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RESEARCH ARTICLE

IN VITRO MULTIPLICATION OF *Pithecellobium dulce* BENTH. - AN IMPORTANT ETHNO-MEDICINAL PLANT

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ABSTRACT

A micropropagation method is described for *Pithecellobium dulce* Benth. using nodal explants. Multiple shoots were induced from axillary buds on Murashige and Skoog's (MS) agar-gelled medium containing different concentrations of 6-Benzylaminopurine (BAP) and Kinetin (Kn). Medium containing 3.0 mgl^{-1} BAP was the most effective for shoot induction .The cultures were amplified by passages on MS medium supplemented with 1.5 mgl^{-1} each of BAP and Kn +0.5 mgl^{-1} Indole-3 acetic acid (IAA). The micropropagated shoots were subsequently transferred for root formation on half-strength MS medium + 2.5 mgl^{-1} Indole-3 butyric acid (IBA) with 94% success rate. These plantlets were hardened in the greenhouse. The established protocol is suitable for true to type cloning of mature plants of *P. dulce*.

INTRODUCTION

Since ancient times, mankind has been dependent on plants for food, flavors, medicinal and many other uses. Ancient written records of many civilizations give strong evidence regarding use of medicinal plant (Patwardhan *et al*., 2005; Micke *et al*., 2009). The World Health Organization (WHO) reported that 80% of people in the developing world use medicinal plants for their primary health care (Vines, 2004). The use of herbal medicines is growing in developed countries, presently 25% of the UK population use herbal medicine (Zhou *et al*., 2006). About 40% of compounds used in pharmaceutical industry are directly or indirectly derived from plants (Rout *et al*., 2000) because chemical synthesis of such compounds is either not possible and/or economically not viable (Oksman and Inzé, 2004). Therefore a large number of plant species (especially medicinal) are under threat of extinction because of their over exploitation (Edwards, 2004). About 10 species of genus *Pithecellobium*, (*P. clypearia, P. dulce, P. monadelphum, P. globosum, P. unguiscati, P. arboreum, P. flexicaule, P. jiringa, P. parviflorum and P. mart*) commonly occur in India. *Pithecellobium dulce* Benth. (Leguminosae), commonly referred as manila tamarind, as its sour taste resembles tamarind (Sugumaran *et al*., 2007). It is a small to medium sized, evergreen, spiny tree up to 18 meters height, native of tropical America and available throughout the plains of India and in the Andamans. It is known as Jangal Jalibi in Hindi and Kodukkapuli in Tamil. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and it is also useful in dermatitis and eye inflammation. The leaves have been reported to possess astringent, emollient, anticonvulsant, antiulcer, abortifacient and antidiabetic properties (Sivakumar and Murugesan, 2005). *P. dulce* leaves are rich source of calcium and ascorbic acid, which are effective against fluoride toxicity in the Thar Desert of India (Sheikh, 2011; Choubisa *et al*., 2011). Ethanolic and aqueous leaf extract of *P. dulce* was studied by Sugumaran *et al*., (2009) for its antidiabetic activity. Prasenjit *et al*., (2011) investigated the beneficial role of the aqueous extract of the fruits of *P. dulce* (AEPD) against carbon tetrachloride (CCl_4)-induced hepatic injury and found it highly useful. A steroid saponin, lipids, phospholipids, glycosides, glycolipids and polysaccharides have been reported from the seeds (Nigam *et al*., 1997). The bark contains 37% of tannins of catechol type. Quericitin, kaempferol, dulcitol and afezilin have been reported from the leaves. Roots have been reported to possess estrogenic activity (Saxena and Singhal, 1998). Alkylated resins have been isolated from seed oil of this plant by Banarjee (2005). *P. dulce* pods are a rich source of vitamin C and can be considered as a functional food (*Nilakshi et al.,* 2011).

