UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF STERILIZATION METHOD AND EFFECT OF 6-BENZYLAMINOPURINE AND α-NAPHTHALENE ACETIC ACID ON SHOOT FORMATION OF *Clinacanthus nutans*

SAW SIEW FEN

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SAW SIEW FEN

FACULTY OF AGRICULTURE

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SERDANG, SELANGOR DARUL EHSAN

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BY

SAW SIEW FEN

A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science

Faculty of Agriculture

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ENDORSEMENT/CERTIFICATION

This project report entitled DETERMINATION OF STERILIZATION METHOD AND EFFECT OF 6-BENZYLAMINOPURINE AND ά-NAPHTHALENE ACETIC ACID ON SHOOT FORMATION OF *Clinacanthus nutans* is prepared by Saw Siew Fen and submitted to Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science.

Student's name: Student's signature

SAW SIEW FEN

……

Certified by:

……..

Assoc. Prof. Dr. Maheran binti Abdul Aziz

Department of Agriculture Technology

Date: ……..

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LIST OF ABBREVIATIONS AND SYMBOLS

2,4-D	2,4-Dichlorophenoxyacetic Acid
ANOVA	Analysis of Variance
BAP	6-Benzylaminopurine
BBTV	Banana Bunchy Top Virus
Benomyl	Methyl [1-[(butylamino)carbonyl]-1 H-benzimidazol-2-yl]carbamate
BMV	Brome Mosaic Virus
cv.	Cultivar
DNA	Deoxyribonucleic Acid
mm	Millimeter
g	Gram
g/L	Gram per Liter
GAE	Gallic Acid Equivalents
HCl	Hydrogen Chloride
$HgCl_2$	Mercury (II) Chloride
IAA	Indole-3-Acetic Acid
IBA	Indole-3-Butyric Acid

mg/L	Milligram per Liter
°C	Degrees Celsius
Mins	Minutes
MS	Murashige and Skoog
NAA	Naphthalene Acetic Acid
NaOCl	Sodium Hypochlorite
NaOH	Sodium Hydroxide
PGRs	Plant Growth Regulators
Picloram	4 Amino-2,5,6-trichloropicolinic acid
PMA	Phorbol-12 myristate 13-acetate
psi	Pound per Square Inch
RCBD	Ramdomized Complete Block Design
SAS	Statistical Analysis System
TDZ	Thidiazuron
µg/ml	Microgram per Milliliter
v/v	Volume per Volume
%	Percentage

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ABSTRACT

The herb plant from the family Acanthaceae called *Clinacanthus nutans* (Burm. f.) Lindau or better known as Sabah Snake Grass has been commercialized recently. It is native to Southeast Asia, including Indonesia, Thailand, and Malaysia. The leaves are believed to be able to treat insect bites, herpes infection, nettle rash, allergic responses and recently it is well known for its healing properties such as a cure for cancer and kidney failure alleviation. The objectives of this study were to establish the best sterilization method and to determine the best combination of BAP and NAA for optimizing the in vitro shoot formation of *C. nutans*. The explants for this study were the nodes. In the determination of sterilization method, treatment with 30% Clorox for 30 min immersion time resulted in the least contamination, however some of the explants were damaged and unable to produce shoots after 4 to 6 weeks of culture. By using 30% Clorox at 20 min immersion time, resulted in 67.67% survived, non-contaminated explants. The protocol needs to be further refined to improve the effectiveness of killing the contaminants. For the shoot regeneration study, almost all the explants (99.96%) produced shoots successfully. Treatment consisting of 10 mg/L BAP without NAA produced the highest mean number of shoots (3.17) per explant, that reached a mean height of 0.73 cm and produced mean number of 13.56 leaves per shoot after 8 weeks of culture.

