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Callus Induction, Anti Microbial Screening and in Vitro Plantlet Regeneration of Adansonia Digitata L.: Anendangered Medicinal Tree

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Abstract- *Adansonia digitata* L. (Bombacaceae) is a majestic tree revered in Africa for its immense medicinal and nutritional value. Commonly called as Baobab, the tree is stem succulent and is native to dry regions of tropical Africa, Australia and Madagascar. The tree can grow up to 25 metres in height, 28 metres in girth and may live for several hundred years. Being one of the largest and world's oldest tree, Baobab was introduced in India by Muslim traders almost 500 years ago. *Adansonia* has very limited distribution in India and in Madhya Pradesh. In India, it is commonly called as **'Kalpavriksha', 'Mandav imli', 'Khursani imli',** etc. The tree is known for its high economic value as well as numerous biological properties including anti-malarial, anti-pyretic, anti-oxidant etc. and almost every part of the tree is found to be useful for treatment of various diseases. At present, it faces a crisis of survival due to lack of knowledge and awareness on the need to protect and conserve it.

The present study was carried out to isolate important bioactive components using cell and suspension cultures, to determine antimicrobial activity and to standardize a protocol for *in vitro* propagation of this rare woody tree species.
Plant growth regulators like BAP, NAA, TDZ were used for callus induction and formation of somatic embryos. Various parts like leaf and fruit pulp were used and their extracts were prepared in methanol and aqueous solvents. The anti-bacterial activity was evaluated against *E. coli* and *Staphylococcus aureus.* Looking into its extremely thin distribution in the Indian sub-continent, tissue culture approaches have been initiated for its future conservation. An *in vitro* protocol was developed for shoot induction using seedling explants and BAP, Kinetin as growth regulators.

Results indicate that the leaves and fruit pulp show remarkable bioactivity against *Staphylococcus aureus*. These can be considered as good potential drug candidates as antibacterial agents. Maximum callus induction occurred in leaf explants with a combination of BAP (2 mg/l) and NAA (7 mg/l). The apical explants were found more responsive to kinetin as compared to BAP and more responsive in MS as compared to WPM medium for the induction of shoots. 70% shoot induction occurred with BAP (0.5 mg/l) and Kinetin (2 mg/l). Thus, a standard protocol was generated for shoot multiplication using tissue culture techniques for conservation of this medicinal tree. The conservation strategy under Indian sub-tropical states will help in studying its medicinal properties for further research on this multipurpose tree species.

Key words: *Adansonia digitata*, Baobab, medicinal, anti-bacterial, *Staphylococcus aureus,* callus, shoot induction, 6-BAP

INTRODUCTION

Adansonia digitata L. (Baobab) is a fascinating tree of African savannas and known for its enormous size and distinctive appearance. According to Wickens and Lowe (2008), Baobab is one of the eight species of the genus Adansonia, but it has until recently been considered the only native species on African mainland. The tree may reach upto 25 m in height and may live for hundreds of years. It is very important in the economy of rural and urban people for food, fodder, medicine, shelter and numerous other products (Gebauer 2003, De Caluwe *et al.,* 2010). *Adansonia* has high water holding capacity and its hollow trunk keeps the water potable for many years and serves as a reservoir during drought. It is also resistant to fire and survives well in dry climate. The leaves bark and fruit are employed as food stuffs and medicinal purposes. It has numerous health benefits which can be related to the presence of bioactive compounds like terpenoids, saponins, tannins etc. that are isolated from its various parts like leaves and fruits (sugandha*et al.,* 2013).

The tree has mythological significance and is known as 'kalpavriksha' in India and elsewhere. Various plant parts such as leaves, bark, and fruit pulp have been traditionally used as immuno-stimulant, anti-inflammatory, analgesic and in the treatment of fever, diarrhea, cough, dysentery, haemoptysis, tuberculosis, microbial infection and worms (Wickens & Lowe, 2008; Vermaak *et al.,* 2011). The tree is thus named as **"The small pharmacy or chemist tree".** The seeds and oil

extracts are also used as food, fuel, cosmetics, medicines and in the tropical treatment of muscle wounds, dandruff and other skin conditions (Chivandi, Davidson and Erlwanger, 2008; Kamatou *et al.,* 2011). Pulp extract is applied as eye-drops in cases of measles (FAO, 1988) whereas leaves form a component or herbal remedies.

