

REFERENCES

- Agrawal, S., and Chandra, N. 1983. Differentiation of multiple shoot buds and Plantlets in cultured in cultured embryos of *Capsicum annum* L., var. Mathania. Curr.Sci. India 52: 645-646
- Ajithakumar, D. and Seeni, S. 1998. Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos* L. Corr., a medicinal tree. Plant Cell Reports. 17: 422-426.
- Anjaneyula, A.S.R., Murty, Y.L.N. and Ramachandra Rao, L. (1981). J. Indian Chem. Soc. 58: 817-818.
- Aranvantis-Zafiris, V Orepoulou, C Tzia and C D Thomopoulos.1992. Utilisation of Orange By-Products-Orange pell Carotenoids.Food Agric.59:77-79.
- Arya, C. and Daniel, M. 1997. Detoxification of flavonoids by fungal pathogens in *Crataeva nurvala* Buch-Ham. and *Nyctanthes arbor-tristis* L. National Academy Science Letters-India 20:3-4.
- Bajaj, Y.P.S.1988. Biotechnology in agriculture and forestry 4: medicinal and aromatic plants I. New York: Springer-Verlag Berlin Heidelberg.
- Blomstedt, C., Cameron, J., Whiteman,P. and Chandler, S.F. 1991. Micropropagation of juvenile *Eucalyptus regnans* (mountain ash). Australain Journal of Botany 39(2): 179-186.
- Bovo, O.A., and Mroginski, L.A. 1986. Regeneration of plants from callus tissue of the pasture legumes *Lotononis bainesii*. Plant Cell Rep. 5: 295-297.

- Chaleff, R.S. 1983. Isolation of agronomically useful mutants from plant cell culture.
Science 219: 676-682
- Chauhan, J.S. and Manju Saraswat. 1978. A new glycoside from the stem of *Nyctanthes arbor-tristis* Linn. J. Indian Chem. 5: 1049-1051.
- Chee, P.P. 1991. Plant regeneration from cotyledons of *Cucumis melo* 'Topmark'. Hort Science 26(7): 908-910.
- Choo, T.M. 1988. Plant regeneration in zigzag clover (*Trifolium medium* L.). Plant Cell Rep. 7:246-248
- Corchete, M.P., Diez, J. J. and Valle, T. 1993. Micropropagation of *Ulmus pumilia* L. from mature trees. Plant Cell Reports 12: 534-536.
- Curl, A.L. 1962. The carotenoids of red bell peppers. J. Agric. Food Chem. 10: 504.
- Day, K.B., Draper, J. and Smith, H. 1986. Plant regeneration and the baine content of plants derived from callus cultures of *Papaver bracteatum*. Plant Cell Rep. 5: 471-474.
- Day, K.B., Draper, J., and Smith, H. 1989. Plant regeneration and thebaine content of plants derived from callus cultures of *Papaver bracteatum*. Plant Cell Rep. 5: 471-474.
- Demeke, T., and Hughes, H.G. 1990. Micropropagation of *Phytolacca dodecandra* through shoot-tip and nodal cultures. Plant Cell Rep. 9: 390-392.
- Deora, N.S. and Shekhawat, N.S. 1995. Micropropagation of *Capparis decidua* (Forsk) Edgew-a tree of arid horticulture. Plant Cell Reports 15: 278-281.

Department of Pharmacy. 1992. Herbal garden of Sirilukkachard. Mahidol University

Ellis, DD., and Bilderbach, D.E. 1984. Multiple bud formation by cultured embryos of *Pinus ponderosa*. J. Plant Physiol. 115: 201-204.

Gayle K. Gregory, Thung-Shan Chen, and Thomas Philip. 1987. Quantitative Analysis of Carotenoids and Carotenoid Esters in Fruits by HPLC: Red Bell Peppers. Journal of Food Science.52(4):1071-1073.

George, E.F., and Sherrington, P.D. 1984. Plant propagation by tissue culture: Handbook and dictionary of commercial laboratories. 709 p. England: Eastern Press.

