
**Faculty of Medicine and Health Sciences**

# Antioxidant, Antimicrobial and Wound Healing Properties of *Clinacanthus nutans* (Burm. f.) Lindau and *Strobilanthes crispus* (L.) Blume Extracts

**Ban Weng Kit**

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# Antioxidant, Antimicrobial and Wound Healing Properties of *Clinacanthus nutans* (Burm.f.) Lindau and *Strobilanthes crispus* (L.) Blume Extracts

Ban Weng Kit

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## DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Signature

Name: Ban Weng Kit

Matric No.: 18020132

Faculty of Medicine and Health Sciences

Universiti Malaysia Sarawak

Date : 10 JUNE 2023

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## ABSTRACT

*Clinacanthus nutans* (*C. nutans*) and *Strobilanthes crispus* (*S. crispus*) both are well-known for their specific beneficial properties against different diseases. *C. nutans* is known to be anticancer and antiviral, especially against colon cancer, *varicella-zoster virus,* and herpes simplex virus. *S. crispus* has proven to be anti-diuretic and antidiabetic by multiple studies. In the previous studies, *S. crispus* extract showed promising results, capable of aiding in the wound healing process. Since *C. nutans* was commonly available as a balm in the traditional market, there is a high possibility that it contains antimicrobial and wound healing properties as well. Thus, this study aimed to confirm the medicinal benefits of *C. nutans* and *S. crispus*, specifically antimicrobial and wound healing properties. The *C. nutans* and *S. crispus* leaves were extracted with different polarity solvents; ethanol, acetone, and chloroform, through cold maceration and kept refrigerated at 2-8°C in the dark. The presence of phytochemicals, such as alkaloids, flavonoids, and saponin in the extracts were screened. These extracts were then assessed for their antioxidant potential by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay using ascorbic acid as standard; determination of Total Phenolic Content (TPC) and Total Flavonoids Content (TFC) using Gallic Acid and Rutin as standard, respectively. Disk Diffusion Assay was performed to determine their antimicrobial properties. Modified scratch assay by co-incubating skin fibroblast with Methicillin-resistant *Staphylococcus aureus* (MRSA) and treated with the *C. nutans* and *S. crispus* extracts to determine their wound healing effects. These extracts were observed at 1, 3, 6, 10, 24, and 48 hours to investigate the cell migration activity depicting the closing up of wound. The phytochemical profiles for both *C. nutans* and *S. crispus* extracts were similar, except the *C. nutans* extracts have a higher saponin level compared with *S. crispus* extracts. The *C. nutans* acetone extract contained the highest level of antioxidant potential and TPC than the other

*C. nutans* extracts. However, the ethanol extract of *C. nutans* showed the highest TFC, which was corresponded to the flavonoid content obtained from the phytochemical screening. The highest TPC value was recorded by the *S. crispus* ethanol extract, while the TFC test revealed similar pattern results among these *S. crispus* extracts. The *C. nutans* extracts showed a zone of inhibition of 14 to 16 mm when treated on *Ps. aeruginosa*, while *S. crispus* extracts showed distinctive zone of inhibition range between 11 to 15 mm on *Ps. aeruginosa*. There was no positive result observed when the extracts were treated on other bacteria. Fibroblasts were sensitive to acetone extracts, and *S. crispus* extracts were proven to have higher toxicity during the MTT assay. During the scratch assay with extracts only, *C. nutans* extracts showed wound healing properties, whereas *S. crispus* extracts seemed to be delayed the wound healing activity. However, when MRSA was added for the co-incubation, *S. crispus* extracts showed inhibition against the growth of MRSA and aided the wound healing activity, but this situation did not demonstrate in *C. nutans* extracts. The *C. nutans* and *S. crispus* extracts exhibited strong antioxidant and antimicrobial properties. *C. nutans* extracts aids in the wound healing activity, but if MRSA was involved, *S. crispus* extracts were capable of inhibiting the growth and aiding in the wound healing activity.