In many areas, particularly in the tropical countries, traditional medicinal plants remain one of the main sources in prevention and treatment of varying ailments through self-medication (Mahomoodally*et al.*, 2012). Earlier studies reveal that aqueous stem bark and aqueous root bark extracts have significant anti-bacterial activity against *B.anthracis* and *B.subtulis* (Masola *et al.*, 2009). The results of phytochemical screening of ethanol and chloroform extracts of *A.digitata* using percolation and Sohxlet extraction method revealed the presence of flavonoids and steroids in the extracts (Yushao*et al.,* 2010). Callus formation is a desirable prerequisite for plant regeneration because callus offers the greatest opportunity for in *vitro* selection and production of genetic variations (Espinasse& Lay 1989, Bregitzer*et al.,* 1995, Chauhan & Kothari 2004, Šerhantová*et al.,* 2004, Nasircilara*et al.,* 2006). Generally, high concentration of auxins and low cytokinins in the medium promote abundant cell proliferation with the formation of callus (Shah *et al.,* 2003).

The present study was undertaken to induce callus for isolation of important bioactive components using cell and suspension cultures and establish an *in vitro* culture using tissue culture methods. Also, the antibacterial activity of *A. digitata* (leaf and fruit pulp) extracts was evaluated against *Staphylococcus aureus.*

MATERIAL AND METHODS

A] Collection and identification of plant material:

Different parts like leaves and fruits of *Adansonia digitata* were procured from the tree located in the centre of the Bhopal city. These were identified by the taxonomists at the Botanical survey of India, Regional Office, Pune. Accession number was assigned to *Adansonia digitata* as (SUSADDI).

B] Callus induction

i) Plant material

In order to preserve this genetic resource of great economic and medicinal value, studies on callus induction of this multipurpose tree species through tissue culture techniques is carried out. Different parts of $1^{1/2}$ month old *in vitro* grown seedlings of *Adansonia digitata* were used as explants i.e leaf segments, nodal segments, hypocotyl, epicotyl, apical,axillary meristems and root segments for callus induction. The above explants were aseptically cut from $1^{1/2}$ month old *in vitro* raised seedlings and inoculated in different media supplemented with different concentrations of plant growth regulators like 6-BAP(0.5 mg/l- 2 mg/l), NAA(0.5- 10.0 mg/l) and TDZ (1.0-1.5 mg/l).

ii) Callus culture establishment:

Sterile explants were cultured on WPM medium supplemented with different concentrations of BAP, NAA and TDZ (as mentioned above) either alone or in combinations for callus induction in light and dark. The inoculated explants were incubated in culture room at 25±2° C for standard 16/8 hrs photoperiod under fluorescent light. The calli were sub-cultured to a freshly prepared medium of same composition and pH=5.5-5.8 after every 30 days. Fresh weight and dry weight of calli was determined and high quality friable calli were transferred into MS liquid medium for suspension culture with different concentrations of NAA (0.1-5mg/l). Data was collected on percent callus induction and morphology of calli were observed. Each experiment was conducted in 10 replicates.

iii) In vitro culture establishment:a) Plant material

Apical and axillary shoots were collected from 5 year old seedlings. The shoots were surface sterilized by immersion in 4% NaOCl containing 0.01% Tween-20 for 15 min, followed by four 5 min washes with sterile distilled water. Shoot apices with 2-3 axillary buds were excised from terminal shoots and cultured on different media.

b) Media and culture conditions

The basal media tested were MS and WPM with different concentrations of BAP and Kinetin. Solidified nutrient medium was prepared using 0.22% phytagel and pH was adjusted to 5.8-6.0. Growth regulators were added before autoclaving and explants were cultured in 150×25 mm glass tubes and phytajars each containing 20 ml and 60 ml of nutrient medium respectively. Cultures were examined for 60 days at 25±2°C and a 16-h photoperiod with white light supplied by cool fluorescent tubes (80 µmol m^{-2} s^{-1}).

c) Shoot multiplication

Shoot apices (0.6-0.8 cm) were grown for 60 days on the test media supplemented with BAP (0.5-5 mg/l) alone or in combination with 0.5-5 mg/l kinetin. In another experiment, explants were maintained in the test media supplemented with 05-5 mg/l kinetin alone.

iii] Preparation of Extracts:

The leaves were washed in running water, cleaned and dried under shade for 20 days. Dried leaves were grinded into fine powdered samples. The fruit pulp was separated from the seeds manually. 7.5 grams of the powdered plant material was dispensed in 75 ml of methanol and water in separate conical flasks, kept for 24 hours with shaking at regular intervals. The content was filtered through Whatman#1 filter paper. The filtrate was evaporated at room temperature. The process was repeated thrice with each solvent.