Gill, M.S., and Bajaj, Y.P.S. 1984. Interspecific hybridization in the genus *Gossypium* through embryo culture. Euphytica 33: 305-311.

Gresshoff, P.M. and Doy, C.H. 1972. Development and differentiation of haploid *Lycopersicon esculentum* (tomato). Planta 107:161-170.

Hammatt, N., and Evans, P.K. 1985. The *in vitro* propagation of an endangered species: *Centaurea junoniana* Svent. (compositae). J. Hort. Sci. 60(1): 93-97.

Hansen, K.C. and Lazarte, J.E. 1984. *In vitro* propagation of pecan seedlings. HortScience 19(2): 237-239.

Hasegawa, P.M. 1979. *In vitro* propagation of rose. Hort Science 14(5): 610-612.

Heirwegh, K.M.G., Benerjee, N., Van Nerum, K., and de Langhe, E.1985. somatic embryogenesis and plant regeneration in *Cichorium intybus* L. (Witloof, compositae). Plant Cell Rep. 4: 108-111.

- Herrera, M.T., Cacho, M.P., Tarrago, J.F. 1990. One step shoot tip multiplication and rooting of *Digitalis thapsi* L. Plant Cell Tiss. Org. Culture 22: 179-182.
- Hosoki, T., and Sagawa, Y. 1997. Clonal propagation of ginger (*Zingiber officinale* Roscoe.) through tissue culture. Hort Science 12:451-452.
- Hussey, G. 1978. *In vitro* propagation of onion *Allium cepa* by axillary and adventitious shoot proliferation. Science Hortic. 9: 227-236.
- Indira, R., Mathuram, V. and Gopinath, P.M. 1998. Establishment of callus cultures of *Nyctanthes arbor-tristis* Linn from juvenile explants and detection of secondary metabolites in the callus. Current Science 74(3): 243-245.
- Jarret, R.L., Hasegawa, P.M., and Erickson, H.T. 1980. Factors affecting shoot initiation from tuber disc of potato (*Solanum tuberosum*). Physiol. Plant 49: 177-184.
- Kartha, K.K., Mroginski, L.A., Pahl, K., and leung, N.L. 1981. Germplasm preservation of coffee (*Coffea arabica* L.) by *in vitro* culture of shoot apical meristems. Plant Sci Lett. 22: 301-307.
- Khan, Z.K., Anita Manglani, Shukla, P.K., Anju Puri, Saxena, R.P. and Tandon J.S. 1995. Immunomodulatory effect of plant extracts and Iridoid Glucosides from *Nyctanthes arbor-tristis* L. against systemic Candidiasis in mice. International Journal of Pharmacognosy 33(4): 297-304.
- Kiran Lata Singh, Raja Roy, Vandita Srivastava, J.S. Tandon. 1995. Arborside D, A Minor Iridoid Glucoside From *Nyctanthes arbor-tristis* L. Journal of Natural Products 58(10): 1562-1564.

- Kirtikar, D.N. 1995. Indian Medicinal Plants 2nd edn., Basu, L.M., Allahabad, pp. 1526-1528.
- Lakshmi Sita, G. 1986. Progress towards the *in vitro* clonal propagation of *Eucalyptus grandis* Plan Tissue Culture and Its Agricultural Application London: Lyndsey A Withers PGALDERSON.
- Latscha, T. 1990. Carotenoids-Their Nature and Significance in Animal Feeds. F. Hoffmann-La Roche Ltd, Animal Nutrition and Health, Basel, Switzerland, 110 p.
- Lester Packer. 1992. Carotenoids Part A Chemistry, Separation, Quantitation and Antioxidation. Methods in Enzymology 213: 3-13.
- Liaaen-Jensen, S. 1990. Marine Carotenoids-Select topics. New Journal of Chemistry 14: 747-759.
- Lloyd, L. and McCown, B.H. 1981. Woody plant medium (WPM)- A mineral nutrient formation for microculture of woody plant species. HortScience 16: 89.
- Maclean, N.C., and Nowak, J. 1989. Plant regeneration from hypocotyls and petiole callus of *Trifolium pratense* L. Plant Cell Rep. 8: 395-398.
- McCann, A.W., Cooley, G., and Van Dreser, J. 1988. A system for routine plantlets regeneration of sunflower (*Helianthus annuus* L.) from immature embryos derived callus. Plant cell Tiss. Org. Culture 14: 103-110.
- Murashige, T and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