**Keywords:** *Clinacanthus nutans*, *Strobilanthes crispus*, antioxidant, antimicrobial, wound healing.

***Sifat-sifat Antioksidan, Antimikrobial dan Penyembuhan Luka* Clinacanthus *nutans* *(Burm.f.) Lindau dan* Strobilanthes crispus *(L.) Ekstrak***

## *ABSTRAK*

*Clinacanthus nutans (C. nutans) dan Strobilanthes crispus (S. crispus) kedua-duanya terkenal dengan ciri-ciri khusus bermanfaat terhadap penyakit yang berlainan. C. nutans dikenali sebagai antikanser dan antivirus, terutama terhadap kanser usus, virus varicella-zoster dan virus herpes simplex. S. crispus telah terbukti anti-diuretik dan antidiabetik oleh pelbagai kajian. Dalam kajian yang lepas, ekstrak S. crispus menunjukkan hasil yang menjanjikan, dapat membantu proses penyembuhan luka. Memandangkan C. nutans biasanya diperolehi sebagai balsem di pasar tradisional, maka ada kemungkinan besar ia juga mengandung sifat antimikrob dan penyembuhan luka. Oleh itu, kajian ini bertujuan untuk membukti kebaikan perubatan C. nutans and S. crispus, khususnya sifat antimikrob dan penyembuhan luka. Daun C. nutans dan S. crispus diekstraksi dengan pelarut pelbagai polariti: etanol, aseton dan kloroform melalui maserasi sejuk dan disimpan dalam peti sejuk pada suhu 2-8 ° C dalam keadaan gelap. Kandungan fitokimia seperti alkaloid, flavonoid dan saponin dalam ekstrak disaring. Ekstrak ini kemudian diuji potensi antioxidan dengan uji pemungutan radikal bebas DPPH (2,2-diphenil-1-picrylhidrazil) dengan asid askorbik sebagai standard, penentuan Jumlah Kandungan Fenolik Total (TPC) dan Jumlah Kandungan Flavonoid (TFC) dengan Asid Gallik dan Rutin masing-masing sebagai standard. Kaedah resapan cakera digunakan untuk menentukan sifat antimikrob mereka. Uji gores yang diubahsuai dengan menginkubasi fibroblas kulit dan* Methicillin Resistant *Staph. aureus (MRSA) dengan ekstrak C. nutans dan S. crispus digunakan untuk menentukan kesan penyembuhan luka. Ekstrak ini diperhatikan pada 1 jam, 3 jam, 6 jam, 10 jam, 24 jam, dan 48 jam untuk menyiasat aktiviti migrasi sel. Profil fitokimia untuk kedua-dua ekstrak C.*

*nutans dan S. crispus adalah serupa, kecuali ekstrak C. nutans mempunyai kandungan saponin yang lebih tinggi berbanding dengan ekstrak S. crispus. Ekstrak C. nutans aseton mempunyai potensi antioksidan dan TPC yang lebih tinggi berbanding dengan ekstrak C. nutans yang lain. Walau bagaimanapun, ekstrak etanol C. nutans menunjukkan TFC tertinggi yang sepadan dengan jumlah flavonoid yang diperolehi dari penyaringan fitikimia flavonoid. Ekstrak C. nutans menunjukkan zon perencatan antara 14 hingga 16 mm ketika dirawat pada* Ps. aeruginosa*, sementara ekstrak S. crispus menunjukkan zon perencatan yang jelas antara 11 hingga 15 mm pada Ps. aeruginosa. Semasa ekstrak merawat pada bakteria lain, tiada keputusan positif diperhatikan. Fibroblas sensitif terhadap ekstrak aseton dan ekstrak S. crispus terbukti mempunyai ketoksikan yang lebih tinggi semasa ujian MTT. Semasa ujian calar dengan ekstrak sahaja, ekstrak C. nutans menunjukkan sifat penyembuhan luka, sedangkan ekstrak S. crispus melambatkan aktiviti penyembuhan luka. Namun, ketika MRSA ditambahkan untuk inkubasi bersama, ekstrak S. crispus menunjukkan perencatan terhadap pertumbuhan MRSA dan membantu aktiviti penyembuhan luka, tetapi keadaan ini tidak ditunjuk dalam ekstrak C. nutans. C. nutans dan S. crispus menunjukkan sifat antioksidan dan antimikroba yang kuat. Ekstrak C. nutans membantu dalam aktiviti penyembuhan luka tetapi jika MRSA terlibat, ekstrak S. crispus mampu merencat pertumbuhannya dan membantu dalam aktiviti penyembuhan luka.*