It is evident that *P. dulce* plant has great potentials in treating a number of ailments where the free radicals have been reported to be the major factors contributing to the disorders (Sivakumar and Murugesan, 2005; Rajasab and Isaq, 2004). Sugumaran *et al*., (2008) evaluated ethano-pharmacological properties of *P. dulce*, they studied the *in vitro* antioxidant activity of ethanolic and aqueous leaf extract of *P. dulce.* The saponin obtained from fruits has been studied against the exudative and proliferative phase of inflammatory reaction (Bhargva *et al*., 1970). In view of the importance of saponins as possible spermicidal agents, the saponins of *P. dulce* were also subjected to tests for spermicidal property. The saponin showed the activity in the dilution of 0.03% against human

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semen (Misra *et al*., 1979). Polyphenols from the aqueous extract of *P. dulce* was tested for their inhibitory activities against *Naja kaouthia* venom. The extract could completely inhibit the lethality of the venom (Pithayanukul *et al*., 2005). The powder, methanolic and aqueous extracts of *P. dulce* seeds have proved fungistatic and possess fungicidal effects against plant pathogens (Gomathi *et al*., 2011)**.** Several triterpene saponins, pitheduloside inhibited *in vitro* mycelial growth of *Rhizopus stolonifer* and *colletotrichum gloeosporioides*, respectively. The less-polar hexane extract and polar methanolic extract of this plant screened against various bacteria and fungi were also found to be active (Bautista *et al*., 2003; Ali *et al*., 2001). The alcoholic extract showed highest activity when comparable with standard drugs like, streptomycin, isoniazid, rifampicin, ethambutol and pyrazinamide (Shanmuga *et al*., 2005, 2006). The potency of alcoholic and aqueous extracts of leaf was compared with that of chlorpromazine. Sugumaran *et al*., (2008) also studied the aqueous and alcoholic extract of *Pithecellobium dulce* leaves for radical scavenging activity. Cerdas *et al*., (1997) developed *in vitro* propagation protocol of *P. saman* (Rain tree) from hypocotyl explants on Murashige and Skoog (1962). Saxena and Gill (1987) regenerated plants from mesophyll protoplasts of the *P. dulce*. Since no literary evidence is available for *in vitro* propagation of *P. dulce* from mature tissues, the main objective of the present investigation is to develop a highly reproducible micropropagation protocol for providing continuous supply of a better source of elite plant to be used as standard material in pharmaceutical industries.

MATERIALS AND METHODS

Collection, surface sterilization, and culture of nodal explants

Field surveys were conducted for identification and selection of mature and superior trees of *P. dulce* along with the East Coast of South India. These include various sites in Tamil Nadu and Pondicherry. The trees were selected on the basis of height, biomass production, number, size and the test of the pods. The fresh shoot sprouts were collected from the field-grown, mature plants and 2 - 3 cm long shoots each with 1 - 2 nodes were used as explants. These explants were surface sterilized with 90% ethanol for 30 - 40 seconds followed by 0.1% (w/v) $HgCl_2$ (disinfectant, Hi-Media, India) for 4 - 5 min and rinsed 6 - 8 times with sterile water. These surface sterilized explants were placed on MS medium (Murashige and Skoog, 1962) with the combinations of cytokinins (BAP and Kn ranging from 0.1 to 5.0 mgl^{-1}) and auxin (IAA ranging from 0.1 to 1.0 mgl^{-1}). The medium was solidified with analytical grade agar (0.8%) (Hi-Media, India). The pH of the medium was adjusted to 5.8 ± 0.02 using 1 N NaOH or 0.1 N HCl prior to autoclaving for 15 min at 15 psi and 121°C. These cultures were incubated in the culture room at 25 ± 2°C, 70% relative humidity (RH) under light intensity of 40 µmol m^{-2} s^{-1} Photosynthetic Photon Flux Density (PPFD) for 14 h per day photoperiod.

Multiplication of the cultures

The *in vitro* regenerated shoots were multiplied by repeated transfer of mother explants and sub culturing of *in vitro* produced shoots on fresh medium. The shoot-clumps were subcultured in the culture flasks. For multiplication of cultures, MS medium supplemented with various concentration and combination of cytokinins (BAP and Kn 0.5 to 3.0 mgl^{-1} and IAA/IBA ranging from 0.1 to 2.0 mgl^{-1}) were used.

Rooting of *in vitro*-produced shoots

At the time of transfer of the shoot clumps on the fresh medium, the elongated (3 - 4 cm long) and thicker shoots were excised carefully and cultured on full, half, and one-fourth strengths of MS media for induction of roots with the incorporation of 200 mgl^{-1} of activated charcoal and auxins (IBA and IAA ranging from 0.5 - 5.0 mgl^{-1}). These cultures were maintained under diffused light (15 µmol m^{-2} s^{-1} PPFD).

