

ANTIOXIDANT, ANTIMICROBIAL AND WOUND HEALING PROPERTIES
OF *Quercus infectoria* GALL EXTRACTS

NUR SYUKRIAH BT AB RAHMAN

UNIVERSITI TEKNOLOGI MALAYSIA

ANTIOXDANT, ANTIMICROBIAL AND WOUND HEALING PROPERTIES OF
Quercus infectoria GALL EXTRACTS

NUR SYUKRIAH BT AB RAHMAN

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Specially for
My beloved father and mother;
My greatest siblings;
Most importantly, my forever husband, Khairul Anwar Alpatihah.

List of Publications and Award

Publications

- 1) Bioactive Compounds from *Quercus Infectoria* (Manjakani) Galls Extract and Their Effects on Antioxidant and Antibacterial Activities.
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- 2) Comparison of Supercritical Co₂ Extraction and Soxhlet Extraction of Bioactive Compound from *Quercus Infectoria*.
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- 5) Effect Of Extraction Solvents On Antioxidant And Antibacterial Activity Of *Quercus Infectoria* (Manjakani) Galls
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- 6) *Quercus Infectoria* Galls Promote Wound Healing Activity: An *In Vitro* Study
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- 7) Quantification Of Gallic Acid And Tannic Acid From *Quercus Infectoria* (Manjakani) And Their Effects On Antioxidant And Antibacterial Activities
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- 8) Effect of Solvent Extraction On Antioxidant And Antibacterial Activities From *Quercus Infectoria* (Manjakani).
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Awards

- 1) Bioactive Compounds from *Quercus Infectoria* (Manjakani) Galls Extract and Their Effects on Antioxidant and Antibacterial Activities.
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ABSTRACT

Quercus infectoria is one of the greatest herbs and contains several bioactive compounds which are believed to possess wound healing property. However, the scientific data to support its wound healing action is very rare and to date, the extraction of local *Q. infectoria* using various solvents is not well-established. Hence, the objective of the present research is to evaluate the antioxidant, antimicrobial and wound healing properties of *Q. infectoria* extract using relevant *in vitro* assay for application in topical wound management. *Q. infectoria* were extracted by using four solvents, namely 100% water, 99.8% and 70% methanol, 70% ethanol, 99.8% acetone and 99.8% ethanol. The extracts were then subjected to high performance liquid chromatography (HPLC) to identify the presence of gallic acid and tannic acid, while the total phenolics content were measured using Folin-Ciocalteu method. The extracts were tested using appropriate *in vitro* models, which were antioxidant (DPPH assay), antimicrobial (disc diffusion assay), suitable dose study (MTT assay), and fibroblast migration to the wounded area (scratch assay). Antimicrobial activity was tested against common bacteria found in wounds, which were *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. In HPLC analysis, gallic acid and tannic acid were found in all extracts in the range concentration of 34.04 until 2975.11 mg/g which might be the responsible compounds for wound healing activity. Water extract showed the highest antioxidant activity of approximately $94.55\% \pm 0.59$, while acetone extract showed the largest inhibition zone for disc diffusion assay (19.00 ± 0.14 mm). All extracts were found to stimulate the growth of the fibroblast at $1 \mu\text{g/ml}$ - $0.001 \mu\text{g/ml}$, induced the migration rate up to $77\% \pm 3.05$, and statistically significant when compared with control ($p < 0.05$). These findings supported the traditional claim of *Q. infectoria* for its potential in wound healing activity and it can be a good herbal to incorporate in the topical wound healing product.