***Kata kunci:*** *Clinacanthus nutans, Strobilanthes crispus, antioksidan, antimikrob, penyembuhan luka*

## TABLE OF CONTENTS


| | | Page |
|---|---|---|
| **DECLARATION** | | **i** |
| **ACKNOWLEDGEMENT** | | ii |
| **ABSTRACT** | | iii |
| **ABSTRAK** | | v |
| **TABLE OF CONTENTS** | | vii |
| **LIST OF TABLES** | | x |
| **LIST OF FIGURES** | | xi |
| **LIST OF ABBREVIATIONS** | | xiii |
| **CHAPTER 1** | **INTRODUCTION** | **1** |
| 1.1 | Study Background | 1 |
| 1.1.1 | Microenvironment on Wounds | 3 |
| 1.1.2 | Antimicrobial Resistance | 4 |
| 1.1.3 | Natural Products for Wound Healing Activities | 4 |
| 1.2 | Problem Statement | 6 |
| 1.3 | Objectives | 7 |
| 1.4 | Chapter Summary | 8 |
| **CHAPTER 2** | **LITERATURE REVIEW** | **9** |
| 2.1 | Acanthaceae Family | 9 |

2.2 *Clinacanthus nutans* (Burm f.) Lindau 11

2.3 *Strobilanthes crispus* (L.) Blume 21

2.3.1 External (Topical) Application of *Clinacanthus nutans* and *Strobilanthes crispus* 28

**CHAPTER 3 MATERIALS AND METHODS 34**

3.1 Plant Collection 34

3.2 Extraction 34

3.3 Phytochemical Screening 35

3.3.1 Alkaloids Test 35

3.3.2 Flavonoids Test 35

3.3.3 Saponin Test 36

3.4 Total Phenolics Content (TPC) 36

3.5 Total Flavonoids Content (TFC) 37

3.6 Antioxidant (2,2-Diphenyl-1-picrylhydrazyl Assay) 37

3.7 Antimicrobial (Disk Diffusion Assay) 38

3.7.1 Nutrient broth (NB) 38

3.7.2 Mueller-Hinton Agar (MHA) 38

3.7.3 Mueller-Hinton Broth (MHB) 39

3.7.4 Antimicrobial Sensitivity Test (AST) 39

3.8 Wound Healing Activity (Scratch Assay) 40

3.8.1 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay 41

3.8.2 Bacteria Preparation 42

3.8.3 Co-incubation *in-vitro* Assay 43

**CHAPTER 4 RESULTS 45**

4.1 Determination of Total Flavonoid and Phenolics Contents. 45

4.2 Determination of Antimicrobial Properties of *C. nutans* and *S. crispus* Plant Extracts against Gram-positive and Gram-negative Bacteria. 48

4.3 Effects of *C. nutans* and *S. crispus* Extracts on Skin Fibroblasts. 50

4.4 Wound Healing Effects of *C. nutans* and *S. crispus* Extracts Treated on Pseudo-wound in the Presence of MRSA 58

**CHAPTER 5 DISCUSSION 66**

5.1 Phytochemical 66

5.2 Total Phenolic Content and Total Flavonoid Content of *Clinacanthus nutans* (*C. nutans*) and *Strobilanthes crispus* (*S. crispus*) 67