Similarly, callus obtained from leaf explants was grinded into fine powder with the help of liquid nitrogen and its methanolic and aqueous extracts were prepared using the above procedure.

C] Phytochemical screening and Anti-microbial activity:

S.No	Name of Test	Phytochemicals
1.	To 0.1 ml of the extract and fractions in a test tube, 2-3 drops of Dragendorff's reagent was added. Orange red precipitate is formed.	Alkaloids (Ciulci, 1994).
2.	To 4 ml of the extract, a piece of magnesium ribbon is added followed by drop-wise addition of concentrated HCl. The color changes from orange to red.	Flavonoids(Sofaw ora, 1993).
3.	2 ml of the extract was diluted with distilled water in separate test tubes. 2-3 drops of 5% ferric chloride ($FeCl_3$) solution was added. A green- black or blue color is formed.	Tannins
4.	50 mg of the extract is diluted with distilled water and made upto 20 ml. This is shaken in a graduated cylinder for 15 min. A 2 cm layer of foam is formed.	Saponins
5.	5 ml of extract was mixed in 2 ml of chloroform. 3 ml of concentrated sulfuric acid was added to form a layer. A reddish brown coloration forms at interface.	Terpenoids (Salkowski test)
6.	5 ml of distilled water was added to the extract. The occurrence of turbidity showed the presence of resins.	Resins

d] Evaluation of Anti-bacterial activity:

Staphylococcus aureus (ATCC 25923) was used to screen the antibacterial activity of leafand callus extracts of *A.digitata.* Agar well diffusion method was employed and the bacterial strain was revived and adjusted for the turbidity of the inoculums suspension using 0.5 McFarland's technique. Mueller-Hinton agar plates were prepared and inoculated by spreading the swab over the surface of the agar plate. Wells were bored on each plate using a standard sterile cork borer of 6mm diameter. Equal volumes of plant extracts were transferred into the wells. Another well was filled with equal volume of ampicillen (1 mg/ml) which served as the standard drug. The plates were allowed for pre-diffusion for 30 minutes and incubated at 37°C. The experiment was carried out in triplicates and mean of zones of inhibition were counted.

RESULTS AND DISCUSSION

Auxins and cytokinins showed significant variation for callus induction from different explants. Callus induction was observed in almost all the explants used supplemented with different concentrations of 6-BAP, NAA and TDZ.The leaf and node segments were equally responsive for induction of callus and gave good results. All media supplemented with higher concentrations (above 1.0 mg/l) of BAP mostly showed callus formation in both light and dark conditions.Two superior plant growth regulator combinations that resulted in 90% of callus induction in leaf and node explants were 2.0 mg/l BAP and 10.0 mg/l NAA (Fig. 1A).. Superiority of BAP over other cytokinins has been demonstrated in other tree species such as *Simarouba glauca* (Rout and Das, 1995), *Simmondsiachinensis* (Chaturvedi and Sharma, 1989), Morus species (Islam *et al.,* 1993; Pattnaik and Chand, 1997). The best result in terms of percentage response of callus induction (90%) and nature of callus obtained on 6-BAP was found in case of nodal segment, hypocotyl and leaf segments, after 28-30 days of inoculation on WPM medium.(Table 2). Lower concentrations of TDZ generally tended to promote the formation of compact, green, nodular calli (Murthy *et al.,* 1998).

Production of calli from fragments of steams, leaves and roots are mainly carried out to determine the culture conditions required by the explants to survive and grow, study cell development, exploit products coming from primary and secondary metabolism and obtain cell suspension in propagation. It can also pave the way for

isolating economically valuable phytochemicals, which can avoid collecting plant materials from natural sources (Gita *et al.,* 2009;Berkov*et al.,* 2009).

