

EFFECT OF NODES EXCISED FROM DIFFERENT POSITION OF Gynura Procumbens MOTHER STOCK PLANT ON REDUCTION OF MICROBIAL CONTAMINATION AND INCREASING IN VITRO EXPLANT SURVIVAL

**NURUL SHAFFIQQA BINTI AZIZ** 

# EFFECT OF NODES EXCISED FROM DIFFERENT POSITION OF Gynura Procumbens MOTHER STOCK PLANT ON REDUCTION OF MICROBIAL CONTAMINATION AND INCREASING IN VITRO EXPLANT SURVIVAL

NURUL SHAFFIQQA BINTI AZIZ

FACULTY OF AGRICULTURE
UNIVERSITI PUTRA MALAYSIA
SERDANG, SELANGOR DARUL EHSAN
2017/2018

# EFFECT OF NODES EXCISED FROM DIFFERENT POSITION OF Gynura Procumbens MOTHER STOCK PLANT ON REDUCTION OF MICROBIAL CONTAMINATION AND INCREASING IN VITRO EXPLANT SURVIVAL

BY NURUL SHAFFIQQA BINTI AZIZ

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

FACULTY OF AGRICULTURE
UNIVERSITI PUTRA MALAYSIA
SERDANG, SELANGOR DARUL EHSAN
2017/2018

#### **ENDORSEMENT**

This project report entitled "Effect Of Nodes Excised From Different Position Of *Gynura Procumbens* Mother Stock Plant On Reduction Of Microbial Contamination And Increasing In Vitro Explant Survival" is prepared by Nurul Shaffiqqa binti Aziz and submitted to the Faculty of Agriculture in fulfilment of requirement of PRT 4999 (Final Year Project) for award of the degree of Bachelor of Agricultural Science.

Student's name:	Student's signature:
Nurul Shaffiqqa binti Aziz.	
	1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1
Certified by:	
Supervisor,	
En. Azmi bin Abdul Rashid,	
Department of Agricultural Technology.	
Date:	

#### ACKNOWLEDGEMENT

I express my greatest thankfulness to the Almighty God for giving me good health, strength, patience and wisdom to complete this final year project successfully. I also show my deepest gratitude to my excellent supervisor, Encik Azmi bin Abdul Rashid for his guidance, assistance, patience, and understanding during the project progression.

I would like to take this opportunity to thank all the staff in the *In Vitro* Laboratory, Department of Agriculture Technology for giving me permission to use lab facilities and preparing apparatus and materials needed for my project. Especially, Puan Rohani and Encik Sharifudin for helping me in media culture guidance until culturing explant technique because this is my first time conducting the tissue culture experiment. In additional, I like to extend my appreciation to my final year project partner, Nur Syazwani for her effort and endless help during the project progression.

I would like to thank my beloved family. Specially, my parents, Encik Aziz bin Harun and Puan Azlina binti Zakaria for their support and encouragement throughout the project. Last but not least, I would like to express my sincere gratitude to my friend, Muhamad Enes Hossien bin Mohd Sies for his endless help and support to me.

## TABLE OF CONTENTS

ENDO	RSEMENT	i
ACKN	OWLEDGEMENT	ii
LIST C	OF TABLES	v
LIST C	OF FIRGURES	vi
LIST C	OF ABBREVIATIONS	vii
ABSTR	RACT	viii
ABSTR	RAK	ix
СНАР	TER 1	
	DDUCTION	1
1.1	Background of Gynura procumbens	
1.1	Problem Statement	 2
1.3	Objective of Experiment	
1.5	Objective of Experiment	
~		
	PTER 2	
LITER	RATURE REVIEW	
2.1	Botany of <i>Gynura procambens</i> and use of the plant	
2.2	In Vitro Culture Technique	
2.3	Contamination of Explant	
2.4	Explant Browning	6
2.5	Explant Sterilization	7
CHAP	TER 3	
METH	IODOLOGY	8
3.1	Stock Solution and Media Preparation	8
3.2	Explant Collection and Selection	
3.3	Aseptic Method	
3.4	Sterilization and Inoculation of Explant	
3.5	Culture Incubation	
3.6	Treatment	
3.7	Parameters observed	
3.8	Experimental Design and Data Analysis	