5.3 Antioxidant Properties 69

5.4 Antimicrobial Properties 72

5.5 Wound Healing Properties 74

**CHAPTER 6 CONCLUSION AND RECOMMENDATIONS 79**

6.1 Conclusion 79

6.2 Recommendations 80

**REFERENCES 81**

**APPENDICES 96**

## LIST OF TABLES


| | | Page |
|---|---|---|
| **Table 2.1:** | The antioxidant potential of *C. nutans* extracts based on its DPPH free radical scavenging activities. | 14 |
| **Table 2.2:** | $IC_{50}$ of different solvent *C. nutans* extracts when treated on different cancer cell lines using various bioassays with their respectively study references. | 18 |
| **Table 2.3:** | Antioxidant activity of *S. crispus* extracts based on DPPH free scavenging assay | 23 |
| **Table 2.4:** | $IC_{50}$ of MTT assays against different cancer cell lines on *C. nutans* solvent extracts. | 27 |
| **Table 2.5:** | The Minimum Inhibition Concentration (MIC) of Gram-positive and Gram-negative bacteria when treated with different extracts of *C. nutans*. | 32 |
| **Table 2.6:** | The Minimum Inhibition Concentration (MIC) of Gram-positive and Gram-negative bacteria when treated with different extracts of *S. crispus*. | 33 |
| **Table 3.1:** | The preparation of culture media with different *C. nutans* and *S. crispus* extracts with MRSA | 44 |
| **Table 4.1:** | The result of the phytochemical screening for different solvents extracts of *Clinacanthus nutans* and *Strobilanthes crispus.* | 46 |
| **Table 4.2:** | The comparison of the antioxidant content (AAEAC), total phenolic content (TPC), and total flavonoids content (TFC) between *C. nutans* and *S. crispus* with different solvents extracts. | 47 |
| **Table 4.3:** | Zone of inhibition when different *C. nutans* and *S. crispus* extracts treated on Gram-positive and Gram-negative bacteria on MHA. | 49 |
| **Table 4.4**: | Cell ViabilityAssay of different concentrations of *C. nutans* extracts applied on the normal human skin fibroblast and incubated for 24 and 48 hours respectively. | 51 |
| **Table 4.5:** | Cell ViabilityAssay of different concentrations of *S. crispus* extracts applied on the normal human skin fibroblast and incubated for 24 and 48 hours respectively. | 55 |
| **Table 4.6:** | The Result of *in vitro* Scratch Assay with *C. nutans* extracts. | 64 |
| **Table 4.7:** | The Result of *in vitro* Scratch Assay with *S. crispus* extracts. | 65 |

## LIST OF FIGURES


**Page**

**Figure 1.1:** Stages of Wound Healing 2

**Figure 2.1:** *Clinacanthus nutans* 11

**Figure 2.2:** Bioactive Compunds in *Clinacanthus nutans* (*C. nutans*) 16

**Figure 2.3:** *Strobilanthes crispus* 21

**Figure 4.1:** Cell Viability Assay of different concentrations of *C. nutans* ethanol extract applied on the normal human skin fibroblast and incubated for 24 and 48 hours respectively. 52

**Figure 4.2:** Cell Viability Assay of different concentrations of *C. nutans* acetone extract applied on the normal human skin fibroblast and incubated for 24 and 48 hours respectively. 53

**Figure 4.3:** Cell Viability Assay of different concentrations of *C. nutans* chloroform extract applied on the normal human skin fibroblast and incubated for 24 and 48 hours respectively. 53

**Figure 4.4:** Cell Viability Assay of different concentrations of *S. crispus* ethanol extract applied on the normal human skin fibroblast and incubated for 24 and 48 hours respectively. 56

**Figure 4.5:** Cell Viability Assay of different concentrations of *S. crispus* acetone extract applied on the normal human skin fibroblast and incubated for 24 and 48 hours respectively. 57

**Figure 4.6:** Cell Viability Assay of different concentrations of *S. crispus* chloroform extract applied on the normal human skin fibroblast and incubated for 24 and 48 hours respectively. 57

**Figure 4.7:** Growth and coverage of MRSA over a duration of 24 hours when different concentration of MRSA were co-incubated with normal human skin fibroblasts. 58

