-	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-	-	-	-	-
1.0	-	-	-	48	-	-	-	45	-	-	-	-
1.5	-	-	-	65	-	-	-	46	-	-	-	-
2.0	-	-	-	64	-	-	-	49	-	-	-	-
3.0	-	-	-	56	-	-	-	58	-	-	-	46
4.0	-	-	-	60	-	-	-	54	-	-	-	48
5.0	-	-	-	66	-	-	-	62	-	-	-	52
10.0	-	-	-	-	-	-	-	65	-	-	-	56
20.0	-	-	-	-	-	-	-	-	-	-	-	-
Type of callus	YG	G	CN	CC	YG	G	CN	CC	YG	G	CC	CC

C: Cotyledon, H: Hypocotyl, E: Epicotyl, L: Leaf -:No response, YG: Yellowish green friable callus, G: Green friable callus, CN: Cream nodular callus, CC: Cream compact callus. MS: Murashiege and Skoog, LS: Linsmaier and Skoog, BS: Gamborg

Table 4: Effect of different explants and media used for benzyl aminopurine (BAP) on callus induction (%)

	MS				LS				B5			
BAP (mg L^{-1})	L	C	H	E	L	C	H	E	L	C	H	E
0.1	-	22	25	-	31	-	-	20	-	42	-	-
0.5	20	32	29	-	40	-	-	35	16	48	8	-
1.0	24	29	32	9	44	54	5	36	25	52	11	-
1.5	30	54	38	14	56	48	8	56	28	65	12	-
2.0	38	62	44	18	53	43	7	49	28	33	14	6
3.0	62	69	42	26	62	26	6	34	46	26	15	8
4.0	88	82	45	34	66	16	12	24	44	15	19	9
5.0	79	68	26	28	65	8	16	21	25	12	17	16
10.0	24	47	20	16	20	5	14	9	20	6	12	18
20.0	15	44	14	10	12	-	6	-	-	-	-	-
Type of callus	YG	G	CN	CC	YG	G	CN	CC	YG	G	CC	CC

C: Cotyledon, H: Hypocotyl, E: Epicotyl, L: Leaf :-No response, YG: Yellowish green friable callus, G: Green friable callus, CN: Cream nodular callus, CC: Cream compact callus. MS: Murashiege and Skoog, LS: Linsmaier and Skoog, BS: Gamborg

was developed along with green callus of hypocotyls explant (Rathore *et al*., 2008). Combination of IBA (0.1 mg L^{-1}) with NAA and IAA increased the percentage of root formation *T. bellerica*.

CONCLUSION

As suggested in the published literature, very less significant work has been done on this plant species. With regard to the tissue culture aspect, already some of work has been done. This experiments are carried out to propagate the selected explants of leaves, cotyledon, hypocotyls and epicotyls segment were used for different medium. The present investigation study of *T. arjuna*. Highly morphogenetic regeneration capacity of leaf explants callus formation of the multiple shoot and root of MS medium in following as LS and B5 medium was very poor callus regeneration capacity. This MS medium with different hormones contain protocol has been used successfully for *in vitro* mass propagation of medicinal plant of *T. arjuna*.

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REFERENCES

Ahmad, M.U., K.B. Mullah, T. Norin and J.K. Ulla, 1983. Terminoic acid, a new trihydroxytriterpene carboxylic acid from bark of *Terminalia arjuna*. Indian J. Chem., 22: 738-740.

Alam, M.S., G. Kaur, A. Ali, H. Hamid, M. Ali and M. Athar, 2008. Two new bioactive oleanane triterpene glycosides from *Terminalia arjuna*. Nat. Prod. Res., 22: 1279-1288.

Ali, A., G. Kaur, H. Hamid, T. Abdullah, M. Ali, M. Niwa and M.S. Alam, 2003a. Terminoside A, a new triterpene glycoside from the bark of *Terminalia arjuna* inhibits nitric oxide production in murine macrophages. J. Asian Nat. Prod. Res., 5: 137-142.

Ali, A., G. Kaur, K. Hayat, M. Ali and M. Ather, 2003b. A novel naphthanol glycoside from *Terminalia arjuna* with antioxidant and nitric oxide inhibitory activities. Pharmazie, 58: 932-934.

Anjaneyulu, A.S.R. and A.V. Rama-Prasad, 1982a. Chemical examination of the roots of *Terminalia arjuna*-characterization of two new triterpenoid glycoside. Indian J. Chem., 21: 530-533.

Anjaneyulu, A.S.R. and A.V. Rama-Prasad, 1982b. Structure of terminic acid, a dihyroxy triterpene carboxylic acid from *Terminalia arjuna*. Phytochemistry, 21: 2057-2060.

Anjaneyulu, A.S.R. and A.V. Rama-Prasad., 1983. Chemical examination of the roots of *Terminalia arjuna*-the structure of arjunoside III and IV, two new triterpenoid glycoside. Phytochemistry, 22: 993-998.

Dwivedi, S. and D. Gupta, 2002. Modification of coronary risk factors by medicinal plants. J. Med. Aromatic Plant, 22: 616-620.

Gamborg, O.L., R.A. Miller and K. Ojima, 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell. Res., 50: 151-158.

Kandil, F.E. and M.I. Nassar, 1998. A tannin anti-cancer promotor from *Terminalia arjuna*. Phytochemistry, 47: 1567-1568.

Kaur, S., I.S. Grover and S. Kumar, 2001. Antimutagenic potential of extracts isolated from *Terminalia arjuna*. J. Environ. Patho. Toxicol. Oncol., 20: 9-14.

Kumar, C., A.K. Singh and S. Nehar, 2010. *In-vitro* propagation and callus formation from seedling of *Terminalia arjuna* Roxb. Bioscan. J. Life Sci., 2: 423-429.

