## Table of Contents

**Page**

Abstract ..…….…..... (1)

Acknowledgements ……... (3)

List of Tables ……..……..…… (7)

List of Figures ……..... (9)

Chapter

1. Introduction ……..... 1

   Objectives ……... 3

2. Review of Literature ……. 4

   1. General data of plants called Hua-Khao-Yen ……. 4
   2. *Dioscorea membranacea* Pierre ……. 6
      - General description of *Dioscorea membranacea* Pierre ……..... 6
      - Chemical compounds and biological activities of *D. membranacea* 7
   3. Cancer …… 12
      - Etiology of cancer ……..…… 14
      - Tumor origin ……..…… 17
      - Diagnosis ……..……. 19
      - Cancer treatment ……..……. 21
   4. Breast cancer and MCF-7 cell line ……...……..…… 24
      - Breast cancer ……..…… 24
      - MCF-7 cell line ……..…… 27
   5. Cytotoxic acitivity test ……...…… 29

**Table of Contents (continued)**


**Page**

6. High performance liquid chromatography (HPLC) ...……. 31
   - Chromatography in the pharmaceutical world ……..…… 32
   - Chromatographic process …….……. 33
   - General separation process ……..... 33
   - HPLC separation modes ……... 35
7. Stability testing …….. 38
   - General principles ……. 38
   - Storage conditions ……. 38

3. Methodology ……..... 41

1. Conceptual frame work …….. 41
2. Plant material ……..…….……..... 42
3. Preparation of plant extracts ……..…..... 43
4. Isolation of dioscorealide B from *D. membranacea* ……..... 45 by column chromatography
5. Quantitative determination of dioscorealide B by HPLC technique .. 47
6. *In vitro* assay for cytotoxic activity of *D. membranacea* extracts….. 51
7. Stability study of ethanolic extract under accelerated conditions ….. 55

4. Findings and Results ……. 57

1. Identification of plant material ……..... 57
2. Preparation of plant extracts ……... 58
   - Percentage of yield of *D. membranacea* extracts ……. 58
3. Isolation of dioscorealide B from *D. membranacea* by ……. 61 column chromatography
   - Isolation of dioscorealide B ……... 61
   - Identification of isolated compound …….. 61

**Table of Contents (continued)**


**Page**

4. Chemical fingerprint analysis of *D. membranacea* …….. 65
   extracts by HPLC technique
   - Validation of HPLC method for dioscorealide B content analysis 66
   - HPLC fingerprints of *D. membranacea* extracts ……..…… 70
   - Quantitative determination of dioscorealide B from …… 74
     *D. membranacea* extracts
5. *In vitro* assay for cytotoxic activity of *D. membranacea* extracts …. 78
6. Stability study of ethanolic extract under accelerated conditions …. 81
   - Stability of dioscorealide B in ethanolic extract ……. 81
   - Cytotoxic activity change of ethanolic extract ……. 84

5. Conclusions and Recommendations ……. 90

References ……..... 94

Appendix

A. Chemical Reagents …… 102

B. List of Abbreviations and Symbols ……..... 104

Vita ……... 109