Hardening and pot transfer of *in vitro*-regenerated plantlets

The rooted plantlets were taken out from the culture vessels, washed thoroughly with sterile water in order to remove the adhered nutrient agar, and potted in bottles containing soilrite moistened with one-fourth strength of the MS salts. *In vitro*- and *ex vitro*-rooted shoots were kept in covered bottles under 60 - 70% humidity in the greenhouse for 25 - 35 days. Subsequently, these plantlets were exposed to *ex vitro* conditions by gradual loosening and finally removing the caps of bottles. When the plantlets became hard, these were transferred to pots containing a mixture of soil and organic manure.

Observation and data analysis

The cultures were regularly sub cultured on fresh medium after 4-5 weeks interval. The observations were taken after every five days of inoculation. The experiments were repeated thrice with ten replicates per treatment. The rate of multiplication represents number of shoots produce per explant on a specific medium after number of days of its inoculation as mentioned in the results. The data were subjected to statistical analysis.

RESULTS

Establishment of the cultures of *P. dulce*

The different types of explants of *P. dulce* exhibited variable responses on the culture media. The axillary shoot apices responded quickly in cultures. From each node 3 to 5 shoots were differentiated within 7 to 10 days on different concentrations of BAP and Kn on MS medium. Maximum number of explants responded on BAP 3.0 mgl^{-1} where 96% explants regenerated shoots from the nodes (Table 1). The effect of concentrations of Kn on response of explants and shoot induction from nodal explants was not impressive (Table 2). Four to five shoots regenerated from each node on MS medium supplemented with 3.0 mgl^{-1} BAP (Fig. 1 A-C). Incorporation of auxin (IAA) in the medium was also not found very impressive in the induction of shoots. Again the MS medium supplemented with BAP + Kn also not impressive as far as the number of shoots regeneration is concern. The explants harvested during November-December responded earlier in the culture (within 10 days) while the explants collected in April-May (responded after15 to 20 days).

Table 1: Effects of BAP on induction of number of shoots in *P. dulce* on MS medium

BAP Concentrations (mgl^{-1})	% of Response	Number of Shoots / Explant ± SD
Control (0.0)	19	0.0 ± 0.00
0.5	48	1.2 ± 0.45
1.0	59	1.6 ± 0.56
1.5	76	2.2 ± 0.78
2.0	82	3.8 ± 0.23
2.5	89	4.2 ± 0.89
3.0	96	4.6 ± 0.78
3.5	82	3.6 ± 0.34
4.0	68	2.2 ± 0.89
4.5	42	1.9 ± 0.45
5.0	36	1.2 ± 0.29

Note: The observations were taken after every twenty days of inoculation. The experiments were repeated trice with ten replicates per treatment.

Table 2: Effects of cytokinin (Kn) on induction of number of shoots from explants of *P. dulce* on MS medium

Kn Concentrations (mgl^{-1})	% of Response	Number of Shoots / Explant ± SD
Control (0.0)	21	0.0 ± 0.00
0.5	28	1.1 ± 0.60
1.0	40	1.5 ± 0.65
1.5	52	2.7 ± 0.87
2.0	69	4.2 ± 0.43
2.5	83	3.5 ± 0.12
3.0	95	3.6 ± 0.89
3.5	78	2.5 ± 0.87
4.0	63	2.2 ± 0.40
4.5	47	2.0 ± 0.27
5.0	29	1.8 ± 0.97

Note: The observations were taken after every twenty days of inoculation. The experiments were repeated trice with ten replicates per treatment

Table 3: Effects of Cytokinins (BAP + Kn) concentrations on multiplication of shoots from *in vitro* regenerated shoots on MS Medium containing 0.5 mgl^{-1} IAA.

