UNIVERSITI PUTRA MALAYSIA

COLLECTION, MORPHOLOGICAL CHARACTERIZATION, BIOACTIVITY EVALUATION AND MICROPROPAGATION OF SELECTED TACCA SPECIES (DIOSCOREACEAE)

MOHD ZULHILMI MISROL

FP 2016 60

COLLECTION, MORPHOLOGICAL CHARACTERIZATION, BIOACTIVITY EVALUATION AND MICROPROPAGATION OF SELECTED *TACCA* SPECIES (DIOSCOREACEAE)

By

MOHD ZULHILMI MISROL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

April 2016

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

COLLECTION, MORPHOLOGICAL CHARACTERIZATION, BIOACTIVITY EVLUATION AND MICROPROPAGATION OF SELECTED *TACCA* SPECIES (DIOSCOREACEAE)

By

MOHD ZULHILMI MISROL

April 2016

Chairman : Associate Professor Thohirah Lee Abdullah, PhD
Faculty : Agriculture

Studies were carried out with the objectives of establishing a germplasm collection of selected *Tacca* species from various accessions in Malaysia, to describe the morphological characters of selected *Tacca* species, to evaluate anti-cancer, anti-histamine and anti-inflammatory activity using various parts of plant crude extract and to improve and develop tissue culture protocol for *T.integrifolia*. *Tacca* (Dioscoreaceae) is a native understorey medicinal plant with a great potential to be developed as an ornamental plant and is considered rare in Malaysia. A germplasm collection consisting of 60 samples representing four species from three states of Peninsular Malaysia and one of Sarawak was established in Field 2 of the University Farm, UPM. *Tacca* species was found growing under various environment conditions where *T. integrifolia, T. chantrieri* and *T .nivea* thrived on the moist soils of the forest floor in well-drained areas, high humidty and under more than 70% shade. In contrast, *T. leontopetaloides* was found in dappled shade under coconut trees and under full sun (0% shade) along coastal areas. The conserved *Tacca* species has been characterized for important vegetative and morphological characters for utilization as an ornamental and medicinal plant.

The most variation among *Tacca* species differed in their seed shape, apices of innermost bracts, bract and bracteoles color. The bract and bracteoles color were purple color in *Tacca integrifolia*, dark purple color in *Tacca chantrieri,* white purplish in *Tacca nivea* and green color in *Tacca leontopetaloides.*Three different groups were determined from group cluster and dendrogram based on 23 qualitative and 12 quantitative characteristics among *Tacca* species using MVSP programme.

Various plant parts of selected *Tacca* species [*Tacca integrifolia* (TI), *Tacca chantrieri* (TC) and *Tacca nivea* (TN)] were extracted and tested for their *in vitro* cytotoxicty in cancer cell lines using MTT assay. Results obtained showed that the rhizome extracts were the most potent among the various parts of the plants. Among the rhizome extracts, TI showed the most promising anti-tumour activity, followed by TN and TC. Further investigation on TI revealed that HCT116 and PC-3 cells were the most

sensitive towards the rhizome extracts, with GI_{50} values of 3.3 ± 1.3 µg/mL and 4.0 ± 0.8 µg/mL respectively. In conclusion, the rhizome extract of TI emerged as the most potent and further study on the plant is warranted.

Antihistamine and nitric oxide (NO) inhibitory activity were carried out using methanolic extract from various plant parts (PPCE) and selected *Tacca* species (TSCE). The antihistamine activity was tested in rat mast cell (RBL-2H3) line. Results obtained shows mild antihistamine activity among PPCE and TSCE without showing cytotoxicity activity on RBL-2H3 cells after 4 h using MTT assay. The NO inhibitory activity was determined in a murin macrophage cell line (RAW 264.7). The results demonstrated weak NO inhibitory activity by *T. integrifolia* rhizome extract (TIR) compared with others TSCE and PPCE. Cell exposed to the extracts showed viability with a range of 60 to 90%.

The sterile seeds for the propagation of sterile seedlings were prepared by surface sterilization of loose seed (LS) in 10% commercial bleach, CLOROX and aseptic removal of sterile seeds from fruits pre-sterilized (FS) by burning using 95% ethanol. The sterile seeds from both sources were cultured on the sterile ½ MS and full MS media containing 30 g/L sucrose, pH 5.6 for 4 months. The highest percentage of germination of *T.integrifolia* seeds were recorded from LS in ½ MS media (56%), follow by FS with ½ MS media (16%), LS with full MS (10%) and FS with full MS media (8%) after four months of cultured.

The effect of *in vitro* vertical cutting and decapitation on efficient shoot multiplication of *T. integrifolia* was investigated via shoot tip in MS medium and MS medium fortified with 1 mg/L of 6-benzylaminopurine (BAP). *In vitro* seedlings (2 to 4 cm in height) were cut directly by vertical and decapitated 0.5-0.7 cm above from cotyledon node. After 12 weeks of culture, plantlets regenerated from decapitation in MS medium fortified with 1 mg/L BAP were able to produce new healthy shoots higher than intact and vertical cutting plantlet in the same medium.

The protocol for *in vitro* propagation of *T. integrifolia* through shoot was carried out. The shoot derived from sterile seedling was used as explants for shoot induction and multiplication. The explants were harvested from 16 weeks old seedling and cultured on solidified MS medium supplemented with various concentration of BAP (0, 1, 2, 3, 4, 5 mg/L) for shoot induction and multiplication. The highest number of new shoots and leaves were obtained in MS medium supplemented with 3 mg/l BAP. MS medium with 0.3 mg/L IBA was recommended for root induction after eight weeks of culture with highest number of healthier root.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KOLEKSI, PENCIRIAN MORFOLOGI, PENILAIAN BIOAKTIVITI DAN MIKROPROPAGASI *TACCA* SPESIS TERPILIH (DIOSCOREACEAE)

Oleh

MOHD ZULHILMI MISROL

April 2016

Pengerusi : Profesor Madya Thohirah Lee Abdullah, PhD
Fakulti : Pertanian

Kajian telah dijalankan dengan objektif untuk mewujudkan koleksi germplasma spesies *Tacca* terpilih dari pelbagai tempat penemuan baharu di Malaysia, untuk menghuraikan ciri-ciri morfologi bagi spesies *Tacca* terpilih, untuk menilai aktiviti anti-kanser, anti-histamine dan anti radang dengan menggunakan pelbagai ekstrak mentah dari bahagian tumbuhan dan untuk penambahbaikkan dan mewujudkan protokol kultur tisu untuk *Tacca integrifolia*. *Tacca* (Dioscoreaceae) adalah tumbuhan ubatan di kaki hutan yang berpotensi besar untuk dibangunkan sebagai tumbuhan hiasan dan sukar ditemui di Malaysia. Satu koleksi germplasma yang terdiri daripada 60 sampel yang mewakili empat spesies daripada tiga negeri di Semenanjung Malaysia dan satu di Sarawak, telah dibangunkan di Lapangan 2, Ladang Universiti Putra Malaysia. Spesies *Tacca* telah ditemui tumbuh di bawah pelbagai keadaan persekitaran di mana *T. integrifolia*, *T. chantrieri* dan *T. nivea* hidup subur di kawasan lantai hutan bertanah lembap dan bersaliran baik, berkelembapan tinggi dan di bawah lebih daripada 70% teduhan. Sebaliknya, *T. leontopetaloides* didapati di bawah naungan belang-belang pokok kelapa dan di bawah sinaran matahari penuh (0% naungan) sepanjang kawasan pantai. Spesies *Tacca* yang dipulihara dikenalpasti ciri-ciri morfologi dan vegetatif yang penting bagi penggunaan sebagai tumbuhan hiasan dan ubatan.

Kebanyakkan variasi bagi spesies *Tacca* telah dikenalpasti dengan ketara pada bentuk biji benih, bentuk hujung pelepah bunga yang terdalam, warna pelepah bunga dan misai pelepah bunga. Warna pelepah bunga dan misai pelepah bunga saling berinteraksi antara satu sama lain di mana warna ungu pada *T. integrifolia*, warna ungu gelap pada *T. chantrieri*, ungu keputihan pada T. nivea dan warna hijau pada *T. leontopetaloides*. Tiga kumpulan yang berbeza telah ditentukan dari kelompok kluster dan dendrogram berdasarkan 23 ciri kualitatif dan 21 ciri kuantitatif antara spesies *Tacca* terpilih menggunakna program MVSP.

Pelbagai bahagian tumbuhan daripada species *Tacca* terpilih [*T. integrifolia* (TI), *T. chantrieri* (TC) dan *T. nivea* (TN)] telah diekstrakkan dan kesitotoksikan *in vitro* diuji dalam tiga bahagian sel kanser [HCT116 (usus), PC-3 (prostat) dan MCF-7 (payudara)] dengan menggunakan ujian MTT. Saringan awal mendapati ekstrak rizom adalah yang

paling berkesan di kalangan pelbagai bahagian tumbuhan untuk perencatan bahagian sel kanser. Di antara ekstrak rizom bagi spesies *Tacca* terpilih, TI menunjukkan aktiviti anti-tumor yang paling menjanjikan, diikuti oelh TN dan TC. Siasatan lanjut mengenai tindak balas dos TI menunjukkan bahawa sel HCT116 dan PC-3 adalah yang paling sensitif terhadap ekstrak rizom, dengan nilai GI_{50} masing masing sebanyak 3.3 ± 1.2 µg/mL dan 4.0 ± 0.8 µg/mL. Kesimpulannya, ekstrak rizom daripada TI muncul sebagai yang paling mujarab dan kajian lanjut mengenai tumbuhan ini adalah wajar dilakukan.

Aktiviti antihistamin dan penghalang nitric oksida (NO) telah dijalankan menggunakan ekstrak methanol dari pelbagai bahagian tumbuhan (PPCE) dan spesies Tacca terpilih (TSCE). Aktiviti antihistamin telah diuji dalam jaluran sel leukemia basofilik tikus (RBL-2H3). Keputusan yang diperolehi menunjukkan aktiviti antihistamin yang lemah di antara PPCE dan TSCE tanpa menunjukkan aktivitik sitotoksik terhadap sel RBL-2H3 selepas 4 jam menggunakan ujian MTT. Aktiviti penghalang NO diukur dalam jaluran sel makrofaj murin (RAW 264.7). Keputusan menunjukkan aktiviti penghalang NO yang lemah oleh ekstrak rizom T. integrifolia (TIR) berbanding dengan TSCE dan PPCE. Sel yang dirawat dengan ekstrak menunjukkan daya maju sel dari 60 hingga 90%.

Biji benih yang steril untuk pembiakan anak benih yang steril telah disediakan dengan pensterilan permukaan biji benih longgar (LS) di dalam 10% peluntur komersial, CLOROX, dan penyingkiran aseptik biji benih yang steril daripada buah (FS) yang terlebih dahulu disterilkan dengan membakar menggunakan 95% etanol. Biji benih steril daripada kedua-dua sumber dikulturkan di atas media ½ MS dan MS penuh yang steril, mengandungi 30 g/L sukrosa, pH 5.6 selama empat bulan. Peratusan tertinggi bagi percambahan biji benih *T. integrifolia* telah direkodkan dari biji benih longgar dalam media ½ MS (56%), diikuti dengan biji benih steril dari buah dengan media ½ MS (16%), biji benih longgar dengan media MS penuh (10%) dan biji benih steril dari buah dengan media MS penuh (8%) selepas empat bulan dikulturkan.