Key words: *Clinacanthus nutans* (Burm. f.) Lindau, sterilization method, shoot regeneration

ABSTRAK

Salah satu tumbuhan herba dari famili Acanthaceae yang dinamakan Clinacanthus nutans (Burm. f.) atau lebih dikenali sebagai Belalai Gajah telah dikomersilkan baru-baru ini. Tumbuhan ini berasal dari Asia Timur Selatan termasuk Indonesia, Thailand, dan Malaysia. Daunnya dipercayai boleh merawat gigitan serangga, jangkitan herpes, ruam jelatang, alergi dan juga terkenal dengan sifat-sifat penyembuhan seperti ubat untuk kanser dan membendung kegagalan buah pinggang. Objektif untuk kajian ini adalah untuk mewujudkan kaedah pensterilan yang terbaik dan untuk menentukan kombinasi terbaik daripada BAP dan NAA untuk mengoptimumkan formasi in vitro pucuk C. nutans. Tunas dipilih sebagai ekplan untuk kajian ini. Bagi kajian untuk kaedah pensterilan, keputusan menunjukkan bahawa rawatan 30% Clorox dengan masa rendaman 30 minit memberikan kadar tercemar yang paling rendah tetapi dengan rawatan ini juga menyebabkan sesetengah ekplan rosak dan tidak dapat mengeluarkan pucuk selepas 4 hingga 6 minggu dikultur. Dengan menggunakan 30% Clorox dengan 20 minit masa rendaman, sebanyak 67.67% kadar ekplan menjadi hidup dan tidak tercemar. Protokol tersebut perlu dimurnikan lagi untuk meningkatkan keberkesanan untuk membunuh bahan cemar. Untuk kajian pertumbuhan pucuk, hampir semua explan (99.96%) dapat mengeluarkan pucuk dengan jayanya. Rawatan dengan 10 mg/L BAP menghasilkan purata nombor pucuk yang paling tinggi (3.17) per ekplan, dan mencapai purata tinggi sebanyak 0.73 cm serta menghasilkan purata nombor daun yang sebanyak 13.56 per pucuk selepas 8 minggu dikultur.

Kata kunci: Clinacanthus nutans (Burm. f.) Lindau, kaedah pensterilan, pembentukan pucuk

CHAPTER 1

INTRODUCTION

Malaysia's state of health is considered lower if compared with other developed country like Japan and Singapore. Statistics from the Ministry of Health Malaysia in year 2011 have shown that on average, in every hundred Malaysians, there are six people diagnosed with diabetic related diseases. In every ten Malaysians, one has kidney related diseases. In every four Malaysians, there is one that faces the risk of any cancer. In every hour, there are six Malaysians hit by stroke. Such diseases remain a major health threats to Malaysian population.

Nowadays, people are more concerned about their health. This causes the upsurge of the consumption of traditional herbal plants in the world. Herb is a plant which is valued for its medicinal properties, flavor, or scent. Herbal plants have been used for health and medical purposes for more than a few thousand years. In Malaysia, traditional herbal plants have been used for treatment of certain diseases as well. Some people would prefer to consume the traditional medicines to treat their diseases rather than chemical medicines because they believed that herbal medicines are considered as natural constituents and safe to consume. In some developing countries, majority of the population still rely on herbal medicines to meet their health needs. The interest on herbal medicines and their utilization have been rising rapidly in recent years even in areas where modern medicine is available.

Clinacanthus nutans (Burm. f.) Lindau is a herb from family Acanthaceae. The Acanthus family is a taxon of dicotyledonous flowering plants. It consists of 250 genera and around 2500 species. In traditional medicine, the fresh leaves of *C. nutans* has been

suggested to be effective in the healing of snake poison and insect bites, burn, allergic reaction, anti-inflammatory (Wanikiat *et al*., 2008) and viral infections (Thawaranantha *et al*., 1992). Its common names are 'Sabah Snake Grass' in Sabah, 'Belalai Gajah' in Malay, 'Dandang gendis' in Indonesia, 'Phaya yor' in Thailand or 'You Dun Cao' in Mandarin.

Besides that, the *C. nutans* was found to inhibit Varicella-zoster virus (Charuwichitratana *et al.*, 1996), Herpes Simplex Virus (Sakdarat *et al.*, 2008), and also able to inhibit early stage infection of Human papillomavirus (HPV) (Yuann *et al*., 2012) as well.

C. nutans Lindau possesses many useful medicinal properties. Besides the leaves that are needed for medicinal purposes the stems and even the roots have also provided medicinal values. Due to its medicinal properties propagation of the plant through tissue culture will provide an opportunity to local growers and breeders to plant and sell this plant commercially.