CHAPTER 4	
RESULT AND DISCUSSION	16
CHAPTER 5	
CONCLUSION	24
APPENDICES	
APPENDIX A	
BASAL STOCK MEDIUM	25
MS (MURASHIGE & SKOOG, 1962) FORMULATION	26
APPENDIX B	
STATISTICAL ANALYSIS	27
BIBLIOGRAPHY	30

# LIST OF TABLES

<b>TABLE</b>	TITLE	PAGE
	The sterilization treatment for different nodal segment	
Table 3.6.1.	explants of Gynura procumbens obtained from the mother	14
	plant.	
	Effect of different node positions on the mother plant on	
Table 4.1.	reduction of fungal and bacterial contamination, explants	19
	browning and explant survival after two weeks of culturing.	

## LIST OF FIRGURES

<b>FIGURE</b>	TITLE	PAGE	
Eigura 2 1 1	The stock solution are prepared according to Murashige &	8	
Figure 3.1.1.	Skoog (1962) formulation.	O	
	The MS medium after autoclaving. The vials are arrange in		
Figure 3.1.2.	a slanting position to allow the medium to slope better	9	
	inoculation of explant.		
Eigene 2.5.1	The node explants cultured in the vials are kept in the	12	
Figure 3.5.1.	incubation room.	12	
Figure 3.6.1	Different position of nodes (shown in numbering label) on	13	
Figure 3.6.1.	the mother plant are used in the study.	13	
Figure 4.1.	Explant on first day of culture in MS medium.	16	
Figure 4.2.	Elongation of bud occurred after five days of culturing the	17	
riguie 4.2.	explant in the medium.	17	
Figure 13	Axillary branching occurs where the axillary buds elongate	17	
Figure 4.3.	and start to branch.	1 /	
Figure 4.4	Multiple buds protrude from the explant after four to five	18	
Figure 4.4. weeks.		10	
	(A) Fungal contamination at the base of the node explant. (B)		
Figure 4.5.	Fungal contamination at the surface of the explant. Fungal	20	
	contamination was observed after culturing the node explant.		
Eigen 4.6	The surface of the culture medium with the mycelium	20	
Figure 4.6.	spreading like a white cotton.	20	
Eigen 47	Bacterial contamination. (A) Bacteria oozes out from the		
	wounded surface at the base of the explant after five days of	21	
Figure 4.7.	culture. (B) It later formed milky colony within two weeks		
	covering the surface of the culture media		
Figure 4.8.	Bacterial colony competing with the explant for growth	22	
	which later caused death of the explant	22	

### LIST OF ABBREVIATIONS

MS Murashige & Skoog

cm centimetre

mg/L milligram per liter

pH Acidity scale °C Degree Celsius

± Plus minus

UV Ultraviolet

kPa kilo Pascal

μmol/m²/s Light intensity unit

#### **ABSTRACT**

Gynura procumbens belongs to the Asteraceae family. This plant grows naturally in tropical Asia countries such as Malaysia and Indonesia. Its common name is longevity spinach or 'sambung nyawa' in Malay. It is a small herbaceous plant with ovate-elliptic leaves and is commonly used as a medical plant. Its leaves contain bioactive compound that can cure several diseases such as cancer, hypertension, diabetes mellitus, kidney discomfort, and inflammation. The common propagation method of this plant is through stem cutting but the number of cuttings that can be excised from a whole plant for propagation is very limited. Therefore, another alternative for mass propagation of Gynura procumbens plants is through tissue culture method. Currently there is no in vitro culture research carried out on Gynura procumbens. This initial study is aimed to reduce microbial contamination and to induce shoot generation from node cultures of Gynura procumbens. The experiment is carried out at In Vitro Laboratory, Department of Agriculture, Universiti Putra Malaysia (UPM) to determine the nodal segment which is less microbial contamination and to evaluate the best concentration and immersion time of explant in Clorox in reducing microbial contamination. The experiment is conducted using Completely Randomised Design (CRD). It was observed that no significant difference was observed on percentage of fungal and bacterial contamination, explants browning and explant survival by the nodes excised from different position on the mother stock plant. Fungal and bacterial contamination in these explants ranged between 16% to 73% and 6% to 20% respectively. The explant survival ranged between 80% and 90% in these explants indicating that these explants are good material for initiating culture establishment and later propagule multiplication.