Kesan pemotongan *in vitro* secara menegak dan pemenggalan dalam penggandaan pucuk *T. integrifolia* yang efisian dikaji menggunakan hujung pucuk dalam media MS dan media MS diperkaya dengan 1 mg/L 6-benzylaminopurine (BAP). Anak benih *in vitro* (2 hingga 4 cm tinggi) dipotong terus secara menegak dan dipenggal 0.5-0.7 cm dari atas ruas kotiledon. Selepas 12 minggu pengkulturan, anak pokok dijana dari pemenggalan di dalam media MS diperkaya dengan 1 mg/L BAP dapat menghasilkan pucuk baru yang sihat, lebih banyak daripada anak pokok yang dipenggan dan utuh di dalam medium yang sama. Protokol bagi pembiakan *in vitroT. integrifolia* melalui hujung pucuk telah dijalankan. Hujung pucuk yang berasal dari anak benih steril digunakan sebagai eksplan untuk penjanaan dan penggandaan pucuk. Eksplan dituai dari anak benih yang berusia 16 minggu dan dikulturkan dalam media MS pejal yang dibekalkan dengan pelbagai kepekatan BAP (0, 1, 2, 3, 4, 5 mg/L) untuk induksi dan penggandaan pucuk. Bilangan tertinggi pucuk dan daun baru telah diperolehi dalam media MS diperbekalkan dengan 3 mg/L BAP. Media MS diperkayakan dengan 0.3 mg/L IBA telah disyorkan untuk induksi akar selepas lapan minggu dikulturkan dengan jumlah akar sihat tertinggi.

ACKNOWLEDGEMENTS

All praises and thanks due to Allah Almight for His Mercy and Grace.

I would like to express my sincere thanks to Assoc. Prof. Dr. Thohirah Lee Abdullah, chairman of my supervisory committee, for her dedicated efforts, support, invaluable advice and intellectual guidance during the accomplisment of this research work. I would also like to thank my supervisory committee members, Prof. Dr. Johnson Stanslas and Assoc. Prof. Dr. Maheran Abd Aziz for their guidance, assistant and encouragement throughout the period of this study. I greatly appreciate all the help they availed to me while pursuing my studies.

I would like to thank the Ministry of Education from which Assoc. Prof. Dr. Thohirah Lee Abdullah obtained this research under Fundamental Research Grant Scheme (FRGS) (01-12-10-982FR). I would like to appreciate and thank Universiti Putra Malaysia, for give financial support under Research Universiti Grant (RUGs) (01-02-12-1692RU) and provided me the two semesters Graduate research Fellowship (GRF).

I am indebted to Mr. Kenny from Penang Spice Garden, Officers from Penang Botanical Garden and National Park, staff from Bako National Park for their useful insights. I am also grateful to the laboratory members of the Pharmaceutical Laboratory, Lim Chee Woei, Wong Chang Churn and Ethel Ravendran for their kind help and assistance. My sincere appreciations also goes to my colleagues and fellow students in Floriculture Laboratory,Department of Crop Science, Universiti Putra Malaysia for their support, encouragement, co-operation and involvements in discussions during the study.

My deepest gratitude goes to my parents, brothers for their love, support and encouragement. Finally, a very heartfelt gratitude and appreciation goes to my best friend, Norsyalina Ramli for her constant encouragement, patience, great companion and moral support throught my graduate program and for being there for me.

I certify that a Thesis Examination Committee has met on 14 April 2016 to conduct the final examination of Mohd Zulhilmi bin Misrol on his thesis entitled "Collection, Morphological Characterization, Bioactivity Evaluation and Micropropagation of Selected *Tacca* Species (Dioscoreaceae)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Adam b Puteh, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Rusea Go, PhD
Associate Professor
Faculty of Sciece
Universiti Putra Malaysia
(Internal Examiner)

Normah Mohd Noor, PhD
Professor
Institute of Systems Biology
Universiti Kebangsaan Malaysia
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 26 July 2016

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Thohirah Lee Abdullah, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Johnson Stanslas, PhD
Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Maheran bt Abd Aziz, PhD
Associate Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Mohd Zulhilmi Misrol, GS32038

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature:_____
Name of
Chairman of
Supervisory
Committee: Thohirah Lee Abdullah, PhD

Signature:_____
Name of
Member of
Supervisory
Committee: Johnson Stanslas, PhD

Signature:_____
Name of
Member of
Supervisory
Committee: Maheran Abd Aziz, PhD

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
BAP	6-benzylaminopurine
CRD	Completely randomized design
^{o}C	Degree celcius
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
FBS	Foetal bovine serum
FS	Seed from fresh fruit
GI_{50}	Concentration of 50% growth inhibition
HCT116	Colon cancer line
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
IC_{50}	Half maximal inhibitory concentration
iNOS	Inducible nitric oxide synthase
LC_{50}	Lethal concentration
LPS	Lipopolysacharide
LS	Loose seed
MCF-7	Breast cancer cell line
µg	microgram
µM	micromolar
MS	Murashige and Skoog's medium
MSH	Murashige and Skoog's medium+1 BAP
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MVSP	Multivariate System Package
N	north
NAA	1-naphthalene acetic acid
NO	Nitric oxide
NOS	Nitric oxide synthase
PBS	Phosphate buffer saline
PC-3	Prostate cancer cell line

PPCE	Plant part crude extract
S	Second
SAS	Statistical analysis system
S.D	Standard deviation
S.E	Standard error
TC	*Tacca chantrieri*
TCR	*Tacca integrifolia* rhizome crude extract
TGI	Concentration that produces total growth inhibition
TI	*Tacca integrifolia*
TL	*Tacca leontopetaloides*
TN	*Tacca nivea*
TSCE	*Tacca* species crude extract
UPGMA	Unweighted pair group method using arithmetic averages
UV	Ultra violet

CHAPTER I

INTRODUCTION

As one of the 12 mega-diversity countries in the world, Malaysia has not less than 15,000 species of vascular plants (Jamadon *et al.,* 2007). *Tacca* comes from the familyDioscoreaceae and is a native understorey ornamental and medicinal plant that has a great potential to be developed as a commercial plant in Malaysia. *Tacca* is an evergreen herbaceous and perennial plant with thick rhizomes or tubers and possess stunning inflorescenses. Typically, *Tacca* inhabits the moist and shaded understory environment in the tropical lowland forest and hilly areas, at an altitude of 1300m above sea level (Chooi, 2004). A great diversity of *Tacca* species can be found in Malaysia where five of the species are distributed in this region namely *Tacca integrifolia, Tacca chantrieri, Tacca nivea, Tacca leontopetaloides* and *Tacca palmata*.

The inflorescense has whisker-like filiform bracteoles and the colour of the two conspicuous inner involucral bracts range from white, green, purple, brown to near black colour. The true flowers of *Tacca* are dark purple, brown, or near black in colour and they are actinomorphic, hermaphroditic with six stamens (Zhang *et al.,* 2005). Unfortunately, *Tacca* species population in tropical forests are threatened by human development and its habitat preservation in many cases is not considered important. The natural habitat of many species have been totally destroyed, and those of many others have been reduced in size and highly fragmented that the species are in imminent danger of extinction. If the natural populations become extinct, *ex situ* conserved population can be used to maintain the evolutionary process of the endangered species, and will be released back to nature for its habitat restoration (Cohen *et al.,* 1991). In such cases, *ex situ* conservation is essential to preserve a species that has left to extinction in nature (Maxted *et al.,* 1997). Thus, *ex-situ* conservation is required to preserve a species that is in danger of extinction in nature (Li *et al.,* 2002).

The *ex situ* conservation of Tacca species is to keep records of the plant characteristics for identification purposes and historical data. It is also for identification of the economically important varieties on their multiplication in order to reintroduce the species into their natural population and for the aim of commercialization so that the industries are less dependent on harvesting this particular species directly from the wild nature.

The abundance of medicinal plants serves as an ideal resource that can unleash new discoveries in the medicine industry. They have proven beneficial because they contain various phytochemicals which are natural molecules produced by plants for protection. These plants serve as an alternative to modern medicine for most local and tribal communities. Traditional use of herbal medicine consist of herbs, herbal material, herbal preparations and finished herbal products that have active ingredients in various plant parts.

The most serious threats to human health in the world is a cancer and chemotherapy is still the standard treatment method. Most of the anticancer drugs presently applied in chemotherapy are cytotoxic to normal cells and cause immunotoxicity which have an effect not only tumor development, but also aggravates patient's recovery. The finding and identification of new antitumor drug with low side effects on immune system has become an important goal in many studies of immunopharmacology (Xu *et al.,* 2009). With this objective, many researches have been carried out to natural compounds in plants, marine organism and microorganisms. Relating to the low side effects of plants and other natural compounds, many researchers and scientists are concerned in working on them to find new medications. Discovery of anticancer agents from plant sources started in the earliest 1950s with the finding and development of vinblastine and vincristine, vinca alkaloid and the isolation of cytotoxic podophyllotoxins (Cragg and Newman, 2005). Rhizomes of *Tacca* have been applied in Chinese folk medicine for the treatment of gastric ulcer, hepatitis and enteritis (Yokosuka *et al.,* 2005). Previously, the structural characterization and isolation of diarylheptanoids, steroidal glycosides and diarylheptanoid glucosides, such as furostan, spirostan, pseudofurostan, withanolide glucoside and pregnane glycosides from the rhizomes of *T. chantrieri* have been carried out, as well as their cytotoxic activities against cultured normal and tumor cells were reported by Yokosuka *et al.* (2002a,b,c,d).

Allergy is a serious health problem worldwide due to disfunction of immune system. This term was emerged initially form 20^{th} century and describe about acute hypersensitive immune responses (anaphylaxis) to allergens by predisposed individuals. Allergen is a substance that causes organ disfunction and tissue inflammation (allergic reaction) such as food, moles spores, cosmetics, animal hairs, dust mites and pollen. Mast cell is an important mediator of allergy, which is constituent of virtually all tissue and organ. Activation of mast cell will results in the release of mediators such as histamine and serotonin within minutes (Matsuda *et al.,* 2004). The enzyme (β-hexosaminidase) was released a histamine and used as a marker of mast cell and basophil degranulation, which are stored in the secretory granules (Kraithep *et al.,* 2008). There are numerous pharmacological agents that available for allergic treatment. The mechanism of drug that effective against allergy was acted on a target, influenced by multiple mediators within the allergy cascade. The establishment of compound as powerful tools for allergy treatment was successed, if the activity of antigen (IgE) intefering compound against a diverse group of allergy mediators was achieved. Therefore, novel approaches are warranted by screening some useful candidates as anti-allergic drugs, so that a few novel therapeutic candidates can be identified.

Mast cells play an important role in the number of physiological process of homeotasis and disease. Nitric oxide (NO), produced by nitric oxide synthase (NOS), is diatomic radical with cytotoxic properties. Many mast cell functions can be influenced by NO including degranulation, leukotriene production, early mediator release and adhesion (Mc Cauley *et al.,* 2005). Nitric oxide might occured as a source of free radicals, leading to infiltration and damage of tissue of lymphocytes and inflammatory reactions. Some medicinal plants significantly showed inhibition of nitric oxide level, are performed as potential candidates for new anti-inflammatory drug. Inflammation is a biological reaction to noxious stimuli such as pathogens, which cause cell and tissue

damage. It is acted as a protective measure by removing harmful stimuli and to start process of healing.

Tissue culture technique has been used for the *in vitro* mass propagation serves the future requirement instead of the conventional propagation method. *In vitro* conservation can be performed by normal *in vitro* culture preservation, slow growth conservation and cryopreservation. Normal preservation and cryo-preservation are not practical due to the frequent subculture requirement (Peschke and Phillips 1992). The micropropagation of *Tacca chantrieri* has been investigated using young leaves and leaf stalks of seedling via callus induction (He *et al.*, 2002). Axillary and adventitious shoots of seedling as explants for *in vitro* propagation of *T. Chantrieri* also have been reported (Choraensub *et al*., 2008). In 2011, the micropropagation *Tacca leontopetaloides* was studied by Brokini *et al*. (2013) using seed embryos.

This study was designed to achieve the following general objectives:

a) To investigate morphological characterization among selected *Tacca* species
b) To evaluate cytotoxicity potential, anti-histamine and anti-inflammatory activities of selected *Tacca* species
c) To establish a tissue culture protocol for *T. integrifolia*

From each experiment, the specific objectives of this study were as follows:

a) To establish a germplasm collection of selected *Tacca* species from Peninsular Malaysia and Borneo.
b) To describe the morphological characters among selected *Tacca* species for identification.
c) To screen cytotoxicity activities of selected *Tacca* species in MCF-7 (Breast), HCT116 (colon) and PC-3 (prostate) cancer cell lines using MTT assay.
d) To screen anti-allergy activity of selected *Tacca* species using antihistamine assay.
e) To screen anti-inflammatory of selected *Tacca* species using Griess assay.
f) To optimize sterilization protocol and concentration of MS medium *in vitro* seed germination of *T. integrifolia*.
g) To improve shoot multiplication technique of *T. integrifolia*.
h) To determine optimum concentration of 6-benzylaminopurine (BAP) for shoot regeneration.
i) To determine optimum concentration of 1-napthaleneacetic (NAA) and indole-3-butric acid (IBA) for root induction.