Tissue culture, an important area of biotechnology is used to conserve or grow plant tissues, cells or organs under sterile conditions on a nutrient culture medium of known composition. This technique can be used to improve the productivity and quality of planting material through enhanced availability of identified planting stock with desired traits (Thorpe, 2007). Nowadays, the plant tissue culture technique has been applied in many areas especially agriculture, horticulture and sciences. Tissue culture has provided many benefits to growers not only from commercial point of view but also for crop upgrading program. The advantages which can be obtained from tissue culture of *C.*

nutans are the production of shoots that are diseases free, faster multiplication rate for mass production on a commercial scale.

Leaves production of *C. nutans* is very important for the healers and growers in term of the medicinal and entrepreneur purposes. Nonetheless, the leaves quantity creates a crucial problem to the people as they might need certain amount of leaves to treat their diseases or for their daily uses. After every harvest, *C. nutans* plants might need a certain period of time to produce new leaves. As a result, growers need to sell the not fully matured leaves to the customers or the customers need to wait for some time to get the leaves of *C. nutans* from the growers. In order to solve the problem, this experiment aims to find out the possibility of tissue culture treatments playing a role in multiple shoot formation, increase leaves growth rate, and thereby produce better shoot development of *C. nutans*. Hence, the specific objectives of this study are:

1. To establish the most effective sodium hypochlorite (Clorox®) concentration and immersion time for sterilization of *C. nutans* explants.
2. To determine the best combination of 6-Benzylaminopurine (BAP) and Naphthalene acetic acid (NAA) for optimizing the in vitro shoot formation of *C. nutans*.

REFERENCES

Aina, O., Quesenberry, K. and Gallo, M. (2012). Thidiazuron-induced tissue culture regeneration from quartered-seed explants of *Arachis paraguariensis*. Crop Sci. 52(3): 1076-1083. doi:10.2135/cropsci2011.07.0367

Ali, R.M., Samah, Z.A., Mustapha, N.M. and Hussein, N. (2010). ASEAN herbal and medicinal plants (pp. 284-286). Jakarta, Indonesia.

Arief, H. (2008). Hariana Tumbuhan Obat dan Khasiatnya. (pp. 56). Penebar Swadaya, Jakarta.

Babu, K.N., Sajina, A., Minoo, D., John, C.Z., Mini, P.M., Tushar, K.V., Rema, J. and Ravindran, P.N. (2003). Micropropagation of camphor tree (*Cinnamomum camphora*). Plant Cell, Tiss. Org. Cult. 74(2):179-183.

Badoni, A. and Chauhan, J.S. (2010). In vitro sterilization protocol for micropropagation of *Solanum tuberosum* cv. 'Kufri Himalini'. Academia Arena 2(4):24-27.

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. and Biotech. 15:121-126.

Banerjee, P., Maity, S. and Banerjee, N. (2011). In vitro propagation of *Arachis hypogaea* L.Var. AK 1224: Direct and Indirect plant regeneration. Plant Cell Biotech. and Mol. Bio.12(1-4): 57-62.

Basu, J. and Yogananth, N. (2011). In vitro anti-inflammatory activity and tissue culture studies on *Andrographis paniculata* (Burm. F.) Wallich Ex. Nees.: A medicinal plant. J. Phar. Research. 4(5):1368-1369.

Bhavisha, B.W. and Jasrai, Y.T. (2003). Micropropagation of an endangered medicinal plant: *Curculigo orchioides* Gaertn. Plant Tissue Cult. 13(1):13-19.

Bhojwani, S.S. and Razdan, M.K. (2004). Plant Tissue Culture: Theory and Practice. Revised Edition, Elsevier Publication, Amsterdam.

Brown, D.C.W. and Thorpe, T.A. (1984). Organization of a Plant Tissue Culture Laboratory. (pp. 1-12). In Cell Culture and Somatic Cell Genetics of Plants, Vol. 1. D.K. Vasil (editor). Academic Press, New York.

Charuwichitratana, S., Wongrattanapasson, N., Timpatanapong, P. and Bunjob, M. (1996). Herpes Zoster: treatment with *Clinacanthus nutans* cream. Inter. J. Dermatal. 35(9): 664-666.