The apical explants were found to be more responsive to kinetin as compared to BAP and also more responsive in MS as compared to WPM medium (Fig.2 A). It was found that the presence of BAP inhibited sprouting percentage and promoted callusing. Maximum sprouting and no. of shoots per explants was found highest with MS medium with 2mg/l kinetin. After 60 days of culture, sprouting percentage was 80%. Concentrations of BAP more than 2 mg/l induced extensive callusing and necrosis of explants (table 5). In tissue culture, cytokinins play a crucial role as promoters of cell division and act in the induction and development of meristematic centers leading to the formation of organs, mainly shoots (Peeters*et al.,* 1991). Kinetin has been used for micropropagation of many ornamental plants (Jain and Ochatt, 2010). *In vitro* shoot proliferation and multiplication are largely based on media formulations containing cytokinins as a major plant growth regulators (Mamidala and Nanna, 2009; Hoque, 2010). In the present study, kinetin was found to be more effective than BAP in shoot multiplication. The favourable effect of kinetin on shoot multiplication has also been reported by Vernosefadrani*et al.,* (2009) who observed in *Gerbera jamesoni* that shoot proliferation rate was maximum in medium containing 2 mg/l kinetin.

Preliminary phytochemical screening reveals the presence of Flavonoids, alkaloids, tannins in leaves while saponins are absent. Similarly, fruit pulp and callus show the presence of nearly all phytochemicals (table 3). The importance of alkaloids, saponins and tannins in various antibiotics used in treating common diseases has been reported by Kubmarawa (2007). Phytochemical tests are helpful in finding and locating chemical constituents which are source of pharmacologically active principles (Pandith, 2012). These compounds have significant application against human pathogens, including those that cause enteric infections and are reported to have curative properties against several pathogens and therefore could suggest their use in the treatment of various diseases (Hassan *et al.,* 2004).

The antibacterial activity of the leaf, fruit pulp and callus extracts of *A digitata* significant reduction in bacterial growth in terms of zone of inhibition. In the present study, maximum growth of inhibition (46 mm) was observed in methanol extract of leaves whereas leaf callus gave 42 mm zone against *S.aureus* (Fig. D). Similarly, in aqueous extracts leaf gave 30 mm zone and leaf callus gave 28 mm zone. The results showed that leaf, fruit and callus extracts possess bioactive compounds with antibacterial activity against many pathogens. It is suggested that the methanol extract reveals a scope to develop a novel broad spectrum of anti-microbial drug formulation and suspension culture can be used to carry out for the production of these bioactive components.

CONCLUSION

Conventional breeding techniques for woody trees are often difficult and slow because of high levels of heterozygosity and the long generation time between successive crosses as reviewed from time to time (Sriskandarajah*et al.,* 1994; Naik*et al.,* 1999; Naik and Chand, 2003; Singh *et al.,* 2007). The current study revealed the presence of many pharmacologically active constituents in *A.digitata* suggesting their strong potential in antimicrobial activity and treatment of diseases. The results obtained are very encouraging and will give impetus to conserve this medicinal tree using micropropagation and also for isolation and characterization of bioactive compounds present in its leaf and fruit pulp for formulation of herbal medicines.

REFERENCES

[1]. Berkov S, Pavlov A, Georgiev V, Bastida J, Burrus M, Ilieva M, et al. Alkaloid synthesis and accumulation in Leucojumaestivum in vitro cultures. Nat Prod Commun 2009; 4(3): 359-364.

[2]. Bregitzer, P., Campbell, R.D., Wu, Y. (1995): Plant regeneration from barley callus: effects of 2,4 dichlorophenoxyacetic acid and ohenylacetic acid. Plant Cell, Tissue and Organ Culture 43: 229-235.

[3]. Chaturvedi, H.C. and M. Sharma, 1989. *In vitro* production of cloned plants of jojoba (*Simmondsiachinensis* L.) through shoot proliferation in long term culture. Plant Sci., 63: 199-20.

[4]. Chauhan, M., Kothari, S.L. (2004): Optimization of nutrient levels in the medium increase the effeciency of callus induction and plant regeneration in recalcitrant indian barley (*Hordeum vulgare* L.) *in vitro. In Vitro* Cellular and Developmental Biology-Plant 40: 520-527.