REFERENCES

Afui, M. M., Kinge, R. T. & Lawrence, M. N. (2008). Morphological characterization of four selections of *Vernonia hymenolepis* A. Rich. (Asteraceae). *World Journal of Agricultural Sciences.* 4 (2): 220-223.

Aggarwal, B. B., Kunnumakkara, A. B., Harikumar, K. B., Tharakan, S. T., Sung, B. & Anand, P. (2008). Potential of spice-derived phytochemicals for cancer prevention. *Planta Medica*. 74: 1560-1569.

Ahmed, M. F., Kantharajah, A. S. & Holford, P. (1997). *In vitro* regeneration of avocado (*Persea americana* Mill.) and transient gene expression. *Acta Horticulturae*. 461: 339-348.

Akdis, C. A. & Simons, F. E. R. (2006). Histamine receptors are hot in immunopharmacology. *European Journal of Pharmaceutical.* 533:69 –76.

Akin_Idowu, P. E., Ibitoye, D. O. & Ademoyegun, O. T. (2009). Tissue culture as plant production technique for plant horticultural crop. *African Journal of Biotechnolo*gy. 8(16): 3782-3788.

Alfred, O. U. & Uchenna, E. O. (2013). Micropropagation and postflask management of sweet potato using locally available materials as substrates fro hardening. *Plant Knowledge Journal*. 2(2): 56-61.

Amin, A. R., Kucuk, O., Khuri, F. R. & Shin, D. M. (2009). Perspectives for cancer prevention with natural compounds. *Journal of Clinical Oncology*. 27(16): 2712-2725.

Ampofo, A. J.,Andoh, A., Tetteh, W. & Bello, M. (2012). Microbiological profile of some Ghanaian herbal preparation-Safety issues and implications for the health professions. *Open Journal of Medical Microbiology*,2: 121-130.

Andre, E.F. (1901). *Tacca chantrieri* Andre. Revue Horticole 73:541.

Ashworth, A., Christopher, L. J. & Reis-Filho, S. (2011). Genetic interaction in cancer progression and treatment. *Cell* 145(1): 30-38.

Autsavakitipong, T., Khonsung, P., Panthong, A., Chiranthanut, N., Kunanusorn, P., Nuntasaen, N., Jaipetch, T., Bunteang, S. & Reutrakul, V. (2008). Preliminary evaluation of the analgesic and anti-inflammatory effects of *Tacca integrifolia* in rodents. *International Journal of Applied Research in Natural Product.* 8 (1): 20-25

Avdagic, N., Zaciragic, A., Babic, N., Hukic, M., Seremet, M., Lepara, O. (2013). Nitric oxide as a potential biomarker in inflammatory bowel disease. *Bosnian Journal of Basic Medical Science.* 13(1):5–9.

Banares, A., Blanca, G., Guemes, J., Moreno, J. C. & Ortiz, S. (2004). *Atlas and Red Book of Threatened Flora of Spain. General Director of Nature Conservation*, Madrid, Spain.

Baldo, B. A. & Pham, N. H. (2013). *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*; Springer: New York, NY, USA.

Barcelo-Munoz, A., Encina, C. L., Simon-Perez, E. & Pliego-Alfaro, F. (1999). Micropropagation of adult avocado. *Plants cell, Tissue and Organ Cultures*. 69: 1-34.

Barrett, B. A. & Kidwell, K.K. (1998). AFLP based genetic diversity assessment among wheat cultivars from Pacific Northwest. *Crop Science.* 38: 1261-1271.

Baskaran, P. & Jayabalan, N. (2005). A simple approach to improve plant regeneration from callus culture of *Sorghum bicolor* for crop improvement. *Journal of Agriculture Biotechnology*. 1: 179-192.

Benz, D., Cadet, P., Mantione, K., Zhu, W. & Stefano, G. Total nitric oxide and health-a free radical and a scavenger of free radicals. *Medical Science Monitor.* 8(1): 1–4.

Berger, W., Hampel, F. Jr., Bernstein, J., Shah, S., Sacks, H. & Meltzer, E. O. (2006). Impact of azelastine nasal spray on symptoms and quality of lfe compared with cetirizine oral tablets in patients with seasonal allergic rhinitis. *Annals of Allergy, Asthma and Immunology*. 97(3): 375-381.

Bhanot, A., Sharma, R. & Noolve, M. N. (2011). Natural sources as potential anticancer agents: review. *International Journal of Phytomedicine* 3: 9-26.

Bhatia, P., Ashwath, N., David, M., 2005. Effect of genotype, explant orientation and wounding on shoot regeneration in tomato. *In Vitro Cellular and Development Biology Plant.* 41, 457–464.

Bhattacharya, P., Dey, S., Das, N. & Bhattacharya, B. C. (1990). Rapid mass propagation of *Chrysanthemum morifolium* by callus derived from stem explants and leaf explants. *Plant Cell Reproduction*. 9: 439-442

Bhau, B. S. & Wakhlu, A. K. (2001). Effect of genotype, explants type and growth regulators on organogenesis in *Morus alba*. *Plant Cell, Tissue and Organ Culture*. 66(1): 25-29.

Bian, K., Doursout, M. F. & Murad, F. (2008). Vascular system: role of nitric oxide in cardiovascular diseases. *Journal of Clinical Hypertension (Greenwich).* 10(4): 304–310.

Block, G. (1991). Epidemiological evidence regarding vitamin C and cancer. *American Journal of Clinical Nutrition*. 32(6): 1310-1314.

Bogdan, C. (2001). Nitric oxide and the immune response. *Nature Immunology*. 2 (10): 907-916.

Bold, H. C., Alexopoulos, C. J. & Delevoryas, T. (1987). *Morphology of Plants and Fungi*, 5th edition, New York: Harper-Collins: 3

Bottini, P.J., 1981. *Methods in plant tissue culture*. Kemtec Educational Corp., Kensington, Maryland.

Brand, M. D. & Nicholls, D. G. (2011). Assessing mitochondrial dysfunction in cells. *Biochemical Journal*. 435: 297-312.

Bressan, P. H., Kim, Y. J., Hyndman, S. E., Hasegawa, P. M. & Bressan, R. A. (1982). Factors affecting in vitro propagation of rose. *Journal of American Society of Horticultre Science*s. 107: 979-990.

Bretting, P. K. & Widrlechner, M. P. (1995). Genetic markers and plant genetic resource management. *Plant Breeding Reviews*. 31: 11-86.

Bringmann, G., Noll, T., Rischer, H., 2002. *In vitro* germination and establishment of tissue cultures of Bulbine caulescens and of two *Kniphofia species* (Asphodelaceae). *Plant Cell Reproduction*. 21: 125–129.

Brito-Arias, M. (2007). Synthesis and Characterization of Glycosides. *Springer*. ISBN 978-0-387-26251-2

Brokini, T.I., Lawyer, E.F., Ayodele, A.E., 2011. *In vitro* propagation on *Tacca leontopetaloides* (L.) Kuntze in Nigeria. *Egyption Journal of Botany*. 13: 51–56.

Bruhns, P., Fremont, S. & Daeron, M. (2005). Regulation of allergy by Fc receptors. *Current Opinion in Immunology*.17: 662–669.

Bryan, N. S. & Grisham, M. B. (2007). Methods to detect nitric oxide and its metabolites in biological samples. *Free Radical Biology and Medicine.* 43(5): 645-657.

Burkill, I. H. (1993). *A Dictionary of the Economic Products of the Malay Peninsula*. 3rd printing. Publication Unit, Ministry of Agriculture, malaysia, Kuala Lumpur. Volume 1 : 1-1240; volume 2 : 1241-2444.

Caddick, L. R., Rudall, P. J., Wilkin, P., Hedderson, T. A. J. & Chase, M. W. (2002). Phylogenetics of Dioscoreales based on combined analyses of morphological and molecular data. *Botanical Journal of the Linnean Society*. 138:123-144.

Canonica G. W. & Blaiss, M. (2011). Antihistaminic, Anti-inflammatory, and antiallergic properties of the nonsedating second-generation antihistamine desloratadine: A review of the evidence. *World Allergy Organization Journal*. 4: 47-53.

Cardellina, J. H., Fuller, R. W., Gamble, W. R., Westergaard, C. & Boswell J. (1999). Evolving strategies for the selection, dereplication and prioritization of antitumor and HIV-inhibitory natural products extracts, In: Bioassay methods in

natural product research and development, Bohlin, L. & Bruhn J. G. *Kluwer Academic Publisher Dordrecht*: 25-36.

Casey, T. E. & Hilderman, R. H. (2000). Modification of the cadmium reduction assay for detection of nitrite production using fluorescence indicator 2,3-diaminonaphthalene. *Nitric Oxide*. 4: 67- 74.

Cervantes, E. & Tocino, A. (2005). Geometric analysis pf Arabidopsis root apex reveals a new aspect of the ethylene signal transduction pathway in development. *Journal of Plant Physiology*. 162: 1038-1045.

Charoensub, R., Thiantong, D., Phansiri, S., 2008. Micropropagation of bat flower plant, *Tacca chantrieri* Andre. *Kasetsart Journal. (Natural Science)*.42(5): 7–12.

Chan, W. R., Taylor, D. R., Wilis, C. R. & Bodden, R. L. (1971). The structure and stereochemistry of neoandrographolide, a diterpene glucoside from *Andrographis paniculata*. *Tetrahedron*. 27: 5081-5091.

Chan, J. T. & Chang, W. C. (2002). Effect of tissue culture conditions and explant characteristics on direct somatic embryogenesis in *Oncidium* "Grower Ramsay". *Plant Cell, Tissue and Organ Culture*. 69(1): 41-44.

Chee, R., Pool, R. M. & Bucher, D. (1984). A method for large scale *in vitro* propagation of *Vitis*. *New York Food Life Science Bulletin*. 109: 1-9.

Chen, Z. L., Wang, B. D. & Chen M. Q. (1987). Steroidal bitter principal from *Tacca plantaginea*. Structure of taccalonolide A and B. *Tetrahedron Letters*. 28: 1673-1678.

Chern, A., Hosskawa, Z., Cherubini, C. & Cline, M. (1993). Effects of node position on lateral bud out growth in the decapitation shoot of *Ipomea nil*. *Journal of Science*. 93(1): 11-13.

Chhajer, B. (2005). *Allergy: symptoms, causes and mechanism, measures to treatments*. Diamond pocket books ltd., New Delhi: 6-7.

Chothani, D. L. & Patel, N. M. (2014). Anti-allergic potential of methanolic extract of leaves and fruits of *Careya arborea*. *Journal of PharmaSciTech.* 4 (1): 29-31.

Chooi, O. H. (2004). *Tumbuhan Liar Khasiat Ubatan dan Kegunaan lain*. Utusan Publication and Distributors Sdn Bhd: 92.

Chweya, J. A. & Mnzava, N. A. (1997). Cat's whiskers, *Cleome gynandra* L. Promoting the conservation and use of underutilized and neglected crops. *Bibliography of the genetic resources of traditional African vegetables*. Institute of plant Genetics and crop plant Research, Gatersleben/IPGRI, Rome, Italy.

Chu, Q., Vincent, M., Logan, D., Mackay, J. & Evans, W. (2005). Taxanes as first-line therapy for advanced non-small cell lung cancer: a systematic review and practice guideline. *Lung Cancer*. 50: 355–74.

Chuakul, W., Prathanturarug, S. & Saralamp, P. (2000). *Encyclopedia of Medicinal plants.* Bangkok: Amarin printing. Mahidol University.

Cirino, G., Distrutti, E. & Wallace, J. L. (2006). Nitric oxide and inflammation. *Inflammation Allergy Drug Targets.* 5(2): 115–119.