- Murashige, T. 1974. Plant propagation through tissue cultures. Ann.Rev.Plant Physiol. 25:135-206.
- Nadkarni, A.K. 1976. Indian Materia Medica. 3rd edn. Popular Prakashan, Bombay.
- Nagarajan, P., McKenzie, J.S., and Walton, P.D. 1986. Embryogenesis and plant regeneration of *Medicago spp.* in tissue culture. Plant Cell Rep. 5: 77-80.
- Noga, G. and Lenz, F. 1983. Separation of citrus carotenoids by reversed phase HPLC. Chromatographia 17(3): 139.
- Patton, D.A., and Meinke, D.W. 1988. High-frequency plant regeneration from cultured cotyledons of *Arabidopsis thaliana*. Plant Cell Rep. 7: 233-237.
- Rai, M.K. and Sushil Upadhyay. 1988. Screening of Medicinal Plants of Chhindwara District against *Trichophyton mentagrophytes*: A Causal Organism of *Tinea Pedis*. Hindustan Antibiotics Bulletin 30: 33-36
- Rasai, S., Kantharajah.A.S. and Dodd, W.A. 1994. The effect of growth regulators source of explants and irradiance on *in vitro* regeneration of atemoya. Australian Journal of Botany.
- Rech, E.L., and Pires, J.P. 1986. Tissue culture propagation of *Mentha spp.* by the use of axillary buds. Plant Cell Rep. 5: 17-18.
- Reinhard Eder.1982. Pigments.Handbook of Food Analysis 1: 937-952.
- Sangwan, R.S., and Harada, H. 1975. Chemical regulation of callus growth, organogenesis, plant regeneration and somatic embryogenesis in *Antirrhinum majus* tissue and cell cultures. J.Exp.Bot. 26(95): 868-881.

- Saxena, R.S., Gupta, B., Saxena, K.K., Singh, R.C., and Prasad, D.N. 1984. Study of anti-inflammatory activity in the leaves of *Nyctanthes arbor-tristis* L. and Indian medicinal plant. Journal of Ehtnopharmacology 11: 319-330.
- Saxena, R.S., Gupta, B., Saxena, K.K., Srivastava, V.K. and Prasad, D.N. 1987. Analgesic, Antipyretic and Ulcerogenic Activity of *Nyctanthes arbor-tristis* L. Journal of Ethnopharmacology 19: 193-200.
- Shim, K.K., HA, Y.M. and Lee, S.K. 1992. Mass propagation of *Betula pendula* "Trast Dwaf" through axillary bud *in vitro*. Journal of the Korean Soceity for Horticultural Science 33(4): 329-336.
- Simpson, K.L., Chichester, C.O. and Phaff, H.J. 1971. The Yeast. 2: 493-515.
- Songstad, D.D., Duncan, D.R., and Widholm, J.M. 1988. Effect of 1-aminocyclopropane-1-carboxylic acid, silver nitrate and nonornadiene on plant regeneration from maize callus cultures. Plant Cell Rep. 7: 262-265.
- Stuppner, H., Muller, E. P., Mathuram, V. and A. B. Kundu. 1993 Iridoid glycosides from *NYCTANTHES ARBOR-TRISTIS*. Phytochemistry 32(2): 375-378.
- Sudha, C.G. and Seeni, S. 1996. *In vitro* propagation of *Rauwolfia micrantha*, a rare medicinal plant. Plant Cell Tissue and Organ Culture 44: 243-248.
- Sugiura, A., Tao, R., Murayama, H. and Tomana, T. 1986. *In vitro* propagation of *Japanese persimmon*. HortScience 21(5): 1205-1207.