[5]. Chivandi, E., Davidson, B. C., & Erlwanger, K. H. (2008). A comparison of the lipid and fatty profiles from the kernels of the fruit (nuts) of *Ximeniacaffra*and *Ricinodendronrautaneni*ifrom Zimbabwe. *Industrial Cropsand Products, 27*, 29-32. http://dx.doi.org/10.1016/j.indcrop.2007.06.002

[6]. Ciulci, I. (1994): Methodology for the analysis of vegetable drugs. Chemical industries branch,

Division of industrial operations. UNIDO, Romania: 24, 26 and 67.

[7]. De Caluwé E, Halamová K, Van Damme P (2010). *Adansonia digitata* L. – A review of traditional uses, phytochemistry and pharmacology. Afrika focus, 23: 11-51.

[8]. Espinasse, A., Lay, C. (1989): Shoot regeneration of callus derived from globular to torpedo embryos from 59 sunflower genotypes. Crop Science 29: 201-205.

[9]. FAO (1988). Traditional food plants. Food and Agriculture Organisation of the United Nations, Rome, 24: 63-67.

[10]. Gebauer J, El-Siddig K, Ebert G (2002). Baobab (*Adansonia digitata* L.): A review on a multipurpose tree with promising future in the Sudan. Gartenbauwissenschaft, 67: 155-160.

[11]. Gita S, Miyazaki J, Godo T, Kato Y. Possibility for selective accumulation of polyphenolics in tissue cultures of senno (LychnissennoSiebold et Zucc.). Nat Prod Commun 2009; 4(3): 377-380.

[12]. Hassan MM, Oyenwale AO, Abduallahi MS, Okonkwo EM. Preliminary phytochemical and antibacterial investigation of crude extract of the root bark of *Datariummicrocarpum*. J ChemSoc Nigeria. 2004; 29: 26-29.

[13]. Hoque ME (2010) In vitro tuberization in potato (Solanumtuberosum L.). Plant Omics J 3 (1): 7-11.

[14]. Jain SM, Ochatt SJ (2010) Protocols for in vitro propagation of ornamental plants. Springer protocols. Humana press.

Kamatou, G. P. P., Vermaak, I., &Viljoen, A. M. (2011). An updated review of *Adansonia digitata*: A commercially important African tree. *South African Journal of Botany, 77*, 908-919.http://dx.doi.org/10.1016/j.sajb.2011.08.010

[15]. Kubmarawa D, Khan ME, Punah AM & Hassan M. (2007). Phytochemical and Antimicrobial Screening of *Ficus platyphylla* against human/animal pathogens. *The Pacific Journal of Science and Technology* Vol. 10, pp. 382-386.

[16]. Mahomoodally, F., A. Mesaik, M.I. Choudhary, A.H.Subratty and A. Gurib-Fakim, 2012. *In vitro* modulation of oxidative burst via release of reactive oxygen species from immune cells by extracts of selected tropical medicinal herbs and food plants. Asian Pacific J. Tropical Med., 5: 440-447. DOI: 10.1016/S1995-7645(12)60075-3.

[17]. Mamidala P, Nanna RS (2009) Efficient in vitro plant regeneration, flowering and fruiting of dwarf tomato cv. Micro-Msk. Plant Omics J 2 (3): 98-102.

[18]. Masola, SN.,Mosha,RD., Wambura PN. 2009):Assessment of antimicrobial activity of crude extracts of stem and root barks from *Adansonia digitata* (Bombacaceae) (African Baobab). African Journal of Biotechnology., Vol.8(19), pp. 5076-5083.

[19]. Murthy BNS,Murch SJ & Saxena PK (1998). Thidiazuron: A potent regulator of in vitro plant morphogenesis. In Vitro Cell Dev. Pl 34: 267-275.

[20]. Naik S.K., Chand P.K. 2003. Silver nitrate and aminoethoxyvinylglycine promote *in vitro* adventitious shoot regeneration of pomegranate (*Punicagranatum*L.). *J. Plant*

[21]. Naik S.K., Pattnaik S., Chand P.K. 1999. *In vitro* propagation of pomegranate (*Punicagranatum*L. cv. Ganesh) through axillary shoot proliferation from nodal segments of mature tree. *Sci.Hort*. 79: 175-183.