Coers, W., Kempinga, C., Klok, P. A., Timens, W. & Moshage, H. (1998). Specificity of antibodies to nitric oxide synthase isoforms in human, guinea pig, rat and mouse tissues. *Journal of Histochemistry and Cytochemistry*. 46:1385–91.

Cohen, J. I., Williams, J. T., Plucknett, D. L. & Shands, H. (1991). *Ex situ* conservation of plant genetic resources : Global development and environmental concerns. Article *SCIENCE*, vol. 253.

Cragg, G. M., Newman, D. J. & Snader, K. M. (1997). Natural products in drug discovery and development. *Journal of Natural Products*. 60: 52-60.

Cragg, G.M. & Newman, D. J. (2005). Plants as a source of anticancer agents. Journal of Ethnopharmacology 100: 72-79.

Das, A. K., Yoshimura, S., Mishima, R., Fujimoto, K., Mizuguchi, H. (2007). Stimulation of histamine H1 receptor up-regulates histamine H1 receptoritself through activation of receptor gene transcription. *Journal of Pharmacological Science.* 103:374 –382.

Davison, G., Ng, P. & Chew, H. H. (2008). *The Singapore Red Data Book : Threathing Plants and Animals of Singapore*. Nature Society of Singapore: 285

De Mesquita, M. L., De Paula, J. E., Pessoa, C., De Moraes, M. O., Costa-Lotufo, L. V., Grougnet, R., Michel, S., Tillequin, F. & Espindola, L. S. (2009). Cytotoxic activity of Brazilian Cerrado plants used in traditional medicine against cancer cell lines. *Journal of ethnopharmacology*, 2009. 123(3): p. 439-445.

De Vita, V. T., Simon, R. M., Hubbard, S. M., Young, R. C., Beard, C. W., Moxley, J. H., Carbone, P. P. & Canellos, G. P. (1980). Curability of advanced Hodgkin's disease with chemotheraphy: long-term follow-up of MOPP treated patients at NCI. *Annals of Internal Medicine.* 92: 587-595.

Debergh, P. C. & Maene, L. J. (1981). A scheme for commercial propagation of ornamental plants by tissue culture. *Scientia Horticulturea*. 14: 335-345

Debnath, S. C. (2004). Clonal propagtion of dwarf raspberry (*Rubus pubescens* Raf.) through *in vitro* axillary shoot proliferation. *Plant Growth Regulation.* 43: 179-186.

Decoteau, D.R., 1990. Tomato leaf development and distribution as influenced by leaf removal and decapitation. HortSci. 25 (6), 681–684.

Devi, C. S. & Srinivasan, V. M. (2006). Studies on various atmospheric microorganism affecting the plant tissue culture explants. *American Journal of Plant Physiology*. 1(2): 205-209.

Dhed'a, D., Dumortier, F., Panis, B., Vuylsteke, D. & De Langhe, E. (1991). Plant regeneration in cell suspension cultures of cooking banana 'Bluggoe'cultivar (*Musa* spp. ABB group). *Fruits*. 46: 125-135.

Ding, Z. & Larsen, K. (2000). Taccaceae. In: Wu, Z. & Raven, P. H. Editors. *Flora of China*, 24. Science Press, Beijing and Missouri Botanical Garden Press, St. Loius, Missuori, USA: 274-276.

Dobutovic, B. (2011). Nitric Oxide and its Role in Cardiovascular Diseases. *The Open Nitric Oxide Journal.* 3(1): 65–71.

Doebley, J. (1994). Morphology, molecules and maize. *In corn and culture in the prehistoric New World*, Johannessen, S. & Hastorf, C. A. Editors. Boulder, Colo.: Westview Press.

Doumbia, I. Z., Akromah, R. & Asibuo, J. Y. (2013). Comparative study of cowpea germplasms diversity from Ghana and Mali using morphological characteristics. *Journal of Plant Breeding and Genetics*. 1(3): 139-147.

Drenth, E., 1972. A revision of the family Taccaceae. *Blumea* 20, 367–406.

Drew, R. A., McComb, J. A. & Considine, J. A. (1993). Rhizogenesis and root growth of *Carica papaya* L. *in vitro* in relation to auxin sensitive phases and use of riboflavin. *Plant Cell, Tissue and Organ Culture.* 33(1): 1-7.

Dudzinski, D. M., Igarashi, J., Greif, D. & Michel, T. (2006). The regulation and pharmacology of endothelial nitric oxide synthase. *Annual Review of Pharmacology and Toxicology.* 46:235–76.

Dumortier, B. C. J. (1829). Analyse des families des plantes, avec I'Indication des principaux genres qui s'y rattachent Tourney: *Journal of Casterman aine*.

Elfadl, E., Reinbrecht, C. & Claupein, W. (2010). Evaluation of phenotypic variation in a worldwide germplasm collection of safflower (Carthamus tinctorius L.) grown under organic farming conditions in Germany. *Genetic Resources and Crop Evolution*. 57: 155-170.

Ehsan, K. (2011). Phytochemical analysis and biological activities of different parts of three varieties of *Labisia pumila* Benth. Thesis of Degree of Doctor Philosophy, Universiti Putra Malaysia.

Emilio, C. & Juana, G. D. (2010). Morphological description of plants: New perspectives in development and evolution. *International Journal of Plant Development Biology*. 4(1): 68-71.

Engelmann, F., 1991. *In vitro* conservation of tropical plant germplasm. *Euphytica.* 57, 227–243.

Fadel, D., Kintzios, S., Economou, A.S., Moschopoulou, G., Constantinidou, H.A., 2010. Effect of different strength of medium on organogenesis, phenolic accumulation and antioxidant activity of spearmint (*Mentha spicata* I.). Laboratory of Plant Physiology, Faculty of Agricultural Biotechnology,

Agricultural University of Athens, 11855 Athens, Greece. *The Open Hotriculture Journal*.3, 31–35

Farah, L., Jamaludin, M., 2008. Isolation and Characterization of Angiotensin Converting Enzyme (ACE) Inhibitory Compound derived from *Tacca integrifolia*. *Proceeding of The International Symposium of Biodiversity-Biotechnology:* 300–304.

Faridah Hanum, I., Shamsul K., 2004. *A Guide to the Common Plants of Ayer Hitam Forest, Selangor, Peninsular Malaysia*. Universiti Putra Malaysia Press, Serdang: 219.

Fechr, W. R. (1987). *Principles of'cultivar development*. hlacniillan. New York.

Feng, H. A. B., Kouya, Y. A , Xiaoyun, T. C., Lianqing, F. D., Ronghua, Z. B., Yu, C., Rie, Y. E., Ken-ichiro, I. E., Hirohisa, T. E. & Shin, Y. A. (2008). Inhibition of the antigen-induced activation of RBL-2H3 cells by sinomenine. *International Immunopharmacology*. 8: 502–507.

Forstermann, U. & Sessa, W. C. (2012). Nitric oxide synthases: regulation and function. *European Heart Journal.* 33(7):829–837.

Fotakis, G. & Timbrell, J. A (2006). In vitro cytotoxicty assays: Compariosn of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. *Toxicology Letters*. 160: 171-177.

Fraguas, C. B., Pasqual, M., Dutra, L.F. & Cazetta, J. O. (2004). Micropropagation of fig (*Ficus carica* L.) 'Roxo de Valinhos' plants. *In Vitro Cellular and Development Biology Plant.*40: 471-474.

Francis, S. V., Senapati, S. K. & Rout, G.R. (2007). Rapid clonal propagation of Curculigo orchioides Gaertn., an endangered medicinal plant. *In Vitro Cellular & Developmental Biology Plant.*43(2): 140-143.

Gamborg, O. L., Miller, R. A. & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research*. 50: 151-158.

Garcia, R., Morán, R., Somonte, D., Zaldúa, Z., López, A. & Mena, C. J. (1999). *Sweet potato (Ipomoea batatas L.) biotechnology: perspectives and progress*. Plant biotechnology and in vitro biology in 21st century. The Netherlands: 143-146.

Garland, P. & Stoltz, L. P. (1981). Micropropagation of Pissardi plum. *Annales of Botany* 48: 387-389.

Garthwaite, J. (1991). Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neuroscience.* 14(2): 60–67.

George, E. F., Hall, M. A. & Klerk, G. J. D. (2008). *Plant propagation by tissue culture.*Springer-Verlag GmbH 3rd edition: 65-113.

George, E. F. & Sherrington, P. D. (1984). *Plant propagation by tissue culture: Handbook and directory of commercial laboratories*. Eversley, England: Exegeticc Ltd.

Golab, F., Kadkhodaee, M., Zahmatkesh, M., Hedayati, M., Arab, H., Schuster, R. (2009). Ischemic and non-ischemic acute kidney injury cause hepatic damage. *Kidney Interntional.* 75(8): 783–792.

Gonzalez-Benito, M.E., Martin, C. (2011). *In vitro* Preservation of Spanish University. *In Vitro Cellular and Development Biology Plant*.47: 46–54.

González-Gallego, J., Sánchez-Campos, S., Tunon, M. (2007). Anti-inflammatory properties of dietary flavonoids. *Nutricion Hospitalaria.* 22: 287-293.

Govindappa, M., Naga, S. S., Poojashri, M. N., Sadananda, T. S. & Chandrappa, C. P. (2011). Antimicrobial, antioxidant and anti-inflammatory acticityof ethanol extract and active phytochemical screening of widelia trilobata (L.) Hitchc. *Journal of Pharmacogonosy and Phytotehrapy*, 3(3): 43-51.

Grant, N. J. & Hammat, N. (1999). Increased root and shoot production during micropropagation of cherry and apple rootstocks: effect of subculture frequency. *Tree Physiology.* 19: 899-903.

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S. & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and nitrate in biological samples. *Analytical Biochemstry*. 126: 131- 138.

Hamad, A. M. & Taha, R. M. (2008). Effect of sequential subcultures on *in vitro* proliferation capacity and shoot formation pattern of pineaplle (*Ananas comosus* L. Merr) over different incubation periods. *Scentia Horticulturae.* 117: 329-334.

Hance, H. F. (1881). *Schizocaspa plantaginea*. *Journal of Botany*. 19: 292.

Hartinie, M. & Jualang, G.A. (2007). *In vitro* germination and plantlet establishment of *Labisia pumila* (Bl.) F. Vill. School of Science and Technology, Universiti Malaysia Sabah, Locked Bag 2073, 88999 Kota Kinabalu, Sabah, Malaysia. Scientia Horticulturae. 115: 91–97.

Hartmann, H. T., Kester, D. E. & Davies, F. T. (1990). *Plant Propagation: Principle and Practices,* 5th Edition. Printed by Prentice-Hall. Inc., A Division of Simon & Schuster, Englewood Cliffs, New Jersey: 461-478.

Hartmann, H. T., Kester, D. E., Davies, F. T. & Geneve, R. L. (2011). *Hartmann and Kester's Plant Propagation: Principle and Practices,* 8th Edition. Pearson Education, Inc. And published by Prentice-Hall, One Lake Street, Saddle River, New Jersey: 649-678.

Hazarika, B. N. (2003). Acclimatization of tissue-cultured plants. *Current Science (India).* 85: 1704-1712.

He, H. Y., Lan, Q. Y. & Zhang, Y. J.(2002). *In vitro* propagation of *Tacca chantrieri*. *Journal of Guanxi Agriculture and Biology Science* **21**, 107-109 (in Chinese with English abstract)

He, H. Y., Wen, B. &Yin, S. H.(2003). The germination and storage characteristics of *Tacca chantrieri* Andre. seeds. *Journal of Central South Forestry University* 23, 120-122 (in Chinese with English abstract).

Heilmann, J., Shwe, H. H., Aye, M., Sein, M. M., Kreitmeier, P. & Reiser, O. (2006). New furostanol glycosides from the rhizomes of *Tacca integrifolia*. *Planta Medica*. 72: 343.

Henderson, M. R. (1954). *Malayan Wild Flowers Monocotyledons*. The Malayan Nature Society Kuala Lumpur.