BAP + Kn Concentrations (mgl^{-1})	Shoot Numbers ± SD	Shoot Length (cm) ± SD
0.1	6.6 ± 0.69	2.4 ± 0.69
0.5	8.7 ± 0.45	2.8 ± 0.34
1.0	9.2 ± 0.23	2.9 ± 0.67
1.5	10.2 ± 0.59	3.6 ± 0.23
2.0	9.6 ± 0.67	3.3 ± 0.55
2.5	7.4 ± 0.31	2.9 ± 0.33
3.0	6.1 ± 0.56	2.5 ± 0.58

Note: The observations were taken after every twenty days of inoculation. The experiments were repeated trice with ten replicates per treatment

Multiplication of the cultures

The shoots were multiplied by following methods, (i) Sub culturing of the *in vitro* produced shoots, and (ii) Repeated transfer of shoot clumps. The *in vitro* regenerated shoots were sub-cultured for further multiplication on fresh medium. It was observed that cytokinin requirement for sub culturing of shoots was low. If the cultures were multiplied in shoot initiation medium they exhibited 'hyperhydricity'. Comparatively low concentration of BAP (1.5 mgl^{-1}) promoted the shoots multiplication and also minimized the hyperhydricity. Sub culturing was essential within 20 to 25 days. The rate of multiplication of shoots through sub culturing of *in vitro* produced shoots was relatively low and 7 to 8 shoots were produced in each culture flask. In order to enhance the rate of multiplication shoot clumps were repeatedly transferred. This proved to be the most effective method of production and amplification of healthy shoots in cultures. After each repeated transfer 10 to 11 shoots were produced in a flask within 20 to 25 days. The cultures were maintained on MS medium + 0.5 mgl^{-1} IAA + 1.5 mgl^{-1} each BAP and Kn at 25±2° C under 30 µmol m^{-2} s^{-1} PPFP (Fig. 1 D & E). The effect of concentrations of BAP and Kn on shoot multiplication is shown in Table **3**. If sub culturing was delayed beyond 4 weeks the shoots of clumps turned yellow and subsequently became brown. This proved to be detrimental for the cultures. It was therefore, necessary to subculture/transfer the shoot clumps on the fresh medium after 25 days in order to maintain the sustained growth of the shoots and rate of multiplication.

Table 4: Effects of Auxin (IBA) on *in vitro* regeneration of root numbers and length from *in vitro* generated shoots on half strength MS medium

IBA Concentrations (mgl^{-1})	% of Response	Number of Roots ± SD	Length of Roots (cm) ± SD
0.5	34	1.2 ± 0.57	1.3 ± 0.37
1.0	44	2.4 ± 0.86	1.7 ± 0.23
1.5	66	2.7 ± 0.25	1.9 ± 0.67
2.0	82	3.8 ± 0.21	2.1 ± 0.14
2.5	94	4.3 ± 0.47	2.2 ± 0.78
3.0	88	3.3 ± 0.23	2.2 ± 0.46
4.0	77	3.2 ± 0.78	1.9 ± 0.23
5.0	69	2.3 ± 0.98	1.5 ± 0.70

Note: The observations were taken after every twenty days of inoculation. The experiments were repeated trice with ten replicates per treatment.

Fig. 1. A-C: Induction of shoots from nodal shoot segments cultured on MS medium
D-E: Multiplication of *P. dulce* cultures *in vitro*.
F: Rooted shoot of *P. dulce* on MS medium + IBA.
G: Pot transferred plantlet in green house for hardening.

Multiplication of the cultures

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Rooting of micropropagated shoots

Rooting of shoots was achieved by *in vitro* methods only. The *in vitro* generated shoots of *P. dulce* rooted on MS Medium. However, the most suitable medium for root induction was found to be half strength MS medium + 2.5 mgl^{-1} IBA. On this medium about 94 % of the shoots rooted (Table 4). The root initiation was visible on 10^{th} day of inoculation (Fig. 1 F). Whereas 56 % of the shoots were rooted on IAA containing medium. The shoots rooted the best at 26±2°C temperature.

Hardening and pot transfer

The hardening of the plants was achieved within 25- 30 days in the bottles containing soil mixture (sand, soilrite, organic manure and black soil in 1:1:1:1 ratio) moistened with one-fourth strength of MS basal salts. The hardened plants were acclimatized in polybags and later on transferred to pots. 91 % success was achieved in the hardening of plants (Fig. 1 G). To achieve 100 % field survival only hardy shoots were harvested for rooting and low moisture was maintained in the bottles by removing the caps for 2-4 hours in the day time.