[22]. Nasircilara, G., Kenan, T., Callan, F. (2006): Callus induction and plant regeneration from mature embryos of different wheat genotypes. Pakistan Journal of Botany 38(2): 637- 645.

[23]. Pattnaik, S. and P.K. Chand, 2000. Morphogenic response of the alginate encapsulated axillary buds from *in vitro* shoot cultures of six mulberries. Plant Cell Tissue Organ Cult., 64: 177-85.

[24]. Peeters AJM, Gerards W, Barendse GWM, Wullems (1991) *In vitro* flower bud formation in tobacco: interaction of hormones. Plant Physiol 97: 402-408. *Physiol.* 160(4): 423-430.

[25]. Rout. G.R., Das. P (1995). An in vitro study on organogenesis in *Tremaorientalis* (Blume)Linn. Plant Sci. 105: 87-94.

[26]. Šerhantová, V., Ehrenbergerová, J., Ohnoutková, L. (2004): Callus induction and regeneration efficiency of spring barley cultivars registered in the Czech Republic. Plant, Soil and Environment 50: 456-462.

[27]. Shah, M.I., Mussarat, J., Ihsan, I. (2003): *In vitro* callus induction, its proliferation and regeneration in seed explants of wheat (*Triticumaestivum*L.) VAR.LU-26S. PakistanJournal of Botany 35(2): 209-217.

[28]. Singh N.V., Singh S.K., Patel V.B. 2007. *In vitro* axillary shoot proliferation andclonal propagation of 'G-137'pomegranate (*Punicagranatum*). *Indian J. Agric.Sci*. 77(8): 505-508.

[29]. Sofowora, A. (1993): Medicinal plants and Traditional Medicines in Africa. Chichester John Wiley & Sons, New York Pp. 34-36.

[30]. Sriskandarajah S., Goodwin P.B., Speirs J. 1994. Genetic transformation of the apple scion cultivar

'Delicious' via *Agrobacterium tumefaciens*. *Plant Cell Tissue Organ Cult.* 36(3): 317-329.

[31]. Sugandha S, Parasharami,V, Shashi, R. (2013).Medicinal uses of *Adansonia digitata* L.: An edangered tree species.

[32]. Vermaak, I., Kamatou, G. P. P., Komane-Mofokeng, B., Viljoen, A. M., & Beckett, K. (2011). African seed oils of commercial importance-Cosmetic applications. *South African Journal of Botany, 77*, 920-933.http://dx.doi.org/10.1016/j.sajb.2011.07.003.

[33]. Wickens, G. E., & Lowe, P. (2008). *The Baobabs: Pachycauls of Africa, Madagascar and Australia*. London:Springer. http://dx.doi.org/10.1007/978-1-4020-6431-9.

Table 2.Effect of 6-BAP, NAA and TDZ in WPM medium on callus induction of leaf and seed explants (observation recorded after 30-35 days)

Conc. of BAP mg/l	Conc.of NAA mg/l	Conc.of TDZ mg/l	Nature of callus	Duration in days	Induction frequency %	Callus type
0	-	-	-	-	-	No response
1.0	-	-	+	35	70	Brown,watery
2.0	-	-	++	35	75	Brown,nodular
5.0	-	-	+++	30	80	White,brownish, hard
0	0	-	-	-	-	No response
1.0	1.0	-	+	10	60	Green friable
1.0	2.0	-	+	10	70	Light brown, nodular
2.0	5.0	-	++	10	70	Watery
2.0	7.0	-	+++	07	90	Hard, nodular callus
2.0	10.0	-	+++	07	90	Hard, nodular callus
0	-	0	-	-	-	-
1.0	-	0.5	+	15	70	Brown, watery
2.0	-	1.0	++	15	80	Brown, watery

No. of culture vessels inoculated for each experiment=10
Intensity of callus: - No response, + low, ++ moderate, +++, high, good quality.