- Talakal, T.S., Dwivedi, S.K. and Sharma S.R. 1995. *In vitro* screening of some indigenous plants against *Trypanosoma evansi*. Indian Journal of Animal Sciences 66(1): 18-21.
- Torres, K.C. and Carlisi, J.A. 1986. Shoot and root organogenesis of *Camellia sasanqua*. Plant Cell Rep. 5: 381-384.
- Vandita Srivastava, Anita Rathore, Syed Mashhood ALI, and J.S. TANDON. 1990. New benzoic esters of loganin and 6 β -hydroxyloganin from *Nyctanthes arbor-tristis* L. Journal of Natural Products 53(2): 303-308.
- Vietez, A.M. and Vieitez, M.L. 1980. Culture of chesnut shoots from buds *in vitro*. J.Hort.Sci. 55(1): 83-84.
- Walender, T. 1977. *In vitro* organogenesis in explants from different cultures of *Begonia x Hiemalis*. Physiol Plant 41: 142-145.
- Xihai, W. 1990. Extracting natural carotene from *Dunaliella salina*. Proceeding of International Seminar on Biotechnology of Saltponds, China, Sep 18-21, pp. 39-46.

APPENDICES

Appendix A

Murashige and Skoog media (1962)

Constituent	Concentration (mg/l)
Macronutrients:	
KNO ₃	1,900
NH ₄ NO ₃	1,650
CaCl ₂ .2H ₂ O	440
MgSO ₄ .7H ₂ O	370
KH ₂ PO ₄	170
Micronutrients:	
H ₃ BO ₃	6.2
MnSO ₄ .H ₂ O	22.3
ZnSO ₄ .H ₂ O	8.6
KI	0.83
Na ₂ MoO ₄ .2H ₂ O	0.25
CuSO ₄ .5H ₂ O	0.025
CoCl ₂ .6H ₂ O	0.025
Iron:	
Na ₂ EDTA	37.25
FeSO ₄ .7H ₂ O	27.85
Organic components:	
Myo-Inositol	100
Thiamine.HCl	0.1
Nicotinic acid	0.5
Pyridoxin HCl	0.5
Glycine	2.0
Sucrose	3%
pH	5.7

Gresshoff and Doy media (1972)

Constituent	Concentration (mg/l)
Macronutrients:	
KNO ₃	1,000
CaCl ₂ .2H ₂ O	150
MgSO ₄ .7H ₂ O	250
(NH ₄) ₂ SO ₄	200
KCl	300
Micronutrients:	
H ₃ BO ₃	3.0
MnSO ₄ .H ₂ O	10.0
ZnSO ₄ .H ₂ O	3.0
KI	0.75
Na ₂ MoO ₄ .2H ₂ O	0.25
CuSO ₄ .5H ₂ O	0.25
CoCl ₂ .6H ₂ O	0.25
GD-P:	
NaH ₂ PO ₄ .H ₂ O	90
NaHPO ₄	30
Iron:	
Na ₂ EDTA	37.25
FeSO ₄ .7H ₂ O	27.85
Organic components:	
Myo-Inositol	10
Thiamine.HCl	1.0
Nicotinic acid	0.1
Pyridoxin HCl	0.1
Glycine	2.0
Glutamine	2.0
Sucrose	3%
pH	5.7