**Figure 4.8:** Scratch Assay on normal human fibroblasts in the presence of *C. nutans* ethanol (E), chloroform (C) and acetone (A) extracts respectively. An *in vitro* wound healing process was observed in a time-dependent manner on normal human fibroblasts in the presence of ethanol (E), chloroform (C) and acetone (A), respectively. 60

**Figure 4.9:** Scratch Assay on normal human fibroblasts and MRSA. An *in vitro* wound healing process was observed in a time-dependent manner on

human fibroblasts in the presence of ethanol (E), chloroform (C) and acetone (A) *C. nutans* extracts respectively. 61

**Figure 4.10:** Scratch Assay on human fibroblasts in the presence of *S. crispus* extracts. An *in vitro* wound healing process was observed in a time-dependent manner on human fibroblasts in the presence of ethanol (E), chloroform (C) and acetone (A) respectively 62

**Figure 4.11:** Scratch Assay on human fibroblasts in the presence of *S. crispus* extracts and MRSA. An *in vitro* wound healing process was observed in a time-dependent manner on human fibroblasts in the presence of ethanol (E), chloroform (C) and acetone (A) respectively. 63

## LIST OF ABBREVIATIONS

| | |
|---|---|
| µg | Microgram |
| µm | Micron |
| ºC | Degree Celsius |
| *B. cereus* | *Bacillus cereus* |
| BHT | Butylated Hydroxytoluene |
| BT-474 | Human Breast Tumour Cell Line |
| Caco-2 | Human Colorectal Adenocarcinoma Cell Line |
| CaOx | Calcium Oxalate Crystals |
| CCD-18Co | Normal Human Colon Fibroblast Cell Line |
| CCK-8 | Cell Counting Kit |
| Chang Liver Cell | Derived HeLa Cell Line |
| $CO_2$ | Carbon Dioxide |
| *C. nutans* | *Clinacanthus nutans* |
| D24 | Human Melanoma Cancer Cell Line |
| DD | Disc Diffusion Method |
| DGDG | Digalactosyl Diglyceride |
| DPPH | 2,2-Diphenyl-1-Picrylhydrazyl |
| DMSO | Dimethyl Sulfoxide |
| *E. coli* | *Escherichia coli* |
| $EC_{50}$ | Half Maximal Effective Concentration |
| FDA | Food and Drug Administration |
| GAE | Gallic Acid Equivalent |
| HCT-116 | Human Colon Cancer Cell Line |

| | |
|---|---|
| HeLa | Human Cervical Cancer Cell Line |
| HepG2 | Human Liver Hepatocellular Carcinoma Cell Line |
| HIV | Human Immunodeficiency Virus |
| HSV-1 | *Herpes simplex* virus 1 |
| HSV-2 | *Herpes simplex* virus 2 |
| HT-29 | Human Colon Adenocarcinoma Cell Line |
| HUVEC | Primary Human Umbilical Vein Endothelial Cells |
| $IC_{50}$ | Half-Maximal Inhibitory Concentration |
| IMR32 | Human Neuroblastoma Cell Line |
| K562 | Human Erythroleukemic Cell Line |
| *K. pneumoniae* | *Klebsiella pneumoniae* |
| Lev 30 | Levofloxacin 30 μg |
| LS-174T | Human Colon Adenocarcinoma Cell Line |
| *M. luteus* | *Micrococcus luteus* |
| MHA | Mueller-Hinton Agar |
| MCF-7 | Human Breast Cancer Cell Line |
| MDA-MB-231 | Human Invasive Ductal Carcinoma Cell Line |
| MGDG | Monogalactosyl Diglyceride |
| MIC | Minimum Inhibitory Concentration |
| MM-418C1 | Human Melanoma Cancer Cell Line |
| MRSA | Methicillin-Resistant *Staphylococcus aureus* |
| MTT | 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl-2H-Tetrazolium Bromide |
| NCI-H23 | Human Non-Small Cell Lung Carcinoma |
| NCI-H460 | Human Non-Small Cell Lung Cancer Cell Line |