Hendra, R., Syahida, A., Ehsan, O., Aspollah, S. & Yunus, M. S. (2011). Antioxidant, Anti-inflammatory and Cytotoxicity of *Phaleria macrocarpa* (Boerl.) Scheff fruit. *BMC Complementary and Alternative Medicine* (11): 110.

Helgi O. & Rolfe, S.A. (2005). The physiology of flowering plants. *Plant physiology*: 191.

Ho, C. K., Jacobs, G. & Donald, D. M. (1995). Effect of sodium hypochlorite, ethanol and culture medium on seed germination of *Poulownia species*. *Seed Science Technology*. 23: 157–163.

Hopkins, W. G. & Huner, N. P. A. (2004). *Introduction to Plant Physiology*. John Wiley & Sons, New Jersey.

Hoy, J. W., Biscoff, K. P., Miligan, S. B. & Gravious, K. A (2003). Effect of tissue culture explant sources on sugar cane yield components. *Euphytica*. 129(2): 237-240.

Hsiao, I. A. (1979). The effect of sodium hypochlorite and gibberellic acid on seed dormancy and germination of wild oats (*Avena fatua*). *Canadian Journal of Botany*. 57: 1729–1739.

Hussain, A., Ahmaed Qarshi, I., Nazir, H. & Ullah, I. (2012). Plant tissue culture: Current status and oppurtunities. *Agricultural and Biological Sciences:* 1-28.

Husain, M. K. & Anis, M. (2009). Rapid *in vitro* multiplication of *Melia azedarach* L. (a multipurpose woody tree). *Acta Physiologiae Plantarum*. 31(4): 765-772.

Iapichino, G. (1996). Micropropagation of globe artichoke (*Cynara scolymus* L.) from underground dormant buds ("ovoli"). In vitro Cellular and Development Biology Plant. 32: 249–252.

Ibrahim, A. S., Waleed, S.K., Manal, M. E. T., Syam, M., Mouna, C., Mahmood, A. A., Mohd Rais, M., Syahida, A., Mohamed Ibrahim, N., Chung, L. Y., Mohd Roslan, S., Rozana, O. & Asfarina, A. A. (2012). *In vitro* and *in vivo* anti-inflammatory activities of columbin through the inhibition of cycloxygenase-2

and nitric acid but not the suppression of NF-κB translocation. *European Journal of Pharmacology*. 678: 61-70.

Ignarro, L. J. (2000). *Nitirc Oxide: Biology and Pathobiology*. Academic Press: A Harcourt Science and Technology Company,San Diego, California, USA: 3 -20

Ikawati, Z., Wayuono, S. & Maeyama, K. (2001). Screening of several Indonesian medicinal plants for their inhibitory effect on histamine release from RBL-2H3 cells. *Journal of Ethnopharmacology*. 75: 249-256.

Imran, M. A., Begum, G., Sujatha, K. & Mallaiah, B. (2012). Effect of adeninde sulphate (Ads)with cytokinins on multiple shoot production in *Carissa carandas* (L.). *International Journal of Pharma and Bio Sciences*. 3(1): 473-480.

Jackson, M. (2007). *Allergy: The History of a Modern Malady*. Reaktion Books ltd., London: UK: 9.

Jafari, N., Othman, R. Y. & Khalid, N. (2011). Effect of benzylaminopurine (BAP) pulsing on in vitro shoot multiplication of *Musa acuminata* (banana) cv. Berangan. *African Journal of Biotechnology*. 10(13): 2446-2450.

Jamadon, B., Zulhairil, A., Salma, I. & Mohd Shukor, N. (2007). *Current Status of Conservation and Utilisation of Tropical Plant Genetic Resources for Food and Agriculture in Malaysia*. International Training Workshop (2007). The Conservation and Utilisation of Tropical/Subtropical Plant Genetic Resources

Jiang, J. H., Yang, H. M., Wang, Y. L. & Chen, Y.G. (2014). Phytochemical and pharmacological studies of the genus *Tacca*: A review. *Tropical Journal of Pharmaceutical Research*. 13 (4): 635-648.

Joshi, I. P., Bisht, P., Sharma, V. K. & Uniyal, D. (2003). Studies on effect of nutrient media for clonal propagation of superior phenotypes of *Dalbergia sissoo* Roxb. through tissue culture. *Silvae Genetica* 52(3/4): 143-147.

Juene, B., Barabe, D. & LAcroix, C. (2006). Classical and dynamic morphology: Toward a synthesis through the space of forms. *Acta Biothoeretica*. 54(4):277-293.

Kadkhodaee, M., Golab, F., Zahmatkesh, M., Ghaznavi, R., Hedayati, M., Arab, H. A. (2009). Effects of different periods of renal ischemia on liver as a remote organ. *World Journal of Gastroenterol.* 15(9): 1113–1118.

Kadhimi, A. A., Alhasnawi, A. N., MOhamad, A., Wan Yusoff, W. M. & Mohd Zain, C. R. (2014). Tissue culture and some of the factors affecting them and the micropropagation of strawberry. *Life Science Journal.* 11(8): 484-493

Kagan, R. S. (2013). Food allergy: an overview. *Environmental Health Perspective*. 111: 223–225.

Kainsa, S., Kumar, P. & Rani, P. (2012). Medicinal plants of Asian origin having anticancer potential: Short review. *Asian Journal of Biomedical and Pharmaceutical Sciences* 2(10): 1-7.

Kalesnikoff, J. & Galli, S. J. (2008). New developments in mast cell biology. *Nature Immunology*, *9*, 1215–1223.

Kashyap, B. & Dhiman, S. (2011). Effect of media on hardening of in vitro multiplied plantlets of gloxinia and saintpaulia under low cost polytunnels. *International Journal of Farm Sciences* 1(2): 63-67.

Kazlowska, K., Hsu, T., Hou, C., Yang, W. &Tsai, G. (2010). Anti-inflammatory propertiesof phenolic compounds and crude extract from Porphyra dentata. *Journal of ethnopharmacology*. 2010, 128:123-130.

Kepczynski, J., Nemoykina, A. & Kepczynska, E. (2006). Ethylene and *in vitro* rooting of rose shoots. *Plant Growth Regulation*.50(1): 23-28.

Kelm, M. (1999). Nitric oxide metabolism and breakdown. *Biochimica et Biophysica Acta.* 1411(2-3):273–289.

Ker-Gawler, J. B. (1812). *Tacca integrifolia* Ker Gawl. Curtis's Botanical Magazine 36: 1488.

Khatri, A., Khan, I. A., Dahot, M. U., Nizamani, G. S., Siddiqui, M. A., Raza, S. & Naqvi, M. H. (2005). Stduy of callus induction in Banana (*Musa sp.*). *Pakistan Journal of Biotechnology*. 2(1): 36-40.

Khatri, A., Dahot, M. U., Khan, I. A. & Nizamani, G. S. (2010). An efficient method of protoplast isolation in banana (*Musa spp*.). *Pakistan Journal of Botany*. 42(2): 1267-1271.

Kim, S.Y. & Mulkey, T.J. (1997). Effect of ethylene antagonists on auxin-induced inhibition of intact primary root elongation in maize (*Zea mays* L.). *Journal of Plant Biology*. 40(4): 256-260.

Kinghorn, A. D., Cui, B., Ito, A., Chung, H. S., Seo, E. K., Long, L. & Chang, L. C. (2000). *Fractionation of plants to discover substances to combat cancer. In: Biologically activenatural products*. Pharmaceuticals. CRC Press LLC. Florida, United State of America: 16-23.

Khazan, M. & Hdayati, M. (2014). The role of nitric oxide in health and diseases. *Scimetri*. 3 (1): 1-10.

Kim, S. H., Choi, C. H., Kim, S. Y., Eun, J. S. & Shin, T. Y. (2005). Anti-allergic effects of *Artemisia iwayomogi* on mast cell mediated allergy model. *Journal of Pharmacology and Experimental Therapeutics*. 230: 82–88.

Kitjaroennirut, N., Jansakul, C. & Sawangchote, P. (2005). Cardiovascular effects of *Tacca integrifolia*Ker-Gawl. extracts in rats. Songklanakarin *Journal of Science and Technology*. 27: 281-289.

Kleschyov, A. L. & Munzel, T. (2002). Advanced spin trapping of vascular nitric oxide using colloid iron diethyldithiocarbamate. Methods in Enzymology. 359: 42-51.

Klimes, L., Klimesova, J. & Cizkova, H. (1999). Carbohydrate storage in rhizomes of Phrarmites australis: the effect of altitude and rhizome age. *Aquatic Botany*. 64: 105-110.

Kobayashi, S., Kato, T., Azuma, T., Hiroe, K. & Abe, K. (2015). Anti-allergenic activity of polymethoxyflavones from *Kaempferia parviflora. Journal of Functional Foods*. 13: 100-107.

Kornova, K., Stephanova, A. & Terzijsky, D. (1993). *In vitro* culture of immature embryos and cotyledons of *Juglans regia* L. Morphological and anatomical analysis of some regenerants. *Acta Horticulturae*. 311: 125–133.

Kraithep, S., Oungbho, K. & Tewtrakul, S. (2008). Anti-allergy activity of Thai medicinal plants used in longetivity formulation. *Songklanakarin Journal Science of Technology*. 30(5): 621-625.

Krauss, B. (1979). *Native Plants Used As Medicine in Hawaii*. 50, Harold L. Lyon Arboretum, University of Hawaii at Manoa.

Krishna, H., Sairam, R., Singh, S., Patel, V., Sharma, R., Grover, M., Nain, L. & Sachdev, A. (2008). Mango explants browning: Effect of ontogenic age, nycorrhization and pre-treatments. *Scientia Horticulturae*. 118(2): 132-138.

Kuntze, C. E. O. (1891). *Tacca leontopetaloides* (L.) Kuntze. Revision Generum Planatrum 2: 704.

Lee, T., Michele, H. E. E & Pua, E. C. (1997). High frequency shoot regeneration from leaf dise explants of garland chrysanthemum (*Chrysanthemum coronarium* L) *in vitro*. *Plant Science*. 126: 219-226

Leifert, C. & Waites, W. (1992). Bacterial growth in plant tissue culture media. *Journal of Applied Microbiology*. 72(6): 460-466.

Leifert, C., Morris, C. E. & Waites, W. M. (1994). Ecology of microbial saprophytes and pathogens in tissue culture and field-grown plants: reason for contamination problems *in vitro*. *Critical Reviews in Plant Sciences*. 13(2): 139-183.

Leifert, C. & Cassells, A. (2001). Microbial hazards in plant tissue and cell cultures. *In Vitro cellular and Development Biology-Plant*. 37(2): 133-138.

Leshem, B., Werker, E. & Shalev, D.P. (1988). The effect of cytokinins on vitrification in melon and carnation. *Annals of Botany*. 62: 271-276

Li, Q., Xu, Z. & He, T. (2002). *Ex situ* genetic conservation of endangered *Vatica guangxiensis* (Dipterocarpaceae) in China. *Biological Conservation.* 106: 151–156.

Li, L., Ni, W., Li, X. R., Hua, Y., Fang, P. L., Kong, L. M., Pan, L. L., Li, Y., Chen, C. X. & Liu, H. Y. (2011). Taccasubosides A-D, four new steroidal glycosides from *Tacca subflabellata.Steroids.* 76: 1037-1042.

Lindsay, G. C. & Ledger, S. E. (1993). A protoplast to plant system for the Chrysanthemum, *Dendrantheme zawadskii* x *D. grandifolra.Plant Cell Reports*. 12: 278-280

Ling, P. P. (1985). Taccaceae. In: Pei Chien & Ting Chin-tsun, editors. *Flora pf Republic Popularis Sinica*. 16: 42-45.

Ling, P. P. & Ding, Z. Z. (1982). Two new species of Taccaceae from China. Acta Phytotaxon. Sin 20: 202 (in Chinese with English abstract)

Luis, A. R., Corpas, F. J., and Barroso, J. B. (2004). Nitric oxide and nitric oxide synthase activity in plants. *Phytochemistry* 65: 783-792.

Locy, R. D., Moses, M. S. & Garton, S. (1984). Large-scale production of plants through tissue culture. BOSTID Developments (OIA/NRC). 4 (3): 8-10.

