

ต้นฉบับ หน้าขาดหาย

TISSUE CULTURE AND ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACT
FROM LEAF AND STEM OF NIGHT BLOOMING JASMINE

Nyctanthes arbor-tristis L.

MR. PRASERT SALIKA

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Biotechnology

Programme of Biotechnology

Faculty of Science

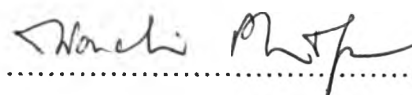
Chulalongkorn University

Academic Year 2000

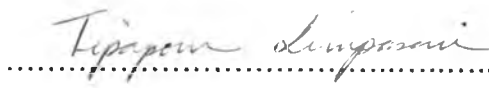
ISBN 974-346-661-4

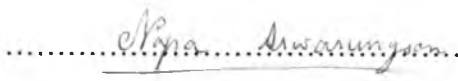
Thesis Title TISSUE CULTURE AND ANTIMICROBIAL ACTIVITY OF
CRUDE EXTRACT FROM LEAF AND STEM OF NIGHT
BLOOMING JASMINE *Nyctanthes arbor-tristis* L.
By Mr. Prasert Salika
Programme Biotechnology
Thesis Adviser Assistant Professor Napa Siwarungson


Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment
of the Requirements for the Master 's Degree


.....Dean of Faculty of Science
(Associate Professor Wanchai Phothiphichitr, Ph.D.)

THESIS COMMITTEE


.....Chairman
(Assistant Professor Tipaporn Limpaseni, Ph.D.)


.....Thesis Advisor
(Assistant Professor Napa Siwarungson)


.....Member
(Associate Professor Wiyada Thephuttee)

ประเสริฐ สาลิกา : การเพาะเลี้ยงเนื้อเยื่อและความสามารถในการต้านเชื้อจุลินทรีย์ของ
สารสกัดจากใบและลำต้นกรรณิการ์ *Nyctanthes arbor-tristis* L.

อ. ที่ปรึกษา : ผศ. นภา ศิวรังสรรค์ 88 หน้า. ISBN 974-346-661-4.

การเพาะเลี้ยงเนื้อเยื่อกรรณิการ์ *Nyctanthes arbor-tristis* L. เพื่อการขยายพันธุ์ในภาวะ
ปลอดเชื้อโดยเพาะเลี้ยงส่วนปล้อง พบว่ามียอดเกิดขึ้นบริเวณตาข้างของข้อ ในอาหารสูตร MS
และ BA ที่ความเข้มข้น 0.4 mg/l และเมื่อเพาะเลี้ยงส่วนยอดในอาหาร MS, BA 0.3, 0.4 mg/l
และ NAA 0.1, 0.2, 0.3, 0.4 mg/l มีการชักนำให้เกิดยอดจำนวนมาก (multiple shoots) ที่ NAA
0.1 mg/l ได้มากที่สุด การชักนำให้เกิดรากพบได้โดยใช้ออกซิน 2,4-D ในอาหาร MS ที่ไม่มีการ
เติมผงถ่านเท่านั้น สารสกัดที่ได้จากใบและลำต้นของกรรณิการ์(จากต้นในธรรมชาติ)สามารถต้าน
เชื้อ(ในอาหารแข็ง) *E. coli* และ *B. subtilis* ที่ความเข้มข้น 0.15 mg/ml และ 0.075 mg/ml ส่วน
เชื้อ *Aspergillus sp.* และ *S. cerevisiae* ต้านได้ที่ความเข้มข้น 0.15 mg/ml เท่านั้น จากการ
เพาะเลี้ยงเนื้อเยื่อสามารถต้านเชื้อ *E. coli*, *B. subtilis* และ *S. cerevisiae* ที่ความเข้มข้น 0.15
mg/ml แต่ไม่สามารถต้านเชื้อ *Aspergillus sp.* ได้การต้านเชื้อในอาหารเหลวพบว่า การต้านเชื้อ
E. coli และ *B. subtilis* เป็นแบบ inhibit growth ส่วน *Aspergillus sp.* และ *S. cerevisiae* เป็น
แบบ partial inhibit growth นอกจากนั้นสามารถสกัดแคโรทีนอยด์ (carotenoid) จากส่วนโคน
กลีบดอกซึ่งมีสีส้มได้และมีค่า $R_f = 0.93$ เมื่อนำมาวิเคราะห์โดย TLC (Thin layer
chromatography)

หลักสูตร...เทคโนโลยีทางชีวภาพ.....
สาขาวิชา.....เทคโนโลยีทางชีวภาพ.....
ปีการศึกษา.....2543.....

ลายมือชื่อนิสิต..... ประเสริฐ สาลิกา.....
ลายมือชื่ออาจารย์ที่ปรึกษา..... ผศ. นภา ศิวรังสรรค์.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....-.....