| | |
|---|---|
| NRU | Neutral Red Uptake |
| $OD_{600}$ | Optical Density at 600nm |
| OH | Hydroxy or Hydroxyl |
| ORAC | Oxygen Radical Absorbance Capacity |
| OUMS-36T-4F | Normal Human Skin Fibroblast |
| OX 1 | Oxacillin 1 μg |
| Pen 10G | Penicillin 10 unit |
| *P. acnes* | *Propionibacterium acnes* |
| PBS | Phosphate-Buffered Saline |
| *Ps. aeruginosa* | *Pseudomonas aeruginosa* |
| PDT | Photodynamic Therapy |
| Raji | Human Continuous Cell Line |
| RE | Rutin Equivalent |
| ROS | Reactive Oxidative Species |
| *Staph. aureus* | *Staphylococcus aureus* |
| *Staph. epidermidis* | *Staphylococcus epidermidis* |
| *Strep. pneumoniae* | *Streptococcus pneumoniae* |
| *Strep. pyogenes* | *Streptococcus pyogenes* |
| *S. crispus* | *Strobilanthes crispus* |
| Saos-2 | Human Osteosarcoma Cell Line |
| SNU-1 | Poorly Differentiated Human Primary Carcinoma of Stomach |
| *spp.* | Species |
| SRB | Sulforhodamine B |
| T25 | $25m^2$ Tissue Culture Flask |
| T-47D | Human Breast Cancer Cell Line |

| | |
|---|---|
| TPC | Total Phenolic Content |
| TFC | Total Flavonoids Content |
| Vero | African Green Monkey Kidney Epithelial Cell Line |
| Van 30 | Vancomycin 30 μg |
| VZV | *Varicella-zoster* virus |

# CHAPTER 1

# INTRODUCTION

## 1.1 Study Background

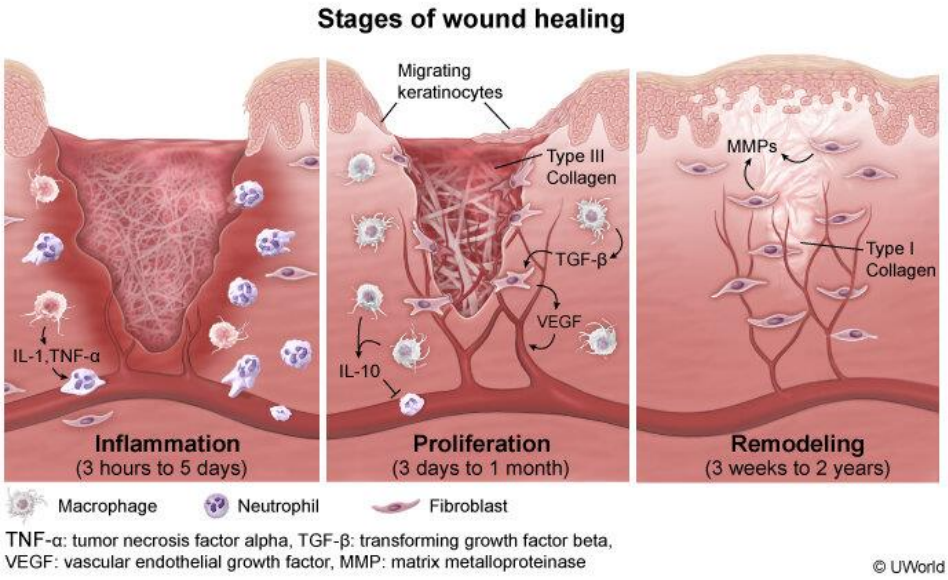
Wound healing, also known as a skin repairing process, is one of the most complicated processes in the human body. To close an open wound, multiple cells and stages are involved, including haemostasis, inflammation, angiogenesis, growth, re-epithelialization, and re-modelling **(Figure 1.1**) (Rodrigues et al., 2019). However, harmful substances or opportunistic microorganisms may act spontaneously and invade the body.