#### **ABSTRAK**

Gynura procumbens tergolong dalam keluarga Asteraceae. Tumbuhan ini tumbuh secara semulajadi di negara Asia tropika seperti Malaysia dan Indonesia. Nama biasa ialah bayam panjang umur atau 'sambung nyawa' dalam Bahasa Melayu. Ia adalah tumbuhan herba kecil dengan daun ovate-eliptik dan biasanya digunakan sebagai tumbuhan perubatan. Daunnya mengandungi sebatian bioaktif yang boleh menyembuhkan beberapa penyakit seperti kanser, tekanan darah tinggi, kencing manis, ketidakselesaan buah pinggang, dan keradangan. Kaedah pembiakan umum tumbuhan ini adalah melalui pemotongan batang tetapi bilangan keratan yang boleh dikeluarkan dari seluruh tumbuhan untuk pembiakan sangat terhad. Oleh itu, satu lagi alternatif pembiakan Gynura procumbens untuk menghasilkan tumbuhan yang banyak adalah melalui kaedah kultur tisu. Pada masa ini tiada penyelidikan *in vitro* dijalankan pada Gynura procumbens. Kajian awal ini bertujuan untuk mengurangkan pencemaran mikrob dan untuk mendorong pembiakan Gynura procumbens daripada tunasnya. Eksperimen ini dijalankan di Makmal In Vitro, Jabatan Pertanian, Universiti Putra Malaysia (UPM) untuk menentukan segmen nod yang kurang pencemaran mikrob dan untuk menilai masa tumpuan dan rendaman yang terbaik dalam Clorox dalam mengurangkan pencemaran mikrob. Eksperimen ini dijalankan menggunakan Reka Bentuk Rawak Lengkap (CRD). Didapati tidak terdapat perbezaan bererti pada peratus pencemaran eksplan oleh kulat dan bakteria dan pemerangan eksplan dan peningkatan keboleh hidupan eksplan. Pencemaran eksplan oleh kulat dan bakteria pada eksplan ini berjulat antara 16% hingga 73% dan 6% hingga 20%. Keboleh hidupan eksplan pula berjulat antara 80% dan 90% pada eksplan ini menunjukkan eksplan ini adalah bahan biak yang baik untuk memulakan aruhan kultur dan seterusnya menggalakkan penggandaan propagul.

#### **CHAPTER 1**

#### INTRODUCTION

#### 1.1 Background of Gynura procumbens

Gynura procumbens (Lour.) Merr. is an essential medicinal plant belonging to the Asteraceae family. It is a native herb in the tropical Asia countries such as Thailand, Vietnam, Myammar, China, Indonesia and Malaysia (Dwijayanti & Rifa, 2015). It has been widely used as a traditional herbal medicine. In Malaysia, the Malays called it as 'Sambung Nyawa' which means 'prologation of life' while the Chinese called it as 'bai bing ca' which means '100 ailments' (Bodeker et al., 2009). This plant is also well known as 'Longevity Spanish'. The distribution of this species is limited to the western part of peninsular Malaysia (Keng et al., 2009).

In this sophisticated era, the chronic ailments are very critical. Thus, people change their lifestyles to become healthier and very sensitive toward health care issues. Most research reported that the risk of varying diseases can be reduced by consuming the medicinal herbs, vegetables and fruits (Deng et al., (2013). The extracts from the plants are the best remedy of curing the diseases. Based on that fact, the traditional medicinal herbs have been widely used as ingredients in pharmaceutical product including *Gynura procumbens*.

Almost all pharmaceutical products used the plant extract as the main ingredient (Zhou & Wu, 2006). *Gynura procumbens* have also been used as ethnoherbal product (Keng et al., 2009). The most useful part of *Gynura procumbens* plant is the leaves. A few decades ago, *Gynura procumbens* leaves are often eaten raw with rice. Dwijayanti & Rifa (2015) stated that the leaves have many benefits in curing various diseases such as eruptive fever, rash, kidney disease, migraine, constipation, hypertension, diabetes mellitus and cancer. Recently, many researches have been carried out to prove the goodness of *Gynura procumbens* for maintaining good health.