DISCUSSION

Numerous medicinal plants and their formulations are used by mankind in ethno-medical practice as well as traditional system of medicine in India (Manna *et al*., 2006, 2007). Plant products play a beneficial role in the management of various disorders (Zhao *et al*., 2009; Lee *et al*., 2009; Kaur *et al*., 2006; Bhattacharjee and Sil, 2007). In the absence of a reliable liver protective drug in the modern medicine, a number of medicinal preparations in ayurveda are recommended for the treatment of liver disorders. *Pithecellobium dulce* is a well-known Indian medicinal plant. It has been commonly used for fencing and tanning, as fodder for feed and pods for food. Infusions of different parts of *P. dulce* have been traditionally used to treat diseases (Touré *et al*., 1998).

In vitro propagation of medicinal and horticultural plants plays a significant role in drug industry. Explant is the material used as initial source of tissue culture. Tissue culture success mainly depends on the age, types and position of explants (Gamborg *et al*., 1976) because not all plant cells have the same ability to express totipotency. In nature the trees/plants experience various kinds of stresses induced by very severe winters and high temperatures and high wind velocities. It seems that the tissues of adult plants accumulate metabolites during seasonal cycle of the year. These prevent bud breaking in culture as such the response of the explants taken from non-pruned/unlopped trees does not respond in culture. (Sasikumar *et al*., 2009). Explants harvested during November-December found most suitable as compared to remaining months of the year for shoot regeneration in *P. dulce* in present study. Growth regulators regulate various physiological and morphological processes in plants (Srivastava, 2002). PGRs are synthesized by plants; therefore many plant species can grow successfully without external medium supplements (Bhavisha and Jasrai, 2003; Baksha *et al*., 2005). Hormones can also be added into cultures to improve plant growth and to enhance metabolite synthesis (Rayns *et al*., 1993). As observed in woody trees, *in vitro* growth and shoot formation was not achieved without adequate concentrations of exogenous hormones (Sasikumar *et al*., 2009). However, inadequate or excessive amount of growth hormones can cause morphological and physiological abnormalities (Bouza *et al*., 1994). As callus formation was observed in *P. dulce* when higher concentration of IAA added in the culture medium. Several re-juvenilization methods for rejuvenation of mature woody trees have been suggested and discussed by Rathore *et al*., (1992) and Deora and Shekhawat (1995). Sanchez *et al*., (1997) suggested many invigoration treatments for the micropropagation of mature chestnut trees. John *et al*., (1997) suggested cold treatment at 15°C for 72 hours for bud breaking to occur after 30-35 days on MS + BAP + Kn + biotin + calcium pantothenate in woody trees. No such treatment was required during our study in this system. For successful plant regeneration by tissue culture first shoots are formed by culture of explants or callus on media containing growth hormones (mainly cytokinins but sometimes auxins also). The best shoot induction was observed in *P. dulce* on MS medium supplemented with 3.0 mgl^{-1} BAP. Basal medium containing 4.0 mgl^{-1} BAP and 1.0 mgl^{-1} of IAA, increased the number and growth rate of multiple shoots in *Aloe barbadensis* (Ujjwala, 2007; Baksha *et al*., 2005). While higher rates of shoot proliferation were also reported in *Momordica dioica* on MS medium containing 0.8 mgl^{-1} of IAA and 2.0 mgl^{-1} of BAP (Shekhawat *et al*., 2011). In case of *Salvadora persica,* MS medium containing 0.5 mgl^{-1} BAP, 0.5 mgl^{-1} Kn with 0.2 mgl^{-1} NAA showed rapid shoot multiplication (Phulwaria *et al*., 2011). MS medium containing 1.0 mgl^{-1} BAP with 0.2 mgl^{-1} IAA reported the best for growth and number of shoots in *Leptadenia reticulata* (Rathore *et al*., 2010). After producing healthy shoots, plantlets transferred to different media for root formation.