Lloyd, G. & McCown, B. (1980). Commercially feasibly micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. *Proceeding of International Plant Propagators Society*. 30: 421-427.

Loria, R., Coombs, J., Yoshida, M., Kers, J. & Bukhalid, R. (2003). A paucity of bacterial root diseases: Streptomyces succeeds where other fail. *Physiological and Molecular Plant Pathology*. 62(2): 65-72.

Lum, H. K., Butt, Y. K. C. & Lo, S. C. L. (2002). Hydrogen peroxide induces a rapid production of nitric oxide in mung bean (Phaseolus aureus). *Nitric oxide: Biology and Chemistry*. 6: 205-213.

MacRae, S. & Van Staven, J. (1990). In vitro of Eucalyptus grandis: Effect of gelling agents on propagation. *Journal of Plant Physiology*. 137(2): 249-251.

Malaysian Biological Diversity Clearing House Mechanism (MyCHM). (www.chm.frim.gov.my).

Manish, G., Thein, W. N., Rahul, S. S., Amaluddin, A., Jegathambigai, R. N. & Ishab, K. (2015). Marketing trends and future prospects of herbal medicine int he treatment of various disease. *World Journal of Pharmaceutical Research*, 4(9): 132-155.

Marana, J. P., Miglioranza, E. & De Faria, R. T. (2009). *In vitro* establishment of *Jacaratia spinosa* (Aubl.) ADC. *Semina-Ciencias Agrarias*. 30(2): 271-274.

Matsuda, H., Tewtrakul, S., Morikawa, T. & Yoshikawa, M. (2004). Anti-allergic activity of stilbenes from *Koreanrhubarb* (*Rheum undulatum* L.): structure requirements for inhibition of antigen-induced degranulation and their effects on the release of TNF-alpha and IL-4 in RBL-2H3 cells. *Bioorganic and Medicinal Chemistry*.12: 4871-4876.

Matu, E., Lindsey, K. & Van Staden, J. (2006). Micropropagation of *Maytenus senegalensis* (Lam.) Excell. *South African Journal of Botany.* 72(3): 409-415.

Maxted, N., Ford-Lloyd, B.V. & Hawkes, J.G. (1997). Complementary conservation strategies. *Plant Genetic Conservation*. Chapman and Hall, London: 15-39.

Mc Cauley, S. D., Gilchrist, M. & Befus, A. D. (2005). Nitric oxide a major determinant of mast cell phenotype and function. *Memorias do Instituto Oswaldo Cruz*.100: 11–14.

Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature.*454: 428–435.

Meenakshi, S., Shinde, B. N. & Suprasnna, P. (2011). Somatic embryogenesis from immature male flowers and molecular analysis of regenerated plants in banana "Lal Kela" (AAA). *Journal of Fruit and Ornamental Plant Research*. 19(2): 15-30.

Merina, N., Chandra, K. J. & Jibon, K. (2012). Medicinal plants with potential anticancer activities: a review. *International Research Journal of Pharmacy.* 3(6): 26-30.

Miller, C. O., Skoog, F., Okomura, F. S., Von Saltza, M. H. & Strong, F. M. (1956). Isolation, structure and synthesis of kinetin, a substance promoting cell division. *Journal of the American Chemical Society*. 78: 1345–1350.

Mitre, E., Nutman, T. B. (2006). Basophils, basophilia and helminth infections. *Chemical Immunology and Allergy*.90: 141–156.

Ministry of Natural Resources and Management.(2009). *Fourth National Report to the Conservation on Biological Diversity*. Ministry on Natural Resources and Environment, Government of Malaysia.

Mohammadi, S.,A. & Prasanna, B. M. (2003). Analysis of genetic diversity in crop plants - salient statistical tools and considerations. *Crop Science*. 43: 1235-1248.

Mohd-Fuat, A. R. , Kofi, E. A. & Allan, G. G. (2007). Mutagenic and cytotoxic properties of three herbal plants from Southeast Asia. *Tropical Biomedicine*. 24: 49-59.

Molnar, Z., Virag, E. & Ordog, V. (2011). Natural substance in tissue culture media of higher plants. *Acta Biologica Szegediensis*. 55(1): 123-127.

Moncada, S., Palmer, R. M. J. & Higgs, E. A. (1989). Biosynthesis of nitric oxide from L-arginine: a pathway fro the regulation of cell function and communication. *Biochemical Pharmacology*. 38: 1709-1715.

Moreno Saiz, J. C., Dominguez Lozano, F. & Sainz Ollero, H. (2003). Recent progress in conservation of threated Spanish vascular flora: a critical review. *Biological Conservation* 113: 419-431.

Morris, J. B. (2009). Characterization of sesame (*Sesamum indicum* L.) germplasm collection. II. Cultivar selection based on traits associated with seed yiled. *Genetic Resources and Crop Evolution*. 56: 651-661.

Moshage, H. (2009). Simple and reliable measurement of nitric oxide metabolite in plasma. *Clinical Chemistry*. 55 (10): 1881-1882.

Moshage, H. (1997). Nitric oxide determinations: much ado about NO-thing?. *Clinical Chemistry*. 43:553– 556.

Moshage, H., Kok, B., Huizenga, J. R. & Jansen, P. L. (1995). Nitrite and nitrate determination in plasma: a critical evaluation. Clinical Chemistry.41: 892-896.

Moustafa, S. M. A., Menshawi, B. M.,Wassel, G. M., Mahmoud, K. & Mounier, M. M. (2014). Screening of some plants in Egypt for their cytotoxicity against four human cancer cell lines. *International Journal of PharmaTech Research.* 6(3): 1074-1084.

Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiology Plant*. 15: 473–497.

Murashige, T. (1974). Plant propagation through tissue cultures. *Annual Review of Plant Physiology*. 25: 135-166.

Nadha, H. K., Kumar, R., Sharma, R. K., Anand, M. & Sood, A. (2011). Evaluation of clonal fidelity of *in vitro* raised plants of *Guadua angustifolia* Kunth using DNA-based markers. *Journal of Medicinal Plant Resources*. 5(23): 5636-5641.

Naseem, K. M. (2005). The role of nitric oxide in cardiovascular diseases. *Molecular Aspects of Medicine.* 26(1-2): 33–65.

Nathan, C. (1992). Nitric oxide as a secretory product of mammalian cells. *The FASEB Journal*. 6: 3051–3064.

National Cancer Institute (NCI). (2014). (http:// www.cancer.gov.) 14th April 2015

Negi, S., Ivanchenko, M. G. & Muday, G. K. (2008). Ethylene regulates lateral root formation and auxin transport in *Arabidopsis thaliana*. *The Plant Journal*. 55(2): 175-187.

Nema, R., Khare, S., Jain, P., Pradhan, A., Gupta, A. & Singh, D. (2013). Natural products potential and scope for modern cancer research. *American Journal of Plant Sciences*. 4: 1270-1277.

Newbury, H. J. & Ford-Lloyd, B. V. (1997). Estimation of genetic diversity. In; Maxted, N., Ford-Lloyd, B. V. & Hawkes, J. G. Editors. *Plant Genetic Conservation: The in situ approach.* Chapman & Hall, London: 3-14

Newman, D. J., Cragg, G. M. & Snader, K. M. J. (2003). Natural products as sources of new drugs over the period 1981-2002. *Journal of Natural Products*. 66:1022-1037.

Newman, D. J. & Cragg, G. M. (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products*. 70(3): 461-477.

Ndouyang, C. J., Nguimbou, R. M., Njintang, Y. N., Scher, J., Facho, B. & Mbofung, C. M. F. (2014). *In vivo* assessment of the nutritional and subchronic toxicity of *Tacca leontopetaloides* (L.) tubers. *Journal of Agricultural Science*: 5-13.

Nitsch, J. P. & Nitsch, C. (1969). Haploid plants from pollen grains. *Science*. 163: 85-87.

Nor Aini, A. S., Merrina, A., Stanslas, J. & Sreramanan, S. (2008) . Cytotoxic Potential on Breast Cancer Cells Using Selected Forest Species Found in Malaysia. *International Journal of Cancer Research*. 4: 103-109.

Norton, M. E. & Norton, C. R. (1986). Change in shoot proliferation with repeated in vitro subculture of shoots of woody species of Rosaceae. *Plant Cell Tissue and Organ Culturae.* 5: 187-197.

Nuanla, R. & Sruamsiri, P. (2000). Insecticidal activity of bat flower plant crude extraction against common cutworm. – *In Seminar Report on Tendency of the Medicinal Plant Improvement* in Thailand. 13-14 September 2000. Office of the National Research Council of Thailand. Bangkok: 200–209.

Odutayo, O. T., Oso, R. T., Akinyemi, B. O. & Amusa, N. A. (2004). Microbial contaminats of cultured Hibiscus cannabinus and Telfaria occidentalis tissues. *African Journal of Biotechnology*. 3(9): 473-476.

Odutayo, O., Amusa, N., Okutade, O. & Ogunsanwo, Y. (2007). Sources of microbial contamination in tissue culture laboratories in southwestern Nigeria. *African Journal of Agricultural Research*. 2(3): 67-72

.

Ohiro, K., Makoto, I. & Ojima, K. (1976). Thiamine requirements of various plant cells in suspension culture. *Plant Cell Physiology*. 17(3): 583-590.

Orlikowska, T., Sabala, I. & Kucharska, D. (2000). The effects of leaf and shoot tip removal and explants orientation on axillary shoot proliferation *of Codiaeum variegatum* Blume *var. pictum* Muell. Arg. Cv. Excellent *Scientia Horticulturae*. 85: 103–111.

Ortega-Loeza, M. M., Salgado-garciglia, S., Gomez-Alonso, C. & Avila-Diaz, I. (2011). Acclimatization of the endangered Mexican epiphytic orchid, Laelia speciosa(H.B.K) Schltr. *European Journal of Environmental Science*, 1 (2): 48-54.

Oskoueian, E., Abdullah, N., Saad, W. Z., Omar, A. R., Ahmad, S., Kuan, W. B., Zolkifli, N. A., Hendra, R., Ho, Y. W.(2011). Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas* Linn. *Journal of Medicinal Research*. 5:49-57

Pacher, P., Beckman, J. S. & Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiologicall Reviews.* 87(1): 315–424.

Parul, R., Kundu, S. K. & Saha, P. (2012). *In vitro* nitric oxide scavenging activity of methanol extracts of three bangladesh medicinal plants. *The Pharma Innovation.* 1 (12): 83-89.

Patcharapisutsin, W. (1990). Somatic embryogenesis and plantlet formation in oil palm tissue culture. *Thesis of Master Science*, Prince of Songkla University.

Patel, R. . & Shah, R. R. (2009). Regeneration of stevia plant through callus culture. Indian Journal of Pharmaceutical Science. 71: 46–50.

Pati, P. K., Rath, S. P., Sharma, M., Sood, A. & Ahuja, P. S. (2006). *In vitro* propagation of rose- a review. *Biotechnology Advances*. 24(1): 94-114.

Pavingero, D., Dostal, J., Biskov, R. & V. (1994). Somatic embryogenesis and Agrobacterium mediated transformation of Chrysanthemum. *Plant Science* 97: 95-101.

Peng, J, Risinger, A. L., Fest, G. A., Jackson, E. M., Helms, G., Polin, L. A. & Mooberry, S. L. (2011). Identification and biological activities of new taccalonolide microtubule stabilizers. *Journal of Medicinal Chemistry*. 54: 6117-6124.

Peng, J. G., Jackson, E. M., Babinski, D. J., Risinger, A.L., Helms, G., Frantz, D. E. & Mooberry, S. L. (2012). Evelynin, a cytotoxic benzoquinone-type retro-dihydrochalcone from *Tacca chantriers*. *Journal of Natural Products*.73: 1590-1592.

Pengklai, C. 1993. *Taccaceae. In Flora of Thailand*, 6(1): 1-9.

Peschke, V. M. & Phillips, R. L. (1992). Genetic implication of somaclonal variation in plants. *Advance in Genetics*. 30: 41–75.