#### 1.2 Problem Statement

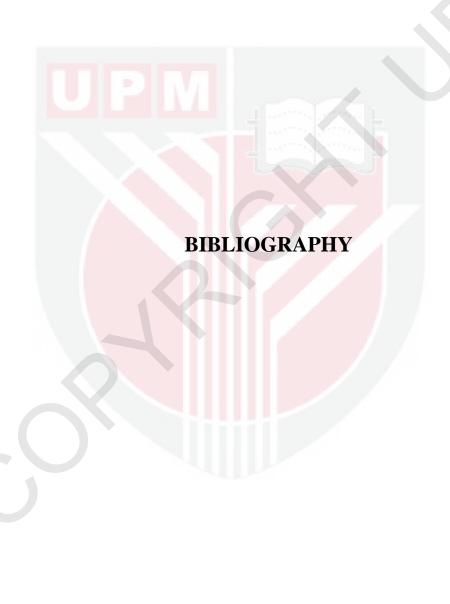
Conventionally, *Gynura procumbens* is propagated by cutting. In the preparation of pharmaceutical products, the demand of the raw material has increase due to its medicinal values. Propagation using cuttings cannot deal with the increasing demand of this plant. This is because of the limited number of stem cutting that can be excised from a whole plant. Therefore, there are less number of planting materials be produced.

In vitro propagation can be another alternative for production of Gynura procumbens planting materials. At present, there is not much research carried out on in vitro culture of Gynura procumbens. This initial study aims to reduce microbial contamination and induce shoot regeneration from Gynura procumbens node culture.

### 1.3 Objective of Experiment

The objectives of this initial study are:

 To see the effect of different node positions on the mother plant on reduction of fungal and bacterial contamination, explants browning and explant survival.



- Aziah, M., Nor Aini, A., Kamis, A., & Mihdzar, A. (2013). Incidence of Fern

  Contamination in Nodal Segment Cultures of Shorea parvifolia Dyer. *Pertanika Journals of Tropical Agricultural Science*, *36*, 67–78.
- Babaei, N., Psyquay, A., Ghizan, S., & Thohirah, L. (2013). Control of contamination and explant browning in Curculigo latifolia in vitro cultures. *J Med Plants Res*, 7(8), 448–454. https://doi.org/10.5897/JMPR012.859
- Bodeker, G., Salleh, H., & Shekar, S. C. (2009). *Health and Beauty from the Rainforest:*Malaysian Traditions of Ramuan.
- Cassells, A. C. (2012). Pathogen and Biological Contamination Management in Plant Tissue Culture: Phytopathogens, Vitro Pathogens, and Vitro Pests. In *Plant Cell Culture Protocols* (pp. 57–80). https://doi.org/10.1007/978-1-61779-818-4\_6
- Çördük, N., & Aki, C. (2011). Inhibition of browning problem during micropropagation of Sideritis trojana bornm., an endemic medicinal herb of Turkey. *Romanian Biotechnological Letters*, *16*(6), 6760–6765.
- Dalga, H. R. (2012). Plant Tissue Culture, (August), 759–767.
- Daud, N. H., Jayaraman, S., & Mohamed, R. (2012). Methods paper: An improved surface sterilization technique for introducing leaf, nodal and seed explants of Aquilaria malaccensis from field sources into tissue culture. *Asia-Pacific Journal of Molecular Biology and Biotechnology*, 20(2), 55–58.
- Deng, Y., Matsui, Y., Zhang, Y., & Lai, Z. C. (2013). Hippo activation through homodimerization and membrane association for growth inhibition and organ size control. *Developmental Biology*, *375*(2), 152–159. https://doi.org/10.1016/j.ydbio.2012.12.017