Auxins are mainly used in root induction and their effect varies with type and concentration used in different plant species (Swamy *et al*., 2002). The excised shoots could be rooted by *in vitro* on half-strength MS medium + 3.0 mgl^{-1} IBA. About 94% of the shoots could be rooted. McClelland *et al*., (1990) investigated the effect of *in vitro* and *ex vitro* root initiation on subsequent microcutting root quality in woody plants *Acer rubrum* and *Betula nigra*. These were distinguished by several important anatomical and morphological traits that continued to regulate both root system and whole plant quality in later stages of production. During hardening about 85-90% of the plants survived. These could be pot transferred. Cerdas *et al*., (1997) developed *in vitro* propagation protocol of *P. saman* (Rain tree) from hypocotyl explants on Murashige and Skoog medium supplemented with BA. The best results were obtained with explants cultured on half-strength MS medium containing 26.6 µM BA, with explants of 5 and 10 days after germination placed horizontally and a 7 day exposure period to BA. Rooting percentages decreased when shoots were exposed for more than 24 hours to indole-3-butyric acid (IBA). Maximum rooting was obtained with 369.0 µM IBA. This indicates the superiority of IBA for root induction. Near about same types of results were also obtained in this investigation. It was first attempt to develop an efficient tissue culture protocol for regeneration of *P. dulce* in cultures from mature tissues. Now, it has been possible to generate clones of *P. dulce* through tissue culture. The process defined is highly reproducible, efficient and can be following for cloning of selected and mature genotypes of *P. dulce* selected for coastal area of South India.

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REFERENCES

Ali M.S., Azhar I., Ahmad F., Ahmad A.U. and Smanghani K.U. (2001) Antimicrobial screening of Mimosaceous plants. *Pharmaceut. Biol.,* 39(1): 43-46.

Baksha R., Jahan M.A.A., Khatum R. and Munshi J.L. (2005) Micropropagation of *Aloe barbadensis* Mill. through *in vitro* culture of shoot tip explants. *Plant tiss. Cult. Biotechnol.,* 15: 121-126.

Banarjee A. (2005) Studies on alkylated resin from Pithecellobium dulce. *J. Indian Chem. Soc.,* 82: 186-187.

Bautista B.S., Garcia D.E., Barrera N.L., Reys C.R. and Wilson C.L. (2003) Seasonal evaluation of the postharvest fungicidal activity of powders and extracts of huamuchil (*Pithecellobium dulce*): Action against *Botrytis cinerea*, *Penicillium digitatum* and *Rhizopus stolonifer* of straw berry fruit. *Postharvest Biol. Technol.,* 29: 81-92.

Bhargva P.K., Gupta M.B. and Chittranjan R. (1970) Anti inflammatory activity of Saponins and other Natural Products. *Indian J. Med. Res.*, 58: 724.

Bhattacharjee R. and Sil P.C. (2007) Protein isolate from the herb, *Phyllanthus niruri* L. (Euphorbiaceae), plays hepatoprotective role against carbon tetrachloride induced liver damage via its antioxidant properties," *Food Chemical. Toxicol.,* 45(5): 817–826.

Bhavisha B.W. and Jasrai Y.T. (2003) Micropropagation of an Endangered Medicinal Plant: *Curculigo orchioides* Gaertn. *Plant tiss. Cult.,* 13: 13-19.

Bouza L., Jacques M. and Miginiac E. (1994) *In vitro* propagation of *Paeonia suffruticosa* Andr. cv. `Mme de Vatry': developmental effects of exogenous hormones during the multiplication phase. *Scientia Horticulturae*, 57: 241-251.

Cerdas L.V., Dufour M. and Villalobos V.M. (1997) *In vitro* propagation of *Pithecellobium saman* (Raintree). *In Vitro* Cell. *Dev. Biol. Plant*, 33 (1):38-42.

Choubisa S.L., Mishra G.V., Sheikh Z., Bhardwaj B., Mali P., and Jarolia V.J. (2011) Food, fluoride, and fluorosis in domestic ruminants in the Dungarpur district of Rajasthan, India. Fluoride, 44(2): 70-76.

Deora N.S. and Shekhawat N.S. (1995) Micropropagation of *Capparis decidua* (Forsk.) Edgew - a tree of arid horticulture. *Plant Cell Rep.,* 15: 278-281.

Edwards R. (2004) No remedy in sight for herbal ransack. New Scientist, 181: 10-11.

Gamborg O.L., Murashige T., Thorpe T.A. and Vasil I.K. (1976) Plant-tissue culture media. *J. Tiss. Cult. Assoc.,* 12: 473-478.