Preparation of stock solution of media

Preparation of stock solution of MS media

Stock	Stock solution	g/l
Stock I	KNO ₃	95.00
	NH ₄ NO ₃	82.50
	KH ₂ PO ₄	8.50
	H ₃ BO ₃	0.31
	MnSO ₄ .H ₂ O	1.115
	ZnSO ₄ .H ₂ O	0.43
	KI	0.0415
	NA ₂ MoO ₄ .2H ₂ O	0.0125
	CuSO ₄ .5H ₂ O	0.00125
	CoCl ₂ .6H ₂ O	0.00125
	(Used 20 ml/l media)	
Stock II	MgSO ₄ .7H ₂ O	37
	(Used 10 ml/l media)	
Stock III	CaCl ₂ .2H ₂ O	44
	(Used 10 ml/l media)	
Stock IV	Na ₂ EDTA	3.73
	FeSO ₄ .7H ₂ O	2.78
	(Used 10 ml/l media)	
Stock V (Organic solution vitamins)	Myo-Inositol	20
	Thiamine.HCl	0.02
	Nicotinic acid	0.1
	Pyridoxin HCl	0.1
	Glycine	0.4
	(Used 5 ml/l media)	

Preparation of stock solution of Gresshoff and Doy media

Stock	Constituent	Concentration (g/l)
Stock I	Macronutrients:	
	KNO ₃	10
	CaCl ₂ .2H ₂ O	1.5
	MgSO ₄ .7H ₂ O	2.5
	(NH ₄) ₂ SO ₄	2.0
	KCl	3.0
	(Used 100 ml/l media)	
Stock II	Micronutrients:	
	H ₃ BO ₃	3.0
	MnSO ₄ .H ₂ O	10.0
	ZnSO ₄ .H ₂ O	3.0
	KI	0.75
	Na ₂ MoO ₄ .2H ₂ O	0.25
	CuSO ₄ .5H ₂ O	0.25
	CoCl ₂ .6H ₂ O	0.25
	(Used 10 ml/l media)	
Stock III	GD-P:	
	NaH ₂ PO ₄ .H ₂ O	9.0
	NaHPO ₄	3.0
	(Used 10 ml/l media)	
Stock IV	Iron:	
	Na ₂ EDTA	3.725
	FeSO ₄ .7H ₂ O	2.785
	(Used 10 ml/l media)	
Stock V	Organic components:	
	Myo-Inositol	10
	Thiamine.HCl	1.0
	Nicotinic acid	0.1
	Pyridoxin HCl	0.1
	Glycine	2.0
Glutamine	2.0	
	(Used 1 ml/l media)	
	Sucrose	3%
	pH	5.7

White Agar medium (Prepare for 1 l.)

Constituent	Amount (g.)
Glucose	10
Peptone	5
Malt Extract	3
Yeast extract	3
pH	6.2
Agar	20

LB medium

Constituent	Amount (g.)
Peptone	10
Yeast extract	5
NaCl	5
pH	7.0
Agar	20

Plant material for tissue culture

The explants were excised from internode for 2-3 nodes with 3-4 cm. long per pieces. Explants were surface sterilized with distilled water 30 min and shaken in distilled water for 30 min. Clean with 10%(v/v) Clorox containing tween20 2-3 drops and shaken with magnetic stirrer 30 min for 3 times. In addition, they were disinfected in 70%(v/v) Ethyl alcohol and tween20 2-3 drops for 1-3 min. In laminar flow cabinet, they continued soaking in 10%(v/v) Clorox with tween20 2-3 drops for 30 min. Followed by rising with sterile distilled water for 5 times. Finally, cleaned explants were cut for 1-2 node with 1-2 cm. long per pieces.

Appendix B

Growth inhibition from crude extract of *Nyctanthes arbor-tristis* L. on *E. coli*

Time (minutes)	Absorbance (OD ₄₂₀)				
	Control	Leaves (mother plants)	Stems (mother plants)	Leaves (tissue culture)	Stems (tissue culture)
0	0.340	0.341	0.338	0.348	0.345
20	0.400	0.428	0.524	0.483	0.579
40	0.460	0.443	0.554	0.498	0.609
60	0.523	0.463	0.584	0.518	0.639
80	0.578	0.473	0.603	0.528	0.658
100	1.169	0.490	0.621	0.545	0.676
120	2.058	0.498	0.644	0.553	0.699
140	2.947	0.503	0.643	0.558	0.698
160	2.948	0.505	0.634	0.560	0.699
180	2.949	0.511	0.632	0.566	0.697
200	2.948	0.514	0.629	0.569	0.694
220	2.949	0.503	0.624	0.558	0.689
240	2.950	0.498	0.613	0.553	0.678
260	2.950	0.495	0.612	0.550	0.677