Table 3:Phytochemical screening of various plant parts

Explant used	Solvent used	Flavonoids	Alkaloids	Tannins	Phenolics	Saponins	Resins
Leaf	a	+	+	+	+	-	+
	b	+	+	+	+	-	+
Fruit Pulp	a	-	+	+	+	+	+
	b	-	+	+	+	+	+
Callus	a	+	+	+	+	+	+
	b	+	+	+	-	+	+

a: methanol, b:aqueous, +- Present, -Absent

Table 4. Antibacterial activity of *A.digitata* leaf, fruit and callus extracts

Plant extract	Zone of inhibition (mm)	
	Staphylococcus aureus	
Methanol	**Leaf**	**Callus**
	46	42
Aqueous	30	28

Table 5: Effect of BAP and Kinetin on the number of shootsinduction and multiplication

Treatment	BAP (mg/L)	Kinetin (mg/l)	% shooting	Days to shooting
T0	0	-	-	-
T1	0.5	-	-	-
T2	1.0	-	-	-
T3	1.5	-	-	-
T4	2.0	-	-	-
T5	5.0	-	-	-
		0.0	-	-
K1	-	0.5	10	14
K2	-	1.0	20	20
K3	-	1.5	40	14
K4	-	2.0	70	14
K5		5.0	20	15

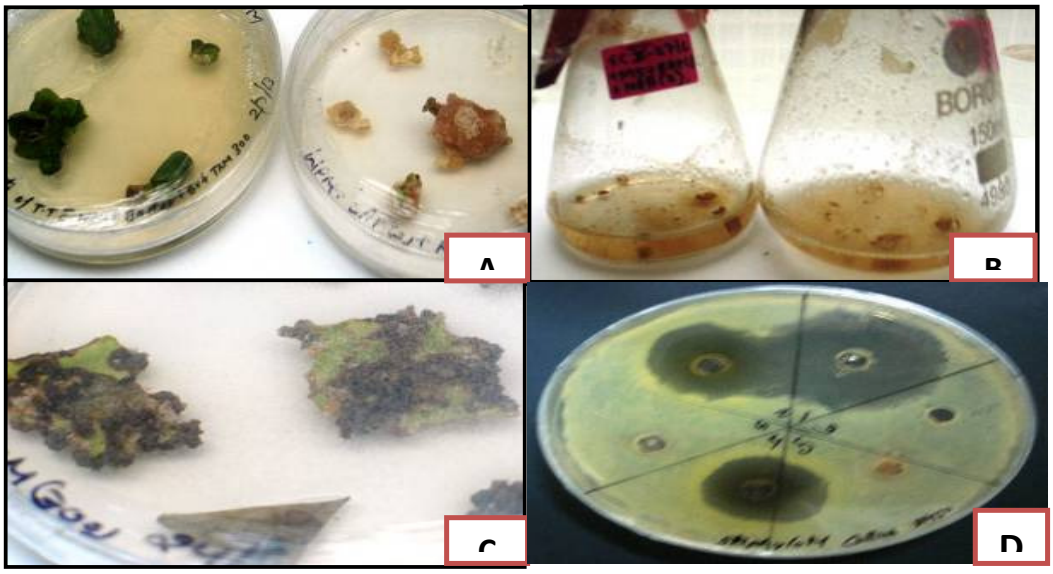

Fig.1A: Callus induction in leaf explants with BAP +NAA
Fig.1 B: Callus in suspension culture
Fig. 1C: Callus induction in leaf explants with BAP and TDZ.
Fig.1D: Anti-bacterial activity of leaf and fruit pulp against *Staphylococcus aureus.*

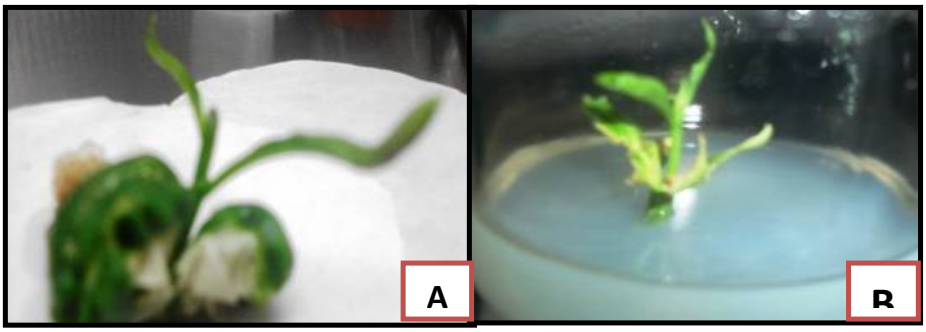

Fig. 2 A: *In vitro* shoot induction
Fig. 2 B: Shoot multiplication