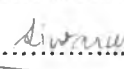
##4072303123 : MAJOR BIOTECHNOLOGY

KEY WORD : *Nyctanthes arbor-tristis* L. / MICROPROPAGATION/

ANTIMICROBIAL

PRASERT SALIKA : TISSUE CULTURE AND ANTIMICROBIAL
ACTIVITY OF CRUDE EXTRACT FROM LEAF AND STEM OF
NIGHT BLOOMING JASMINE *Nyctanthes arbor-tristis* L. THESIS
ADVISOR : ASSISTANT PROFESSOR NAPA SIWARUNGSON 8 8pp.
ISBN 974-346-661-4

Tissue of *Nyctanthes arbor-tristis* L. was cultured *in vitro* for multiplication shown successive shoot induction from internode cultured in MS medium containing 0.4 mg/l BA. Multiple shoot could be obtained culture from internode segments on MS medium supplemented with 0.4 mg/l BA and 0.1 mg/l NAA. Supplementation of 2,4-D at 0.1 mg/l in MS medium without non activated charcoal could induce root formation. Crude extracts from leaf and stem of mother plants from *Nyctanthes arbor-tristis* L. were shown to inhibit growth of *E. coli* and *B. subtilis* at 0.15 mg/ml, 0.075 mg/ml while antifungus activity in *S. cerevisiea* and *Aspergillus sp.* were shown at 0.15 mg/ml only. The extracts of leaves and stems from tissue culture showed microbial activity in *E. coli*, *B. subtilis* and *S. cerevisiea* at 0.15 mg/ml while in *Aspergillus sp.* were not found. After that, Crude extracts of *Nyctanthes arbor-tristis* L. from mother plants and tissue culture (leaves and stems) showed inhibition of growth of bacteria: *E. coli*, *B. subtilis* while they showed partial inhibition of growth in *S. cerevisiea* and *Aspergillus sp.* Extraction of carotenoid from corolla tube of *Nyctanthes arbor-tristis* L. were detected by TLC with Rf = 0.93.

Program.....Biotechnology..... Student's signature..... 
Field of study.....Biotechnology..... Advisor's signature..... 
Academic year.....2543..... Co-advisor's signature.....-

ACKNOWLEDGEMENT



I wish to especially be grateful to my advisor, Assist. Prof. Napa Siwarungson for her suggestions throughout my study and I would also like to express my appreciation to Dr. Pattamarat Rodkachane for her great helps, guidance, suggestion in laboratory.

I would also wish to accredit Assist. Prof. Dr. Tipaporn Limpaseni and Assoc. Prof. Wiyada Thephuttee for their consultations, corrections and evaluations my thesis.

Finally, I would like to express my deepest appreciation and gratefulness to my parents, sisters and brother for their warmest love, care, understanding and cheerfulness through my study.

CONTENTS

| | Page |
|--------------------------------|------|
| THAI ABSTRACT..... | iv |
| ENGLISH ABSTRACT..... | v |
| ACKNOWLEDGEMENT..... | vi |
| CONTENT..... | vii |
| LIST OF TABLES..... | viii |
| LIST OF FIGURES..... | ix |
| LIST OF ABBREVIATIONS..... | xii |
| CHAPTER | |
| I. INTRODUCTION..... | 1 |
| II. MATERIALS AND METHODS..... | 20 |
| III. RESULTS..... | 30 |
| IV. DISCUSSION..... | 61 |
| V. CONCLUSION..... | 66 |
| REFERENCES..... | 68 |
| APPENDICES | |
| APPENDIX A..... | 77 |
| APPENDIX B..... | 82 |
| APPENDIX C..... | 86 |
| BIOGRAPHY..... | 88 |

LIST OF TABLES

| Table | Pag |
|-------|---|
| 1.1 | <i>In vitro</i> regeneration studies on some plants.....7 |
| 1.2 | Distribution of iridoids in some families of plants.....13 |
| 3.1 | Effect of growth regulator of various BA and NAA on shoot induction from internodes in MS medium.....31 |
| 3.2 | Effect of growth regulator of various BA and NAA on shoot induction from internodes in GD medium.....31 |
| 3.3 | Growth effect by regulator such as various BA and NAA, on shoot induction in MS medium for multiple shoots.....33 |
| 3.4 | Influence of MS medium with growth regulator for roots induction.....33 |
| 3.5 | Influence of MS medium with 2,4-D for roots induction.....34 |
| 3.6 | Yield of crude extract in mother plants of <i>Nyctanthes arbor-tristis</i> L.....41 |
| 3.7 | Yield of crude extract from tissue culture of <i>Nyctanthes arbor-tristis</i> L.....41 |
| 3.8 | Result of antibacterial activity from crude extract on <i>E. coli</i>55 |
| 3.9 | Result of antibacterial activity from crude extract on <i>Bacillus substilis</i>55 |
| 3.10 | Result of antiyeast from crude extract on <i>Saccharomyces cerevisiae</i>56 |
| 3.11 | Result of antifungus from crude extract on <i>Aspergillus sp.</i>56 |