Growth inhibition from crude extract of *Nyctanthes arbor-tristis* L.
on *Bacillus subtilis*

Time (minutes)	Absorbance (OD ₄₂₀)				
	Control	Leaves (mother plants)	Stems (mother plants)	Leaves (tissue culture)	Stems (tissue culture)
0	0.352	0.362	0.358	0.357	0.354
20	0.42	0.448	0.503	0.544	0.599
40	0.48	0.463	0.518	0.574	0.629
60	0.543	0.483	0.538	0.604	0.659
80	0.598	0.493	0.548	0.623	0.678
100	1.189	0.51	0.565	0.651	0.696
120	2.078	0.518	0.573	0.671	0.729
140	2.967	0.523	0.579	0.663	0.725
160	2.968	0.525	0.594	0.669	0.732
180	2.969	0.545	0.598	0.692	0.735
200	2.968	0.551	0.615	0.695	0.745
220	2.969	0.558	0.632	0.705	0.749
240	2.97	0.565	0.638	0.721	0.748
260	2.97	0.572	0.645	0.725	0.746

Growth inhibition from crude extract of *Nyctanthes arbor-tristis* L.
on *Saccharomyces cerevisiae*

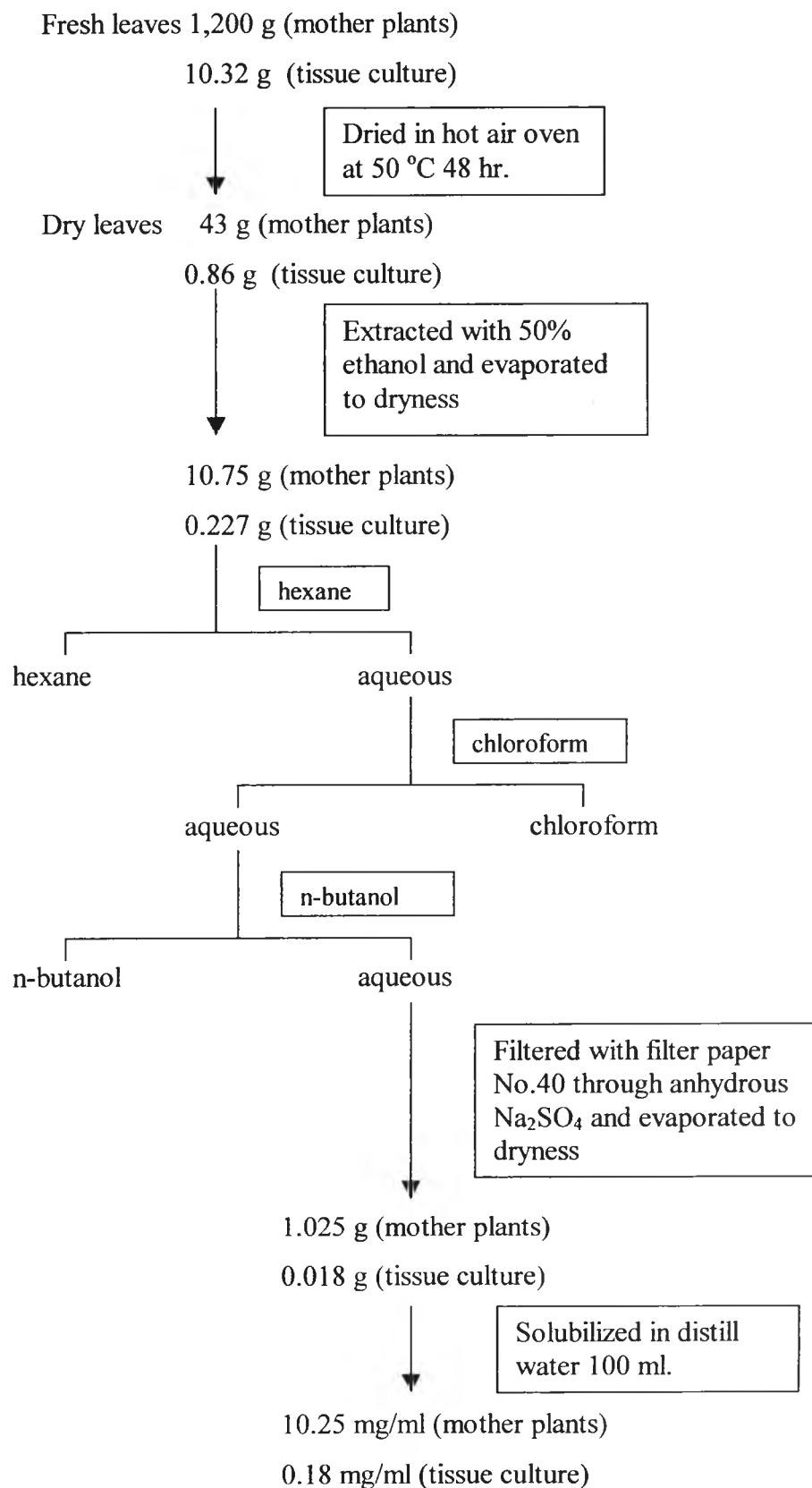
Time (hr.)	Absorbance (OD ₅₃₀)				
	Control	Leaves (mother plants)	Stems (mother plants)	Leaves (tissue culture)	Stems (tissue culture)
0	0.302	0.318	0.308	0.312	0.305
3	0.352	0.395	0.410	0.414	0.428
6	0.429	0.432	0.460	0.475	0.496
9	0.506	0.469	0.510	0.518	0.538
12	0.583	0.506	0.560	0.572	0.596
15	1.169	0.806	0.856	0.875	0.962
18	2.054	1.306	1.359	1.385	1.596
21	2.941	1.311	1.362	1.425	1.625
24	2.947	1.314	1.412	1.429	1.632
27	2.949	1.318	1.420	1.436	1.636
30	2.948	1.320	1.428	1.439	1.643
33	2.944	1.321	1.436	1.442	1.648
36	2.952	1.323	1.444	1.459	1.653
39	2.951	1.326	1.452	1.468	1.657

Growth inhibition from crude extract of *Nyctanthes arbor-tristis* L. on *Aspergillus* sp.