According to Grice and Segre (2011), bacteria such as *Propionibacterium acnes* (*P. acnes*), *Streptococcus pyogenes* (*Strep. pyogenes*), *Staphylococcus aureus* (*Staph. aureus*), *Enterococcus spp.* and *Pseudomonas aeruginosa* (*Ps. aeruginosa*) can be commonly isolated from the skin surface. They are usually the normal flora. Once the skin barrier is breached, persistent inflammation in the absence or delay of wound healing, persistent inflammation ensues, eventually leading to chronic wound.

A chronic wound is defined as an open wound that does not undergo an orderly and timely skin repair process. This process usually takes more than three months (Lanau-Roig et al., 2017). This situation commonly happens to people who have a weak immune system or patients with Human Immunodeficiency Virus (HIV). For example, diabetes is a chronic disease, and due to its high glucose level, the blood flow rate decreases. Thus, abrasions, open wounds, or other injuries tend to recover slower than to a healthy person (Chhibber et al., 2018). A chronic wound is a major public health challenge that presents multifactorial risk factors and complications that hinder the effective use of human resources, deplete the

healthcare system, and inadvertently emergence of resistance strain due to inappropriate use of antibiotics. The presence of methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most challenging issues when dealing with chronic wounds as colonization and proliferation of MRSA at the site of the wound have been found to delay the wound healing process (Manzuoerh et al., 2019).

Based on the market study done by the Fortune Business Insight (2021), chronic wound management incurs a weighty burden on the healthcare system and a hefty cost to the patient. The value of 2021 global chronic wound care market was approximately USD 11.61 billion and the projected to grow estimated USD 1.0 billion until 2029. Therefore, health care professionals are constantly looking for new approaches or medicine in chronic wound management.




(Adapted from Melissa, 2020)

**Figure 1.1:** Stages of Wound Healing

### 1.1.1 Microenvironment on Wounds

Skin is the largest organ in the human body. It is the outer defence system against environment factors and microbes. There is a plethora of microbes including opportunistic pathogens, commensal microbes, fungi, and viruses that human are exposed to every day. A cutaneous wound becomes a perfect entry site for these infectious agents (Kirchner et al., 2020). Besides these active infections, biofilm formation on the wound by certain pathogens will most likely delay the wound healing process and causes chronic wound.

Even though the initial triggered inflammation process causes the fibrin clot formation (Weisel & Litvonov, 2017) to achieve haemostasis and then the recruitment of immune cells to the site, the microbes have their own means to overcome the situation. According to the study by Thammavongsa (2015), *Staph. aureus* was commonly found on the skin and nasal area, it was capable of producing beta-barrel-forming toxins and promoting immune cells' lysis such as macrophages, neutrophils, and monocytes. Besides, the biofilm formation by the *Pseudomonas* is also reported to be one of the main issues of delaying the wound's closure and preventing the infiltration of immune cells towards the wound site (Ruffin & Brochiero, 2019).

Besides, fungi and viruses also affect the wound healing process through different mechanisms. *Candida albicans*, one of the common fungi found on the skin surface, once entered the body, can compromise the macrophage function, thus killing the immune cells (Uwamahoro et al., 2014). A few studies were performed on viruses in the skin ecosystem. Skin-tropic papillomaviruses could take over the wound healing process and infecting the wounded keratinocytes (Hufbauer & Akgul, 2017).

### 1.1.2 Antimicrobial Resistance

The emergence of antimicrobial resistance can be attributed to a lack of awareness, and improper use of antibiotics, and genetic mutation over time (Akova, 2016). Antimicrobial resistance is often associated with high morbidity and mortality. It has always been a worldwide challenge after the discovery of antibiotics. It is because of the improper usage of antibiotics in the early days when the healthcare system still lacks proper procedures to identify of causative microbes (Akova, 2016). Until the year of 2000, only a systematic review first reported proper wound care management, which included the use of antibiotics (O'Meara et al., 2000).