Gomathi S., Ambikapathy V. and Pannerselvam A. (2011) Antimicrobial activity of some medicinal plants against *Pythium dearyanum* (Hesse). *J. Microbiol Biotech. Res.,* 1(2):8-13.

John C.K., Nadgauda R.S. and Mascarenhas A.F. (1997) Tissue culture of economic plants. CS & T of the non-aligned and other developing countries, New Delhi & CSC, London, pp. 82-107.

Kaur G., Jabbar Z., Athar M. and Alam M.S. (2006) *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. *Food Chemical Toxicol.,* 44(7): 984–993.

Lee J.R., Park S.J. and Lee H.S. (2009) Hepatoprotective activity of licorice water extract against Cadmium-induced toxicity in rats," *Evidence-Based Complementary and Alternative Medicine*, 6(2): 195–201.

Manna P., Sinha M. and Sil P.C. (2006) Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. *BMC Complementary and Alternative Medicine*, 6, Article ID 33.

Manna P., Sinha M. and Sil P.C. (2007) Galactos amine-induced hepatotoxic effect and hepatoprotective role of a protein isolated from the herb *Cajanus indicus* L. *in vivo*. *J. Biochem. Molecul. Toxicol.,* 21: 13–23.

McClelland M.T., Smith M.A.L. and Carothers Z.B. (1990) The effects of *in vitro* and *ex vitro* root initiation on subsequent microcutting root quality in three woody plants. *Plant Cell Tiss. Org. Cult*., 23: 115-123.

Micke O., Hubner J. and Munstedt K. (2009) Ayurveda. *Der Onkologe,* 15: 792-798.

Misra G., Nigam S.K., Singh S., Nigam S.G. and Saxena R.C. (1979) Steroid and Triterepenoid Saponins as Spermicidal agents. *Indian Drugs,* 17(1): 6-8.

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

Nigam S.K., Misra G., Uddin R., Yoshikawa K., Kawamoto M. and Arihara S. (1997) Pithedulosides A-G, Oleanane

glycosides from *Pithecellobium dulce*. *Phytochem.,* 44 (7): 1329-1334.

Nilakshi V. G. and Bhaskar V.V. (2011) HPTLC analysis of vitamin c from *Pithecellobium dulce*, Benth (Fabaceae). *J. Pharmacy Res.,* 4 (4): 41-47.

Oksman C.K.M. and Inzé D.(2004) Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites. *Trends in Plant Sci.,* 9: 433-440.

Patwardhan B., Warude D., Pushpangadan P. and Bhatt N. (2005) Ayurveda and traditional Chinese medicine: A comparative overview. *Evidence-Based Complementary and Alternative Medicine,* 2: 465-473.

Phulwaria M., Ram K., Gahlot P., and Shekhawat N.S. (2011) Micropropagation of *Salvadora persica*-a tree of arid hortculture and forestry. New Forest DOI 10.1007/s11056-011-9254-z.

Pithayanukul P., Ruenraroengsak P., Bavovada R., Pakmanee N., Suttisri R. and Saenoon S. (2005) Inhibition of *Naja kaouthia* venom activities by plant polyphenols. *J. Ethano Pharmacol.*, 97(3): 527-533.

Prasenjit M., Bhattacharyya S., Das J., Ghosh J. and Sil P.C. (2011). Phytomedicinal Role of *Pithecellobium dulce* against CCl4-mediated Hepatic Oxidative Impairments and Necrotic Cell Death. Evidence-Based Complementary and Alternative Medicine. doi:10.1093/ecam/neq065. Article ID 832805.

Rajasab A.H. and Isaq M. (2004) Documentation of folk knowledge on edible wild plants of north Karnataka. *Indian J. Tradit. Knowl.*, 4: 419-429.

Rathore M.S., Shekhawat N.S. (2010) *Ex vivo* implications of phytohormones on various in vitro responses in *Leptadenia reticulata* (Retz.) Wight. & Arn. an endangered plant. Environ. Exp. Bot. (in press).

Rathore T.S., Deora N.S. and Shekhawat N.S. (1992) Cloning of *Maytenus emarginata* (Willd.) Ding Hou- a tree of the Indian Desert, through tissue culture. *Plant Cell Rep.,* 11: 449-451.