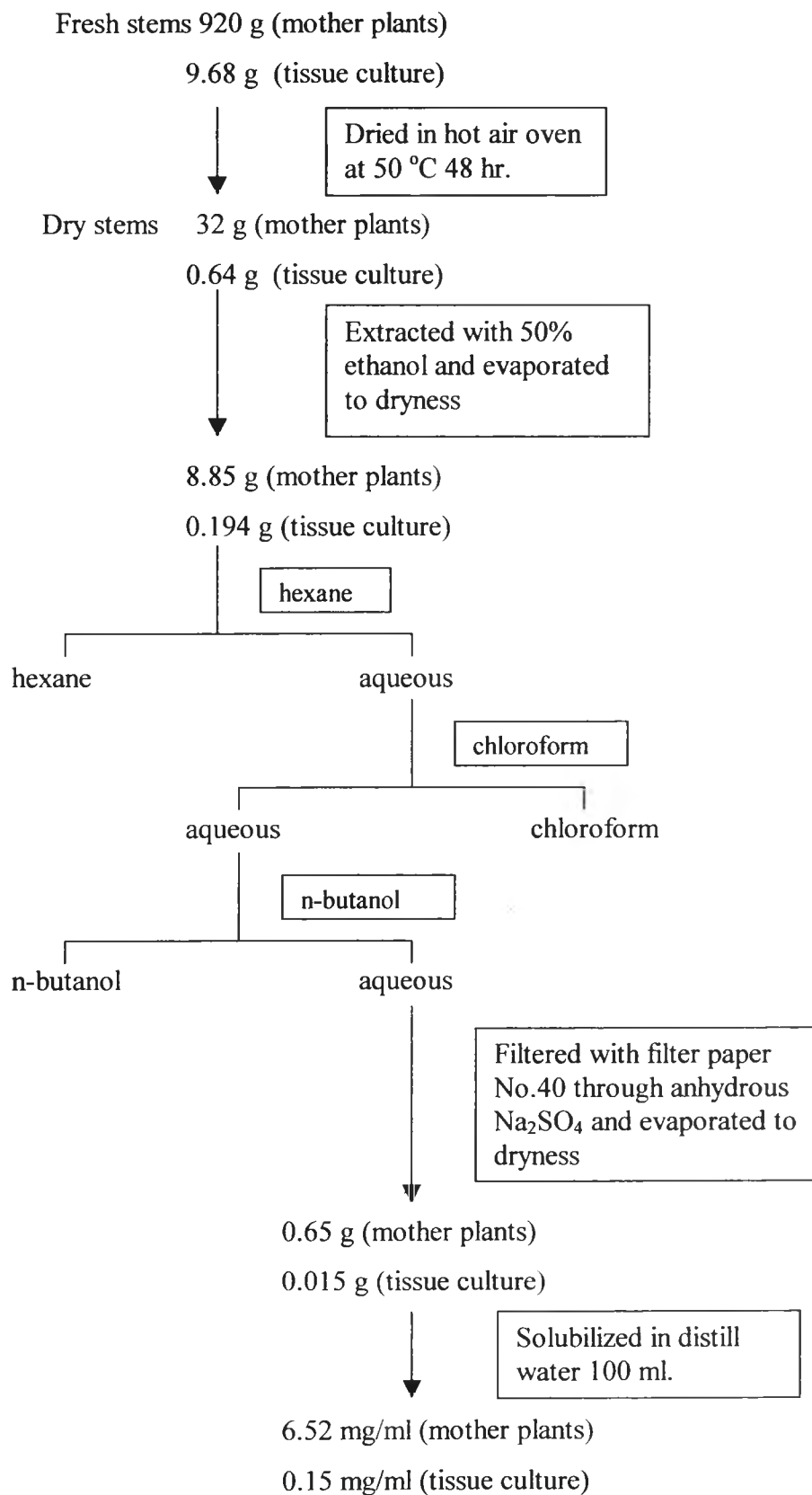
Times (Day)	Absorbance (OD ₅₃₀)				
	Control	Leaves (mother plants)	Stems (mother plants)	Leaves (tissue culture)	Stems (tissue culture)
0	0.512	0.517	0.514	0.519	0.513
1	0.589	0.594	0.599	0.604	0.609
2	0.639	0.644	0.649	0.654	0.659
3	0.685	0.656	0.661	0.666	0.671
4	0.742	0.721	0.726	0.731	0.736
5	0.796	0.753	0.758	0.763	0.768
6	0.856	0.815	0.820	0.827	0.835
7	1.674	1.016	1.125	1.132	1.145
8	2.598	1.457	1.541	1.586	1.626
9	3.314	1.786	1.972	1.987	2.012
10	3.316	1.798	1.984	1.991	2.024
11	3.321	1.815	1.995	2.014	2.032
12	3.325	1.824	1.998	2.021	2.038
13	3.327	1.831	2.012	2.023	2.041

Appendix C

Extraction of leaves from mother plants and tissue culture



Extraction of stems from mother plants and tissue culture



Biography

Prasert Salika was born on August 24, 1972 in Phuket, Thailand. He graduated with the degree of Bachelor of Science in Plant Science from the Songklanagarin University in 1995. In 1997, he has studied in Master degree of Science at the multidiscipline of Biotechnology, Chulalongkorn University.