LIST OF FIGURES

| Figure | Page |
|---|------|
| 1.1 Stem of <i>Nyctanthes arbor-tristis</i> L..... | 2 |
| 1.2 Flowers of <i>Nyctanthes arbor-tristis</i> L..... | 3 |
| 3.1 Shoots induction from internode explants of <i>Nyctanthes arbor-tristis</i> L. supplemented with MS and BA 0.4 mg/l within 8 weeks..... | 35 |
| 3.2 Multiple shoots of <i>Nyctanthes arbor-tristis</i> L. supplement with MS, BA 0.4 mg/l and NAA 0.1 mg/l within 4 weeks from shoots induction | 36 |
| 3.3 Subculture of multiple shoots within 4, 6, 8, and 12 weeks from <i>Nyctanthes arbor-tristis</i> L. supplement with MS, BA 0.4 mg/l and NAA 0.1 mg/l..... | 37 |
| 3.4 Subculture of multiple shoots within 6, 8, 12, and 16 weeks from <i>Nyctanthes arbor-tristis</i> L. supplement with MS, BA 0.4 mg/l and NAA 0.1 mg/l..... | 38 |
| 3.5 Roots induction of <i>Nyctanthes arbor-tristis</i> L. supplement with MS and 0.1 mg/l 2,4-D within 8 weeks from shoots culture..... | 39 |
| 3.6 Plantlet of <i>Nyctanthes arbor-tristis</i> L. from tissue cultures in nursery..... | 40 |
| 3.7 TLC for crude extracts of <i>Nyctanthes arbor-tristis</i> L..... | 43 |
| 3.8 Chromatogram of carotenoids extraction from <i>Nyctanthes arbor-tristis</i> L..... | 44 |
| 3.9 Inhibition zone of crude extract (leafs) from mother plants on <i>E. coli</i> | 47 |

| | | |
|------|---|----|
| 3.10 | Inhibition zone of crude extract (stems) from mother plants on <i>E. coli</i> | 47 |
| 3.11 | Inhibition zone of crude extract (leafs) from tissue culture on <i>E. coli</i> | 48 |
| 3.12 | Inhibition zone of crude extract (stems) from tissue culture on <i>E. coli</i> | 48 |
| 3.13 | Inhibition zone of crude extract (leafs) from mother plants on <i>Bacillus substilis</i> | 49 |
| 3.14 | Inhibition zone of crude extract (stems) from mother plants on <i>Bacillus substilis</i> | 49 |
| 3.15 | Inhibition zone of crude extract (leafs) from tissue culture on <i>Bacillus substilis</i> | 50 |
| 3.16 | Inhibition zone of crude extract (stems) from tissue culture on <i>Bacillus substilis</i> | 50 |
| 3.17 | Inhibition zone of crude extract (leafs) from mother plants on <i>Saccharomyces cerevisiae</i> | 51 |
| 3.18 | Inhibition zone of crude extract (Stems) from mother plants on <i>Saccharomyces cerevisiae</i> | 51 |
| 3.19 | Inhibition zone of crude extract (leafs) from tissue culture on <i>Saccharomyces cerevisiae</i> | 52 |
| 3.20 | Inhibition zone of crude extract (stems) from tissue culture on <i>Saccharomyces cerevisiae</i> | 52 |
| 3.21 | Inhibition zone of crude extract (leafs) from mother plants on <i>Aspergillus sp</i> | 53 |
| 3.22 | Inhibition zone of crude extract (stems) from mother plants on <i>Aspergillus sp</i> | 53 |
| 3.23 | Inhibition zone of crude extract (leafs) from tissue culture on <i>Aspergillus sp</i> | 54 |

| | | |
|------|--|----|
| 3.24 | Inhibition zone of crude extract (stems) from tissue culture on <i>Aspergillus sp.</i> | 54 |
| 3.25 | Kinetic inhibition from crude extract of <i>Nyctanthes arbor-tristis</i> L. on <i>E. coli</i> | 57 |
| 3.26 | Kinetic inhibition from crude extract of <i>Nyctanthes arbor-tristis</i> L. on <i>Bacillus subtilis</i> | 58 |
| 3.27 | Kinetic inhibition from crude extract of <i>Nyctanthes arbor-tristis</i> L. on <i>Saccharomyces cerevisiae</i> | 59 |
| 3.28 | Kinetic inhibition from crude extract of <i>Nyctanthes arbor-tristis</i> L. on <i>Aspergillus sp.</i> | 60 |

LIST OF ABBREVIATIONS

| | |
|--------------------|-------------------------------------|
| 2,4-D | = 2,4 Dichlorophenoxyacetic acid |
| BA | = 6-Benzylaminopurine |
| cm | = centimetre |
| GD | = Gresshoff and Doy media (1972) |
| l | = liter |
| lb/in ² | = Pound per square inch |
| mg | = milligram |
| min | = minute |
| ml | = millilitre |
| mm | = millimetre |
| MS | = Murashige and Skoog media (1962) |
| NAA | = α -naphthalene acetic acid |
| nm | = nanometre |
| $^{\circ}$ C | = Degree Celsius |
| Rf | = Rate of flow in chromatography |
| RPM | = round per minute |
| SD | = Standard deviation |
| TLC | = Thin layer chromatography |