Phatak, S. V. & Heble, M. R. (2002). Organogenesis and terpenoid synthesis in *Mentha arvensis*. *Fitoter*. 73: 32–39.

Phillips, R., Arnott, S. & Kaplan, S. (1981). Antibiotics in plant tissue culture: rifampicin effectively controls bacterial contaminants without affecting the growth of short-term explants cultures of *Helianthus tuberosus*. *Plant Science Letters*. 21(3): 235-240.

Podani, J. & Schmera, D. (2006). On dendrogram-based measures of functional diversity. *Oikos* 115: 179-185.

Pornkamon, S., Tueanjit, K., Ubon, C., Chadamas, P., Pote, S., Puangrat Y. & Patcharee, B. (2011). Antioxidant activity and bioactive phytochemical contents of traditional medicinal plants in northeast Thailand. *Journal of Medicinal Plants Research*. 5(31): 6822-6831.

Prozesky, E. A., Meyer, J. J. M. & Louw, A I. (2001). *In vitro* antiplasmodial activity and cytotoxicity of ethnobotanically selected South African plants. *Journal of Enthopharmacology.* 76: 239-245.

Quah, B. A. (1995). Cytotoxic activity of Goniothalamin on different types of cancer cell lines. *Thesis of Bachelor of Science*, Biotechnology, Universiti Putra Malaysia.

Raina, H., Soni, G., Jauhari, N., Sharma, N. & Bharadvaja, N. (2014). Phytochemical importance of medicinal plants as potential sources of anticancer agents. *Turkish Journal of Botany* 38: 1027-1035.

Raj, N. R., Jain, S. K., Raj, C. N. & Panda, A. B. (2010). Various screening methods fro anti-allergic activity: an overview. *International Journal of Pharmaceutical Sciences and Nanotechnology.* 3(2): 906-911.

Ramanayake, S., Meemaduma, V. & Weerawardene, T. (2006). *In vitro* shoot proliferation and enhancement of rooting for the large-scale propagation of yellow bamboo (*Bambusa vulgaris* 'Striata'). *Scientia Horticulturae*.110(1): 109-113.

Ridley, H. N. (1924). *Flora of the Malay Peninsular*. Volume III. London: L. Reeve & Co.

Risinger, A. L., Jackson, E. M., Polin, L. A., Helms, G. L., Le Boeuf, D. A., Joe, P. A., Hopper-Borge, E., Luduena, R. F., Kruh, G. D. & Mooberry, S. L. (2008). The taccalonolides: microtubule stabilizers that circumvent clinically relevant taxane resistance mechanisms. *Cancer Research*. 68: 8881-8888.

Risinger, A. L., Li, J., Peng, J. & Mooberry, S. L. (2012). Potent new microtubule stabilizers with unique biochemical and cellular effects show promise for cancer treatment. *Planta Medica*. 78: 1405.

Rodrigo, G. J., Rodrigo, C. & Hall, J. B. (2004). Acute asthma in adults. *Ches*t. 125: 1081-1102.

Rodrigues, P. H. V., Lima A. M. L. P. Ambrosano, G. M. B. & Dutra, M. F. B. (2006). Acclimatization of micropropagated *Heliconia bihai* (Heliconiaceae) plants. *Science Agriculture* (Piracicaba, Brazil). 62 (3): 299-301.

Romitelli, F., Santini, S. A., Chierici, E., Pitocco, D., Tavazzi, B., Amorini, A. M. (2007). Comparison of nitrite/nitrate concentration in human plasma and serum samples measured by the enzymatic batch Griess assay, ion-pairing HPLC and ion-trap GC-MS: the importance of a correct removal of proteins in the Griess assay. *Journal of Chromatography B, Analytical Technoloiges in the Biomedical and Life Science*. 851(1-2):257-267.

Rumbaugh, M. D., Graves, W. L., Caddel, J. L. & Mohammad, R. M. (1988). Variability in a collection of alfalfa germplasm from Morocco. *Crop Science*. 28: 605-609.

Saad, A. I. M. & Elshahed, A. M. (2012). Recent advances in plant in vitro culture. *Agricultural and Biological Sciences*: 29-40

Sadik, K., Rubaihayo, P.R., Magambo, M.J.S. & Pillay, M. (2007). Generation of cell suspensions of East African highland bananas through scalps. *African Journal of Biotechnology*. 6: 1352-1357.

Sakuma, S., Higashi, Y., Sato, N., Sasakawa, T., Sengoku, T., Ohkubo, Y. & Amaya, T., Goto, T. (2001). Tacrolimus suppressed the production of cytokines involved in atopic dermatitis by direct stimulation of human PBMC system. (Comparison with steroids). *International Immunopharmacology*. 1: 1219–1226.

Sanatombi, K. & Sharma, G. J. (2007). Micropropagation of *Capscicum annuum* L. Notulae *Botanicae Horti Agrobotanici Cluj-Napoca*. 35(1): 57-64

Sargent, J. M. & Taylor, C. G. (1989). Appraisal of the MTT assay as a rapid test of chemosensitivity in acute myeloid lekaemia. *British Journal of Cancer*. 60: 206-210.

Sauer, A., Walther, F. & Preil, W. (1985). Different suitability for *in vitro* propagation of rose cultivars. *Gartenbauwiss.* 3: 133–138.

Saw, L. G. 1993. *Tacca*: flowering and fruiting behavior. *Nature Malaysiana* 18: 3- 6.

Scadding, G. (2005). Predicting and establishing the clinical efficacy of a histamine H1- receptor antagonist: desloratadine, the model paradigm. *Clinical Drug Investigation.* 25:153–164.

Schenk, R. V. & Hildebrandt, A. C. (1972). Medium and techniques for induction and growth of monocotyledonous plant cell cultures. *Canadian Journal of Botany*. 50: 199-204.

Schoofs, H., Pani, B. & Swennen, R. (1998). Competence of scalps for somatic embryogenesis in *Musa*. *Acta Horticulturae*. 490: 475-483.

Shands, H. L. & Weisner, L. E. (1992). *Use of Plant Introduction in Cultivar Development*, Part II. CSSA Special publication no. 20, CSSA and ASA, Madison, Wisconsin.

Sharma, J. N., Al-Omran, A. & Parvathy, S. S. (2007). Role of nitric oxide in inflammatory diseases. *Inflammopharmacology.*15(6): 252–259.

Sharma, B. V., Rowland, N. S., Clouse M. M., and Rice, N.A. (2014). An improved assay for measuring low levels of nitric oxide in cultured pulmonary myofibroblasts. *Advance in Biological Chemistry* 2: 214-221.

Shirani, S., Sariah, M., Zakaria, W. & Maziah, M. (2010). Scalp induction rate responses to cytokinin on proliferating shoot-tips of banana cultivars (*Musa* spp.). *American Journal of Agricultural and Biological Sciences.*5 (2) : 128-134

Shoeb, M., Celik, S., Jaspars, M., Kumarasamy, Y., MacManus, S., Nahar, L., Kong, T. L. P. & Sarker, S. D. (2005). Isolation, structure elucidation and bioactivity of schischkiniin, a unique indole alkaloid from the seeds of *Centaurea schischkinii*.

Tetrahedron. 61: 9001-9006.

Sholi, N. J. Y., Agrawal, A. C. A. & Sarin, N. B. (2009). ABA enhances plant regeneration of somatic embryos derived from cell suspension cultures of plantain cv. Spambia (*Musa sp*.). *Plant Cell, Tissue and Organ Culture*. 99: 133-140.

Shwe, H. H., Aye, M., Sein, M. M., Htay, K. T., Kreitmeier, P., Gertsch, J., Reiser, O. & Heilmann, J. (2010). Cytotoxic steroidal saponins from the rhizomes of *Tacca integrifolia*.*Chemistry Biodiversity*. 7: 610-622.

Silveira, R. C., Andrade, L. N., Oliveira, R. R. B. & Sousa, D. P. (2014). A review on anti-inflammatory activity of phenylpropanoids found in essential oils. *Molecules.* 19: 1459-1480.

Simons, F. E. R. (2004). Advances in H1-antihistamines. *The New England Journal of Medicine.* 351: 2203–2217.

Singh, K. K. & Gurung, B. (2009). *In vitro* propagation of *Rhododendron maddeni* Hook. F. an endangered Rhododendron species of Sikkim Himalaya. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. 37(1): 79-83.

Singh, A., Holvoet, A. & Mercenier, A. (2011). Dietary polyphenols in the prevention and treatment of allergic diseases. *Clinical and Experimental Allergy.* 41(10): 1346 1359.

Skirvin, R. M. & Chu, M. C. (1984). The effect of light quality on root development on in vitro grown miniature roses. *Horticultural Science.* 19: 575

Smith, W. A. (1981). The aftermath of the test tube. *Proceeding International Plant Propagation Society*, 31: 47-49.

Sohn, E. H., Jang S. A., Joo, H., Park, S., Kang, S. C., Lee, C.H., and Kim, S.Y. (2011). Anti-allergic and anti-inflammatory effect of butanol extract from *Articum lappa* L. *Clinical and Molecular Allergy*. 9: 4.

Stevigny, C., Bailly, C. & Quetin-Leclercq, J. (2005). Cytotoxic and antitumor potentialities of Aporphinoid alkaloids. *Current Medicinal Chemistry – AntiCancer agents* 5: 173-182.

Sudha, C.G., Krishnan, P.N., Pushpanga

dan, P. & Seeni, S. (2005). *In vitro* propagation of *Decalepis arayalpathra*, a critically endangered ethnomedicinal plant. *In Vitro Cellular and Development Biology Plant*. 41: 648-654.

Sudre, C. P., Leonardecz, E., Rodrigues, R. & Amaral Junior, A. T. (2007). Genetic resources of vegetable crops: a survey in the Brazilian germplasm collections pictured through papers published in the journals of the Brazilian Society for Horticultural Science. *Horticultura Brasileira*. 25: 496-503.

Suffness, M. & Pezzuto, J. M. (1990). Assays related to cancer drug discovery. In: Hostettmann, K. (Ed). *Methods in Plant Biochemistry: Assays for Bioactivity*, vol. 6. Academic Press, London: 71-133.

Sugimura, T. (2000). Nutrition and dietary carcinogens. *Carcinogenesis*. 21: 387-395.

Sukkaew, T., Chareonsap, P. & Ramasoot, S. (2012). Effect of culture medium, genotype and kinds of zygotic embryo on germination of bat flowers. *Proceedings of 38th Congress on Science and Technology of Thailand*: 13.

Sun, J., Zhang, X., Broderick, M. & Fein, H. (2003). Measurement of nitric oxide production in biological systems by using Griess reaction assay. *Sensors*. 3: 276-284.

Supawadee, T. & Sompong, T. (2010). Effect culture medium and genotype on germinationof hybrid oil palm zygotic embryos. Department of Plant Science, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand. *ScienceAsia.* 36: 26–32.

Szamosi, C., Solmaz, I., Sari, N. & Barsony, C. (2009). Morphological characterization of Hungarian and Turkish watermelon (*Citrullus lanatus* (Thunb.) Matsum et. Nakai) genetic resources. *Genetic Resources and Crop Evolution*. 56: 1091-1105.

Tetsumura, T., Matsumoto, Y. & Sato, M. (2008). Evaluation of basal media for micropropagation of four highbush blueberry cultivars. *Scientia Horticulturae*. 119: 72–74.

Tewtrakul, S. & Subhadhirasakul, S. (2007). Anti-allergenic activity of some selected plants in the Zingiberaceae family. *Journal of Ethnopharmacology*. 109: 535-538.

Theoharides, T. C. & Kalogeromitros, D. (2006). The critical role of mast cells in allergy and inflammation. *Annals of the New York Academy of Science*. 1088: 78-99.

Tiamjan, R., Panthong, A., Taesotikul, T., Rujjanawate, C.,Taylor, W. C. & Kanjanapothi, D. (2007). Hypotensive activity of *Tacca chantriers* and its hypotensive principles. *Pharmaceutical Biology*. 45: 481-485.

Thirunavoukkarasu, M., Panda, P. K., Nayak, N., Behera, P. R. & Satpathy, G. B. (2010). Effect of media type and explants source on micropropagation of *Dalbergia sissoo* Roxb-An important multipurpose forest tree. *International Journal of Plant Sciences*. 1: 155-162.