Kuo, P.L., Y.L. Hsu, T.C. Lin, L.T. Lin, J.K. Chang and C.C. Lin, 2005. Induction of cell cycle arrest and apoptosis in human non-small cell lung cancer A549 cells by casuarinin from the bark of *Terminalia arjuna* Linn. Anticancer Drugs, 16: 409-415.

Kusumoto, I.T., T. Nakabayashi, H. Kida, M.M. Hattori, T. Namba and Shimotohno, 1995. Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus-I (HIVI) protease. Phytother. Res., 9: 180-180.

Linsmaier, E.M. and F. Skoog, 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant., 18: 100-127.

Mishra, D. and A. Broker, 2009. An ethnomedicinal study among the Gond of Chhattisgarh: India. Antrocom, 5: 61-65.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Nagpal, A., L.S. Meena, S. Kaur, I.S. Grover, R. Wadhwa and S.C. Kaul, 2000. Growth suppression of human transformed cells by treatment with bark extracts from a medicinal plant, *Terminalia arjuna*. *In vitro* Cell Dev. Biol. Anim., 36: 544-547.

Nishi, K., U. Jaiswal and V.S. Jaiswal, 1998. Introduction of somatic embryogenesis and plants regeneration from leaf callus at *Terminalia arjuna*. Curr. Sci., 75: 1052-1085.

Orwa, C., A. Mutua, R. Kindt, R. Jamnadass and S. Anthony, 2009. Agroforestree Database: A Tree Reference and Selection Guide. Version 4.0. International Centre for Research in Agroforestry, Nairobi, Kenya, pp: 6.

Ramesh, M., U. Pavan, S. Prasad, A.V. Rao and A. Sadanandam, 2001. *Terminalia arjuna*: Break through in micropropagation. Indian Silk, 17: 25-28.

Rastogi, R.P. and B.N. Mehrotra, 1993a. Compendium of Indian Medicinal Plants. Vol. 1, CDRI, Lucknow and ID, New Delhi, India.

Rastogi, R.P. and B.N. Mehrotra, 1993b. Compendium of Indian Medicinal Plants. Vol. 2, CSIR, New Delhi.

Rastogi, R.P. and B.N. Mehrotra, 1993c. Compendium of Indian Medicinal Plants. Vol. 3, CSIR, New Delhi.

Rathore, P., R. Suthar and S.D. Purohit, 2008. Micropropagation of *Terminalia bellerica* Roxb. from juvenile explants. Indian J. Biotechnol., 7: 246-249.

Row, L.R., P.S. Murty, G.S.R. Subba-Rao, C.S.P. Sastry and K.V.J. Rao, 1970a. Chemical examination of *Terminalia arjuna*: Part-XII: Isolation and structure determination of arjunetin from *Terminalia arjuna* bark. Indian J. Chem., 8: 772-775.

Row, L.R., P.S. Murty, G.S.R.Subba Rao, C.S.P. Sastry and K.V.J. Rao, 1970b. Chemical examination of *Terminalia arjuna*: Part-XII: Isolation and structure determination of arjunic acid, a new trihydroxytriterpene carboxylic acid from *Terminalia arjuna* bark. Indian J. Chem., 8: 716-721.

Shaila, H.P., S.L. Udupa and A.L. Udupa, 1997. Hypolipidemic effect of *Terminalia arjuna* in cholesterol fed rabbits. Fitoterapia, 68: 405-409.

Sharma, P.N., A. Shoeb, R.S. Kapil and S.P. Popli, 1982. Arjunolone: A new flavone from stem bark of *Terminalia arjuna*. Indian J. Chem., 21B: 263-264.

Sharma, P.V., 1996. Classical Uses of Medicinal Plants. Chaukhambha Visvabharati, Varanasi, India.

Singh, B., V.P. Singh, V.B. Pandey and G. Rucker, 1995. A new triterpene glycoside from *Terminalia arjuna*. Planta Med., 61: 576-577.

Takahashi, S., H. Tanaka, Y. Hano, K. Ito, T. Nomura and K. Shigenobu, 1997. Hypotensive effect in rats of hydrophilic extract from *Terminalia arjuna* containing tannin related compounds. Phytother. Res., 11: 424-427.

Tripathi, V.K., V.B. PAndey, K.N. Udupa and G. Rucker, 1992. Arjunolitin, a triterpene glycoside from *Terminalia arjuna*. Phytochemistry, 31: 349-351.

Upadhyay, R.K., M.B. Pandey, R.N. Jha, V.P. Singh and V.B. Pandey, 2001. Triterpene glycoside from *Terminalia arjuna*. J. Asian Nat. Prod. Res., 3: 207-212.

Wang, W., Z. Ali, X.C. Li, Y. Shen and I.A. Khan, 2010a. 18, 19-secooleanane type triterpene glycosyl esters from the bark of *Terminalia arjuna*. Planta Med., 76: 903-308.

Wang, W., Z. Ali, X.C. Li, Y. Shen and I.A. Khan, 2010b. Triterpenoids from two *Terminalia* species. Planta Med., 76: 1751-1754.

Wang, W., Z. Ali, X.C. Li, Y. Shen and I.A. Khan, 2010c. Ursane triterpenoids from the bark of *Terminalia arjuna*. Fitoterapia, 81: 480-484.

Yadav, R.N. and K. Rathore, 2001. A new cardenolide from the roots of *Terminalia arjuna*. Fitoterapia, 72: 459-461.

Yadava, R.N. and K. Rathore, 2000. A new cardenolide from the seeds of *Terminalia arjuna* (W and A). J. Asian Nat. Prod. Res., 2: 97-101.