Besides, the natural ability of *Staph. aureus* for survival, the environment can be one of the causes of antimicrobial resistance development. Furthermore, the low-level mupirocin resistance has increased from 0 % to 19 % when chlorhexidine baths and intranasal mupirocin were frequently applied to decolonize the MRSA in a 14-bed surgical ICU. (Cho et al., 2016)

### 1.1.3 Natural Products for Wound Healing Activities

There are many types of anecdotal medical plants available in different regions of the world, and numerous indigenous people have been using certain plants as medicine or herbal remedies for centuries. Yet, these are not scientifically documented as an official medicine registered under Food and Drug Administration (FDA). This triggers the initiatives of scientists to delve deeper into bioactive phytochemicals and study their pharmacological properties. In order to identify a plant that is efficacious in providing health benefits, there

are some properties required in the selection process, for example, antioxidant, antimicrobial, and wound healing properties.

Recently, there has been a strong drive towards naturally derived phytochemicals to address the above three different stages of wound healing. *Clinacanthus nutans (Burm.f.) Lindau* (*C. nutans*) and *Strobilanthes crispus* (L.) Blume (*S. crispus*) are among these plants that attracted much interest from researchers due to their medicinal properties (Ong et al., 2020).

*Clinacanthus nutans* (Burm.f.) Lindau (*C. nutans*), also known as Sabah snake grass, is well-known traditional medicine for natives in Southeast Asia, especially in Thailand and Malaysia. The locals in Malaysia usually sun-dried the *C. nutans* leaves and brewed them in hot water to serve as tea. It is believed that daily consuming of *C. nutans* tea can cure diabetes, dysuria and diarrhoea (P'ng et al., 2012). According to Wanikiat (2008), *C. nutans* was used to cure oxidative stress-related diseases and skin lesions *in vivo* caused by herpes simplex virus and *varicella-zoster* virus. Other than that, some colon cancer survivors testified that consuming *C. nutans* over the long period of time could ease the chemotherapy effects and aid in the cancer illness recovery process. Subsequently, *C. nutans* was officially recognized as the principal medicinal plant for primary healthcare by the Ministry of Public Health in Thailand (Shim et al., 2012).

*Strobilanthes crispus* (L.) Blume (*S. crispus*) originated in Madagascar and slowly distributed to the Malay Archipelago region (Burkill, 1935). *S. crispus* is a shrub with darker green colour on its upper surface than underneath the leaf. Because of this characteristic, the Chinese called it "Black-Faced General". In addition, the underside of the leaves is rough, contrary to the upper surface which is smooth hence the Malays named it "Jin Batu" or

"Pecah Beling". *S. crispus* is traditionally used as an antidiabetic, antilytic, laxative, anticancer and diuretic agent. Due to its high calcium carbonate content, when *S. crispus* is brewed in water, it causes the water to be slightly alkaline and capable of easing urination (Nurraihana et al., 2013). In Malaysia, the natives chew and swallow the fresh *S. crispus* leaves to strengthen their immune system (Samuel et al., 2010).

Many studies have been conducted on *C. nutans* and its properties such as antioxidant, antiviral, and anti-inflammatory properties. *C. nutans* is commonly applied externally and available as a balm in the traditional market (Shin et al., 2013). Nevertheless, the studies on antimicrobial properties of *C. nutans* in relation to wound healing properties are minimal. A histological study on *S. crispus* reported by Al-Henhena and team (2011) demonstrated the ethanolic leaves extract of *S. crispus* capable of accelerating the wound healing process in rats (Al-Henhena et al., 2011). As such, this study aimed to assess the efficacy of *C. nutans* and *S. crispus* antimicrobial properties in relation to wound healing.

## 1.2 Problem Statement

Many studies have shown that different extraction methods and solvent polarity affect the outcome of the different bioassays (Khoo et al., 2018). There was little information on the use of a solvent with different polarity correlated in the same bioassays for *C. nutans* and *S. crispus*, respectively. Previous studies on *C. nutans* and *S. crispus*, both had demonstrated their high antioxidant contents and antimicrobial properties against some microbes such as *Staph. aureus* and *Strep. pneumoniae* (Lim et al., 2015; Ong et al., 2020). *S. crispus* was also reported to aid wound healing activity (Al-Henhena et at., 2011). However, only a few intensive studies on wound healing properties (Khoo et al., 2018;