Chen,W.L. and Yeh, D.M. (2006). Elimination of In vitro Contamination, Shoot Multiplication, and Ex vitro Rooting of *Aglaonema.* HortSci. 42(3): 629-632.

Cherdchu, C., Poopyruchpong, N., Adchariyasucha, R. and Ratanabanangkoon, K. (1977). The absence of antagonism between extracts of *Clinacanthus nutans* Burm and *Naja naja siamensis* venom. Southeast Asian J. Trop. Med. Public Health. 8: 249-254.

Chu, I.Y.E. (1986). The application of tissue culture to plant improvement and propagation in the ornamental horticulture industry. In Zimmerman, R.H., Griesbach, R.J., Hammerschlag, F.A. and Lawson, R.H. (Eds.). Tissue culture as a plant production system for horticulture crops. (pp. 15-33.) Netherland: Martinus Nijhoff Publishers.

Chuenjib, L. and Nathorn, C. (2011). Efficacy of *Clinacanthus nutans* extracts in patients with herpes infection: Systematic review and meta-analysis of randomized clinical trials. Complement. Ther. Med. 19: 47-53.

El-Dougdoug, K.A. and El-Shamy, M.M. (2011). Management of viral diseases in banana using certified and virus tested plant material. Afr. J. Microbiol. Res. 5(32): 5923-5932. doi: 10.5897/AJMR11.966

Fossard, R.A. de. (1976). Tissue culture for plant propagators. (pp.1-7) Department of Botany, The University of New England, Australia.

Garcia, G.R., Quiroz, K., Carrasco, B. and Caligari, P. (2010). Plant tissue culture: Current status, opportunities and challenges. Cien. Inv. Agr. 37(3): 5-30.

Gopitha, K., Bhavani, A.L. and Senthilmanickam, J. (2010) Effect of the different auxins and cytokinins in callus induction, shoot, root regeneration in sugarcane. Int. J. Pharm. Bio. Sci. 1(3): 1-7.

Gunasekaran, U. (2014). Callus induction and plant regeneration studies of *Clinacanthus nutans* (Sabah Snake Grass), Degree Thesis, Universiti Tunku Abdul Rahman.

Hatice, C., Ufuk, K., Gülnur, T. (2011). Influence of different sterilization methods on callus initiation and production of pigmented callus in *Arnebia densiflora* Ledeb. Turk. J. Biol. 35: 513-520. doi:10.3906/biy-0911-161

Health Facts (July 2012), Malaysian Ministry of Health. Retrieved 24 February 2014 from http://www.moh.gov.my/images/gallery/stats/heal_fact/health_fact_2012_page_by_page.pdf

Hu, C.C., Deng, Y.F. and Thomas, F.D. (2011). Flora of China. Retrieved 24 February 2014 from http://flora.huh.harvard.edu/china/mss/volume19/Flora_of_China_Volume_19_Acanthaceae.pdf

Hussain, A., Qarshi, I.A., Nazir, H. and Ullah, I. (2012). Plant Tissue Culture: Current Status and Opportunities. In: Leva, A. and Rinaldi, L.M.R. (Eds.). Recent advances in plant in vitro culture. INTECH, Pakistan. doi: 10.5772/50568

Ines, M., Krunoslav, D., Vesna, T., Marija, V., Ankica, P., Zlatko, C., Boris, P. and Zorica, J. (2013). In vitro sterilization procedures for micropropagation of 'Oblačinska' sour cherry. J. Agricult. Sci. 58(2): 117-126. doi: 10.2298/JAS1302117M

Janarthanam, B. and Sumathi, E. (2010). In vitro Regeneration of *Justicia gendarussa.* Burm. f. Libyan Agricult. Res. Cent. J. Int. 1(5): 284-287.

Karen, W.M. and Edzard, E. (2003). Antiviral agents from plants and herbs: a systemic review. Antivir. Ther. 8:77-90.

Kneifel, W. and Leonhardt, W. (1992). Testing different antibiotics against Gram-positive and Gram-negative bacteria isolated from plant tissue culture. Plant Cell Tiss. Org. Cult. 29: 139-144.

Kuhlmann, I. (1996). The prophylactic use of antibiotics in cell culture. Cytotechnol. 19: 95-105.