Thurmond, R. L., Gelfand, E. W. & Dunford, P. J. (2008). The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. *Nature Review Drug Discovery.*7:41–53.

Tilkat. E., Onay, A., Yildirim, H. & Ayaz, E. (2009). Direct plant regneration from mature leaf explants of pistachio, *Pistacia vera* L. *Scientia Horticulturae*. 121(3): 361-365.

Tom Dawn Communication Ltd. (2007). The Seed Germination Resource. http://www.dawncomms. co.uk/plants/default.htm, 26th February 2015.

Torres, K. C. (1989). *Tissue Culture Techniques for Horticultural Crops.* New York, London: Chapman and Hall.

Trigiano, R. N. & Gray, D. J. (1999). *Plant Tissue Culture Concepts and Laboratory Exercises*. CRC Press LLC, 2000 New York. ISBN 0-8493-2029-1.

Tripathi, R. D. & Tiwari, K. P. (1981). Phytochemical investigation of the roots of *Tacca aspera*. *Planta Medica*41: 414-415.

Tsikas, D. (2005). Methods of quantitative analysis of the nitric oxide metabolites nitrite and nitrate in human biological fluids. *Free Radical Research.* 39(8): 797-815.

Tundis, R., Bonesi, M., Deguin, B., Loizzo, M.R., Menichini, F., Conforti, F., Tillequin, F. and Menichini, F. (2009). Cytotoxic activity and inhibitory effect on nitric oxide production of triterpene saponins from the roots of *Physospermum verticillatum* (Waldst & Kit) (Apiaceae). *Bioorganic and Medicinal Chemistry*. 17(13), 4542-4547.

Tyagi, D. K. (2005). *Pharma Forestry: Field Guide to Medicinal Plants*. 276, Atlantic, New Delhi.

Ubalua, A. O. & Okoroafor, U. E. (2013). Micropropagation and postflask management of sweet potato using locally available materials as substrates for hardening. *Plant Knowledge Journal* 2(2): 56-61.

Ubwa, S. T., Anhwange, B. A. & Chia, J. T. (2011). Chemical analysis of *Tacca leontopetaloides* peels. *American Journal of Food Technology* 6 (10): 932-938.

Udomdee, W., Wen, P. J., Chin, S. W. & Chen, F. C. (2012). Shoot multiplication of *Paphiopedilum* orchid through in vitro cutting methods. *African Journal of Biotechnology*. 11(76): 14077-14082

Ukpabi, U. J., Ukenye, E. & Olojede, A. O. (2009). Raw material potentials of Nigerian wild polynesian arrowroot (*Tacca leontopetaloides*) tubers and starch. *Journal Food Sceince and Technology*. 7: 135-138.

Vasil, I. K.& Thorpe, T. A. (1998). *Plant cell and Tissue Culture*. Dordrecht: Kluwer Academy Publisher.

Varjda, R. & Varjda, T. (2001). The effects of cytokinin type and concentration and number of subcultures on the multiplication rate of some decorative plants. *Proceedings of the Estonian Academy of Science, Biology and Ecology.* 50: 22-32.

Verma, A., Prasad, K. N., Singh, A. K., Nyati, K. K., Gupta, R. K. & Paliwal, V. K. (2010). Evaluation of the MTT lymphocyte proliferation assay fro the diagnosis of neurocycticernosis. *Journal of Microbiological Methods*. 81: 175-178

Vieittez, A. M., Sanchez, M. C., Amo-Marco, J. B & Ballester, J. B. (1994). Forced flushing of branch segments as a method fro obtaining reactive explants of

mature *Quercus robur* trees for micropropagation. *Plant Cell Tissue Organ Culture*. 37: 287-295

Vikran, T. & Rashid, A. (2002). Somatic embryogenesis from immature and mature embryos of a minor millet *Paspalum scrobiculatum* L. *Plant Cell, Tissue and Organ Culture*. 69: 71–77.

Vujovic, T., Ruzic, D. J. & Cerovic, R. (2012). *In vitro* shoot multiplication as influenced by repeated subculturing of shoots of cotemporary fruit roostocks. *Horticultural Science* (Prague) 3: 101-107.

Vuylsteke, D. R. (1989). Shoot-tip culture for the propagation, conservation and exchange of *Musa* germplasm. *International Board for Plant Genetic Resources*, Rome.

Vuylsteke, D. & Langhe, D. (1985). Feasibility of *in vitro* propagation of bananas and plantains. *Tropical Agriculture* (Trinidad). 62: 323-328.

Wagley, L. M., Gladfelter, H. J. & Phylips, G. C. (1987). *De novo* shoot organogenesis of *Pinus elderica* Medv. *In vitro*. II. Macro-photographic evidence of *de novo* regeneration. *Plant Cell Reproduction*. 6: 167–171.

Wang, Y. H., Tache,´ Y., Harris, A. G., Kreutner, W., Daly, A. F. & Wei, J. Y. (2005). Desloratadine prevents compound-induced mast cell degranulation: visualization using a vital fluorescent dye technique. *Allergy*. 60:117–124.

Wang, W., Zhou, Q., Liu, Lu. & Zou, K. (2012). Anti-allergic activity of emodin on IgE-mediated activation in RBL-2H3 cells. Pharmacological Reports, 64: 1216-1222.

Watmough, N. J., Butland, G., Cheesman, M. R., Moir, J. W. B., Richardson, D. J. & Spiro, S. (1999). Nitric oxide in bacteria: synthesis and consumption. *Biochim Biophys Acta* 1411: 456-474.

Webster, S., Mitchell, S. & Ahmad, M. (2003). A Novel Surface Sterilization Methods for Reducing Microbial Contamination of Field Grown Medicinal Explants Intended for in vitro Culture. Paper presented at the *17th Annual National Conference of Science and Technology*, Biotechnology Centre, UWI, Mona, Kingston, Jamaica, West Indies.

Wen, B., He, H. Y., Yang, X. Y. & Lan, Q. Y.(2002). Characteristics of seed storage and germination of *Tacca chantrieri*. *Journal of Plant Resources and Environment* 11, 16-19 (in Chinese with English abstract).

Wendell, K. L., Wilson, L. & Hordan, M. A. (1993). Mitotic block in HeLa cells by vinbalstine: Ultra structural changes in kinetochore-microtubule attachement and in centrosomes. *Journal of Cell Science*. 104: 261-274.

White, P. R. (1963). *The Cultivation and Plant and Animal Cells*. 2nd edition. Ronald Prss, New York.

Wiart, C. (2006). *Book of Medicinal Plant of The Asia-Pacific -drugs for the future?*.World Scientific Publishing Co. Pte. Ltd.

Wilkins, H. F. (1988). Techniques to maximize cutting production. *Acta Horticulturae*. 226: 137–143.

Wojtania, A., Pulawska, J & Gabryszewka, E. (2005). Identification and elimination of bacterial contaminants from Pelargomium tissue cultures. *Journal of Fruit and Ornamental Plant Research*, 13(1): 101-108.

World Checklist of Selected Plant Families, Kew Royal Botanic Gardens. (http://app.kw.org/wcsp/home.do).

World Health Organization (WHO). (http://www.who.int/mediacentre/factsheets/fs297/en) 14th April 2015.

Wroblewska, K. (2012). The influence of adeninde and benzyladenine on rooting and development of *Fuchsia* hybrid cuttings. *Acta Agrobotanica*. 65(4): 101-108.

Wu, G. & Meininger, C. J. (2002). Regulation of nitric oxide synthesis by dietary factors. *Annual Review of Nutrition.*22:61–86.

Xie, H. & He, S. H. (2005). Roles of histamine and its receptors in allergic and inflammatory bowel diseases. *World Journal of Gastroenterol*. 11: 2851-2857.

Yamada, T., Saito, H. & Fujieda, S. (2014). Present state of Japanese cedar pollinosis: The national affliction. *The Journal of Allergy and Clinical Immunology*. 133: 632-639.

Yang, F., Troncy, E., Francoeur, M., Vinet, B., Vinay, P., Czaika, G. & Blaise, G. (1997). Effect of reducing agents and temperatures on conversion of nitrite and nitrate to nitric oxide and detection of NO by chemiluminescence. *Clinical Chemistry*. 43: 657-662.

Yasodha, R., Kamala, S., Kumar, S., Kumar, P.D. & Kalaiarasi, K. (2008). Effect of glucose on *in vitro* rooting of mature plants of *Bambusa nutans*. *Scientia Horticulturae*. 116(1): 113-116.

Yokosuka, A., Mimaki, Y. & Sashida, Y. (2002a). Spirostanol saponins from the rhizomes of *Tacca chantrieri* and their cytotoxic activity. *Phytochemistry*. 61: 73-78.

Yokosuka, Y., Mimaki, Y., Sakagami, H. & Sashida, Y. (2002b). New diarylheptanoids and diarylheptanoid glycosides from the rhizomes of *Tacca chantrieri* and their cytotoxic activity. *Journal of Natural Products* 65: 283–289.

Yokosuka, A., Mimaki, Y. & Sashida, Y. (2002c). Two new steroidal glycosides from *Tacca chantrieri*. *Natural Meidicine* (Tokyo). 56: 208–211.

Yokosuka, A., Mimaki ,Y. & Sashida, Y. (2002d). Steroidal and pregnane glycosides from the rhizomes of *Tacca chantrieri*. Journal of Natural Product. 65: 1293–1308.

Yokosuka, A., Mimaki, Y. & Sashida, Y. (2003). Chantriolides A and B, two new withanolide glucosides from the rhizomes of *Tacca chantrieri*. *Journal of Natural Products*. 66: 876–878.

Yokosuka, A., Mimaki, Y., Sakuma, C. & Sashida, Y. (2005). New glycosides of the campesterol derivative from the rhizomes of *Tacca chantrieri*. *Steroids.*70: 257–265.

Yokosuka, A., Mimaki, Y. & Sashida, Y. (2006). Spirostanol saponins from the rhizomes of *Tacca chantrieri*and their cytotoxic activity. *Phytochemistry*.61: 73–78.

Zhang, X. & Broderick, M. (2003). *Electrochemical NO sensors and their applications in biomedical research*. Biomedical Significance of Nitric Oxide 2003, International Scientific literature, Inc.

Zhang, L., Spencher, C. H. B., Gao, J. Y., Jin, C., Cole, W. W., Liu, Y., Bai, Z. L. & Li Q.J. (2005). Predicting Mating Pattern from Pollination Syndromes: The case of "Sapromyiophily" in *Tacca chantrieri* (Taccaceae). American Journal of Botany 92(3): 517- 524. *American Journal of Botany* 92: 517-524.

Zhang, L., Liu, J. Y., Xu, L. Z. & Yang, S. L. (2009). Chantriolide C, a new withanolide glucoside and a new spirostanol saponin from the rhizomes of *Tacca chantriers*. *Chemical and Pharmaceutical Bulletin*. 57: 1126-1128.

Zhang, L., Li, H. T., Gao, L. M., Yang, J. B., Li, D. Z., Cannon, C. H., Chen, J., Li, Q. J. (2011). Phylogeny and Evolution of Bracts and Bracteoles in *Tacca* (Dioscoreaceae). *Journal of Integrative Plant Biology*. 53(11): 901–911.

Zimmerman, R. H. (1986). Propagation of fruit, nut and vegetable crops-overview. In *Tissues Culture as a Plant Production System for Horticultural Crops*. Zimmerman, R. H., Griesbach, R. J., Hammerschlag, F. A. & Lawson, R. H. Editors. Dordretch: MArtinus Nijhoff Publishers: 183-200.

Zimmerman, R. H. & Broome, D. C. (1980). Apple cultivar micropropagation. *USDA, agriculture Research Results ARR-NE-11*: 54-63.

Zuco, V., Supino, R., Righetti, S.C., Cleris, L., Marchesi, E., Passerini, C.G. & Formelli, F. (2002). Selective cytotoxicity of betulinic acid on tumour cell lines, but not on normal cell. *Cancer Letters*. 175:17-25.