Rayns F.W., Fowler M.R. and Hunter C.F. (1993) Media design and use. *In Vitro* Cultivation of Plant Cells, Butterworth-Heinemann Ltd. Oxford, pp 43-64.

Rout G.R., Samantaray S. and Das P. (2000) *In vitro* manipulation and propagation of medicinal plants. *Biotech Advances*, 18: 91-120.

Sanchez M.C., Ballester E.A. and Vieitez A.M. (1997) Reinvigoration treatments for the micropropagation of mature chestnut trees. *Ann. Sci. For.,* 54: 359-370.

Sasikumar S., Raveendar S., Premkumar A., Ignacimuthu S. and Agastian P. (2009) Micropropagation of *Baliospermum montanum* (Willd.) Muell. Ara.-A threatened medicinal plant. *Ind J. Biotechnol.,* 8: 223-226.

Saxena P.K. and Gill R. (1987) Plant regeneration from mesophyll protoplasts of the tree legume *Pithecellobium dulce* Benth. *Plant Sci.,* 53(3): 257-262.

Saxena V.K. and Singhal M. (1998) Novel prenylated flavonoid from stem of *Pithecellobium dulce*. *Fitoterapia*, 70(1): 98-100.

Shanmuga K.S.D., Amerjothy S. and Balakrishna K. (2006) Pharmacognostical, Antibacterial and Antifungal Potentials of the Leaf extracts of *Pithecellobium dulce* Benth. *Phcog. Mag.,* 7(2): 163-167.

Shanmuga K.S.D., Amerjothy S., Balakrishna K. and Kumar M.S.V. (2005) Antimycobacterial properties of leaf extracts of Pithecellobium dulce Benth. *Indian Drugs,* 42(6): 392-395.

Sheikh Z. (2011) Prevention of fluorosis in domestic animals. *Curr Sci.,* 101 (9): 1124-1125.

Shekhawat M.S., Shekhawat N.S., Harish, Kheta R, Phulwaria M., Gupta and Amit K. (2011) High frequency plantlet regeneration from nodal segment culture of female *Momordica dioica* (Roxb.) *J Crop Sci Biotech.,* 14(2): 133-137.

Sivakumar A. and Murugesan M. (2005) Ethnobotanical studies on the wild edible plants used by the tribals of Anaimalai hills, the western ghats. *Ancient Sci. Life*, XXX (2): 69-73.

Srivastava L.M. (2002) Plant Growth and Development: Hormones and Environment Academic Press, New York, pp 140-143.

Sugumaran M., Vetrichelvan T. and Quine S.D. (2009) Antidiabetic potential of aqueous and alcoholic leaf extracts of *Pithecellobium dulce*. *Asian J. Research Chem.,* 2(1): 83-87.

Sugumaran M., Vetrichelvan T., and Quine S.D. (2008) Locomotor Activity of Leaf extracts of *Pithecellobium dulce* Benth. *Ethnobotanical Leaflets,* 12: 490-493.

Swamy S.L., Puri S. and Singh A.K. (2002) Effects of auxins (IBA and NAA) and seasons on rooting of juvenile and mature hardwood cuttings of *Robinia pseudoacaia* and *Grewia optiva*. *New Forest,* 23: 143-157.

Touré S.F., Michalet B.D., Traoré, E., Friot D. and Richard D. (1998) Occurrence of digestive interactions in tree forage-based diets for sheep. *Animal Feed Sci. Technol.,* 74(1): 63–78.

Ujjwala J.S. (2007) *In vitro* Regeneration of *Aloe barbadensis. Biotech.,* 6: 601-603.

Vines G. (2004) Herbal Harvest with future: towards sustainable source for medicinal plants. Plant life International, www.plantlife.co.uk.

Zhao J., Liu T. and Ma L. (2009) Antioxidant and preventive effects of extract from *Nymphaea candida* flower on *in vitro* immunological liver injury of rat primary hepatocyte cultures. Evidence-Based Complementary and Alternative Medicine. (Epub ahead of print).

Zhou L.G. and Wu J.Y. (2006) Development and application of medicinal plant tissue cultures for production of drugs and herbal medicinals in China. *Natural Prod. Rep.,* 23: 789-810.

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