Leifert, C. (2000). Quality Assurance system for plant cells and tissue culture, the problem of latent persistence of bacterial pathogens and Agrobacterium-based transformation vector systems. In Vitro Cell Del. Bio.-Plant 37(2): 133-138.

Maxwell, J. (2001). Vegetation in the Siphandone wetlands. In Daconto, G. (Ed.) Siphandone wetlands. CESVI, pp.178. Bergamo, Italy.

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

Nalawade, S.M., Sagare A.P., Lee, C.Y., Kao, C.L. and Tsay, H.S. (2003). Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. Bot. Bull. Acad. Sinica. 44 : 79-98.

Neama, A.A.A., Mohamed, E.R., Salah, E.M.E. and Hussein, S.E. (2013). In vitro studies on cassava plant micropropagation of cassava (*Manihot Esculenta* Crantz). J. Appl. Sci. Res., 9(1): 811-820.

Ng, L. Y. (2013). Establishment of axenic explants and callus culture of *Clinacanthus nutans* (Rumput Belalai Gajah), Degree Thesis, Universiti Malaysia Sarawak.

Odutayo O.I., Amusa N.A., Okutade O.O., Ogunsanwo Y.R., (2007). Sources of microbial contamination in tissue culture laboratories in southwestern Nigeria. Afr. J. Agricult. Res. 2: 67-72.

Ortola, A.G., Monerri, C. and Guardiola, J.L. (1991). The use of naphthalene acetic acid as a fruit growth enhancer in Satsuma mandarin: a comparison with the fruit thinning effect. Sci. Hortic. 47: 15-25. doi: 10.1016/0304-4238(91)90023-R

Peret, B., De Rybel, B., Casimiro, I., Benkova, E., Swarup, R., Laplaze, L., Beeckman, T., Bennett, M.J. (2009). *Arabidopsis* lateral root development: an emerging story. Trends Plant Sci. 14:399-408.

Perilli, S., Moubayidin, L. and Sabatini, S. (2010). The molecular basis of cytokinin function. Curr. Opin. Plant Biol. 13: 21-26.

Prakash, S. and Staden, J. V. (2007). Micropropagation of *Hoslundia opposita* Vahl--a valuable medicinal plant. S. Afr. J. Bot. 73:60-63.

Rafiq, M., Dahot, M.U., Mangrio, S,M,, Naqvi, H.A. and Qarshi, I.A. (2007). In vitro clonal propagation and biochemical analysis of field established *Stevia rebaudiana Bertoni*. Pak. J. Bot. 39(7): 2467-2474.

Sakdarat, S., Shuyprom, A., Ayudhya, T.D.N., Waterman, P.G. and Karagianis, G. (2008). Chemical composition investigation of the *Clinacanthus nutans* Lindau leaves. Thai J. Phyto. Pharm. 13(2):2549-2551.

Sarfaraj,M.H., Fareed, S. and Saeed, M. (2012). Current approaches toward production of secondary plant metabolites. J. Pharm. Bioallied Sci. 4(1): 10-20.

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

Satayavivad, J., Bunyaoraphatsara, N., Kitisiripomkul, S. and Tanasomwang, W. (1996). Analgesic and anti-inflammatory activities of extract of *Clinacanthus nutans* Lindau". Thai J. Phyto. Pharm. 3: 7-17.

Setterfield, G. (1963). Growth regulation in excised slices of Jerusalem artichoke tuber tissues. Sym. Soc. Exp. Biol. 17: 98-126.

Sharifkhani, A., Halimi, M.S. and Maheran, A.A. (2011). An alternative safer sterilization method for explants of *Aloe vera barbadensis* Mill. 2nd International Conference on Chemical Engineering and Applications IPCBEE vol. 23, IACSIT Press, Singapore.

Sharma, G and Nautiyal, A.R. (2009). Influence of explants type and plant growth regulators on In vitro multiple shoots regeneration of a Laurel from Himalaya. Nat. Sci. 7(9): 1-7.

Siddiqui, M., Bhattacharjya, A., Chakraborty, I. and Dhua, R.S. (2011). 6-Benzylaminopurine improves shelf life, organoleptic quality, and healthy-promoting compounds of fresh-cut broccoli florets. J. Sci. Ind. Res. 70(6): 461-465.