- Dwijayanti, D. R., & Rifa, M. (2015). THE JOURNAL OF TROPICAL LIFE

  SCIENCE OPEN ACCESS Freely available online Gynura procumbens Ethanolic

  Extract Promotes Lymphocyte Activation and Regulatory T Cell Generation In

  Vitro. *Life. Science*, 5(1), 14–19. https://doi.org/10.3390/molecules15129008
- Eksomtramage, L., Jornead, S., Kaewnam, W., & Mama, W. (2010). Karyotypic studies in four species of, 90–94.
- Filiz, A., Betuuml I, B. ruuml n, & Nurettin, Ş. (2010). Fungal contaminants observed during micropropagation of Lilium candidum L. and the effect of chemotherapeutic substances applied after sterilization. *African Journal of Biotechnology*, 9(7), 991–995. https://doi.org/10.5897/AJB08.090
- George, E. F., Hall, M. A., & Klerk, G. J. D. (2008). Plant propagation by tissue culture. Volume 1: the background. Plant propagation by tissue culture. Volume 1: the background. https://doi.org/10.1016/0304-4238(85)90041-X
- Hand, C. R., Wada, N., Stockwell, V., & Reed, B. M. (2016). Node position influences viability and contamination in hazelnut shoot explants. *In Vitro Cellular and Developmental Biology Plant*, 52(6), 580–589. https://doi.org/10.1007/s11627-016-9791-4
- Keng, C. L., Yee, L. S., & Pin, P. L. (2009). Micropropagation of Gynura procumbens (Lour .) Merr. an important medicinal plant. *Journal of Medicinal Plants*, *3*(3), 105–111.
- Maung Lwin, K. (2011). *Gynura procumbens (Lour.) Merr*. FAME Publishing House.

  Retrieved from

  http://nebula.wsimg.com/11e1eda408f83bc09a0ab44c2eae5f0a?AccessKeyId=618

3DC1A959AC9A2AA88&disposition=0&alloworigin=1

- Moura, M., Candeias, M. I., & Silva, L. (2009). In vitro propagation of viburnum treleasei Gand., an Azorean endemic with high ornamental interest. *HortScience*, 44(6), 1668–1671.
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant*, *15*, 473–497. https://doi.org/DOI: 10.1111/j.1399-3054.1962.tb08052.x
- Nobre, J., Santos, C., & Romano, A. (2000). Micropropagation of the Mediterranean species Viburnum tinus. *Plant Cell, Tissue and Organ Culture*, 60(1), 75–78. https://doi.org/10.1023/A:1006405700121
- Rana, M., & Khan, S. (2016). Study of Bacteria and Fungi Isolate from Contaminated

  Banana Tissue Culture. Retrieved from

  https://www.ijirset.com/upload/2016/march/11\_Study.pdf
- Ru, Z., Lai, Y., Xu, C., & Li, L. (2013). Polyphenol Oxidase (PPO) in Early Stage of Browning of Phalaenopsis Leaf Explants. *Journal of Agricultural Science*, *5*(9). https://doi.org/10.5539/jas.v5n9p57
- Sabaz Ali Khan, Hamid Rashid, M. F. C. and Z. C. (2007). Optimization of sterilization conditions in sugarcane.pdf. *Pakistan J. Agric. Res*.
- Smith, R. H. (2013). Plant Tissue Culture. Plant Tissue Culture. https://doi.org/10.1016/C2011-0-04367-3
- Sood, A., Nadha, H., Salwan, R., Kasana, R., & Anand, M. (2012). Identification and elimination of bacterial contamination during in vitro propagation of Guadua angustifolia Kunth. *Pharmacognosy Magazine*, 8(30), 93. https://doi.org/10.4103/0973-1296.96547

- Tan, H. L., Chan, K. G., Pusparajah, P., Lee, L. H., & Goh, B. H. (2016). Gynura procumbens: An overview of the biological activities. *Frontiers in Pharmacology*, 7(MAR). https://doi.org/10.3389/fphar.2016.00052
- Zheng, R., Wang, W., Tan, J., Xu, H., Zhan, R., & Chen, W. (2017). An investigation of fungal contamination on the surface of medical herbs in China. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5209813/
- Zhou, L. G., & Wu, J. Y. (2006). Development and application of medicinal plant tissue cultures for production of drugs and herbal medicinals in China. *Natural Product Reports*, 23(5), 789. https://doi.org/10.1039/b610767b