Sidhu, Y. (2010). In vitro micropropagation of medicinal plants by tissue culture. The Plymouth Student Scientist, 4(1): 432-449.

Singh, R., Kharb, P. and Rani, K. (2011). Rapid micropropagation and callus induction of *Catharanthus roseus* in vitro using different explants. World J. Agricult. Sci. 7(6): 699-704.

Singh, S., Rathod, Z. and Saxena, O. P. (2010). Multiple shoot regeneration from the callus culture of *Centella asiatica* under the influence of various concentration of PGRs. J. Pure Appl. Sci. 18: 18-20.

Skoog, F. and Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissue culture grown in vitro. Symp. Soc. Exp. Boil. 11:118.

Sookmai, W., Ekalaksananan, T., Pientong, C., Sakdarat, S. and Kongyingyoes, B. (2011). The anti-papillomavirus infectivity of *Clinacanthus nutans* compounds. Srinagarind Med. J. 26:240-242.

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

Su, Y.H., Liu, Y.B. and Zhang, X.S. (2011). Auxin-cytokinin interaction regulates meristem development. Mol. Plant 4(4): 616-625.

Tejavathi, D. H. and Indira M. N. (2013). Regeneration of shoots from leaf callus cultures of *Drymaria cordata* (L) Willd Ex Roem and Schult. Indian J. Fund. Appl. Life Sci. 3(1): 111-115.

Thawaranantha, D., Balachandra, K., Jongtrakulsiri, S., Chavalittumrong, P., Bhumiswasdi, J. and Jayavasu, C. (1992). In-vitro antiviral activity of *Clinacanthus nutans* on Viracella-zoster virus. Siriraj Med. J. 44(4): 285-291.

Thiart, S. (2003). Manipulation of growth by using tissue culture techniques. Combined Proceedings International Plant Propagators' Society. 53: 61-67.

Thorpe, T. (2007). History of plant tissue culture. J. Mol. Microbial Biotechnol. 37: 169-180.

Ting, I.P. (1982). Plant Physiology. Addison-Wesleyn Reading, Massachusetts.

Torres, K.C. (1989). Tissue culture techniques for horticulture crops. (pp. 1-51) New York: Van Nostrand Reinhold.

Tuntiwachwuttikul, P. and Pootaeng, O.Y. (2004). Cerebrosides and a monoacylmonogalactosylglycerol from *Clinacanthus nutans*. Chem. Pharm. Bull. 52(1): 27-32.

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

Vanneste, S. and Friml, J. (2009). Auxin: a trigger for change in plant development. Cell 136: 1005-1016. doi: 10.1016/j.cell.2009.03.001.

Wanikiat, P., Panthong A., Sujayanon, P., Yoosook, C., Rossi, A. G. and Reutrakul, V. (2008). The inflammatory effects and the inhibition of neutrophil responsiveness by *Baeleria lupulina* and *Clinacanthus nutans* extracts. J. Ethnopharmacol. 116(2): 234-244.

Watson, R., Ronald, R.W. and Victor, R.P. (2008). Botanical Medicine in Clinical Practice (pp. 819). Cambridge, CAB International.

Wongrattanapasson, N., Charuwichitratana, S., Timpatanapong, P. and Bunjob, M. (1996). Herpes zoster: treatment with *Clinacanthus nutans* cream. Int. J. Dermatal. 35: 665-666.

Yadav, K.and Singh, N. (2012). Factors influencing in vitro plant regeneration of Liquorice (*Glycyrrhiza glabra* L.). Iranian J. Biotechnol. 10: 161-167.

Yoosook , C., Panpisutchai, Y., Chaichana, S., Santisuk, T., Reutrakul, V. (1999). Evaluation of anti-HSV-2 activities of *Barleria lupulina* and *Clinacanthus nutans*. J. Ethnopharmacol. 67:179-87.

Yuann, J. P., Wang, J., Jian, H., Lin, C. and Liang, J. (2012). Effects of *Clinacanthus nutans* (Burm.f) Lindau leaf extracts on protection of plasmid DNA from riboflavin photoreaction. MC-Transaction on Biotechnol. 4(1): 45-58.