ABSTRAK

Quercus infectoria adalah salah satu herba yang hebat dan mengandungi beberapa sebatian bioaktif yang dipercayai mempunyai aktiviti penyembuhan luka. Namun data saintifik yang menyokong aktiviti penyembuhan luka amat jarang dikaji dan setakat ini pengekstrakan *Q. infectoria* tempatan menggunakan pelbagai jenis pelarut belum pernah dikaji. Oleh itu, objektif kajian ini adalah untuk mengkaji potensi *Q. infectoria* terhadap antioksidan, antimikrob dan sifat penyembuhan luka dengan menggunakan ujian *in vitro* untuk diaplikasi dalam pengurusan luka. Pengekstrakan *Q. infectoria* telah dijalankan menggunakan empat pelarut, iaitu 100% air, 99.8% dan 70% metanol, 70% etanol, 99.% aseton dan 99.8% etanol. Ekstrak diuji dengan kromatografi cecair prestasi tinggi (HPLC) untuk mengesan kehadiran asid galik dan tanik, jumlah kandungan fenolik diukur dengan kaedah Folin-Ciocalteu. Ekstrak diuji dengan model penyembuhan luka *in vitro* yang sesuai, iaitu antioksidan (analisis DPPH), antimikrob (analisis penyebaran cakera), kajian dos sesuai (analisis MTT) dan penghijrahan *fibroblast* ke kawasan yang cedera (analisis luka). Aktiviti antimikrob telah diuji terhadap mikrob yang biasa ditemui pada luka seperti *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* dan *Staphylococcus aureus*. Dalam analisis HPLC, asid galik dan tanik ditemui dalam kesemua ekstrak dengan julat kepekatan dari 34.04 hingga 2975.11 mg/g, dan berkemungkinan berfungsi bagi aktiviti penyembuhan luka. Ekstrak dari 100% air menunjukkan aktiviti antioksidan yang tertinggi kira-kira $94.55\% \pm 0.59$, manakala ekstrak aseton menunjukkan zon perencatan terbesar bagi antibakteria (19.00 ± 0.14 mm). Semua ekstrak telah merangsang pertumbuhan *fibroblast* pada $1 \mu\text{g/ml}$ - $0.001 \mu\text{g/ml}$, mendorong kadar penghijrahan sehingga $77\% \pm 3.05$, dengan statistik yang signifikan berbanding dengan kawalan ($p < 0.05$). Kajian ini menyokong pernyataan tradisional bahawa *Q. infectoria* mempunyai potensi dalam aktiviti penyembuhan luka dan merupakan herba yang baik untuk diaplikasikan dalam produk penyembuhan luka.

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LIST OF ABBREVIATIONS

ROS	-	Reactive Oxygen Species
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
MTT	-	(3-(4,5-dimethyl-thiazol-2y) 2,5-diphenyl-tetrazolium bromide
FCR	-	Folin-Ciocalteu Reagent
DAPI	-	4'6-diaminido-2-phenylindole
PBS	-	Phosphate Buffer Saline(PBS),
MEM	-	Modified Eagle's Medium
HPLC	-	High Performance Liquid Chromatography
MEM	-	Modified Eagle's Medium
DMSO	-	Dimethyl Sulfoxide
FBS	-	Fetal Bovine Serum
ECM	-	Extra Cellular Matrix
TPC	-	Total Phenolic Content
CO ₂	-	Carbon Dioxide
HSF	-	Human Skin Fibroblast

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

According to the Wound Healing Society, wounds can be described as physical injuries that results in an opening or breaking of the skin that caused disturbance in the normal skin anatomy and function (Strodtbeck, 2001).

Wounds represent a momentous burden not only to patients but also to health care professionals around the world. In addition, they also affected millions of patients toward the physical and mental health and imposed significant cost on them. Kumar *et al.* (2007) stated that almost 6 million people worldwide suffer from chronic wound. In addition, current statistics showed that 10.5 per 1,000 population of the community were found to have acute wounds (Gupta *et al.*, 2004). However, Balick and Cox (1996) reported that in Western pharmacopoeia, only 1-3% of drugs were listed for used in the wounds and skin. In contrast, at least one third of herbal remedies are used for multiple purposes. Basically, both western and traditional medicines used for wound healing suffer from lack of resources and awareness. These require wider publications and advocacies in search for the relevant remedy to be studied. In broad terms, the relevant headings for considerations are utilization, safety and efficacy (Kumar *et al.*, 2007).

Constantly unhealed wounds will result in inflammatory mediators that produce pain, swelling at the wound site and prolong the recovery of the injured

patients (Nagori and Solanki, 2001). The healing process of the wound is a normal biological response to the injury and although it is a familiar process, its underlying biology is very complex and only partially understood (Sherratt and Dallon, 2002). Furthermore, untreated wounds will lead to chronic wounds, which in turn can cause multiple organ failure or death of the patients. Thus, chronic wounds have prompted the health care professionals to begin a search for unresolved underlying causes.

Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of using modern method through current technology, traditional knowledge also gives better clues to the discovery of valuable drugs (Buenz *et al.*, 2004). The usage of the traditional plant as medicinal plant had been well-documented. In addition, traditional plants are often cheaper, locally available and easily consumable. One of the valuable traditional plants in Malaysia is *Quercus infectoria* (*Manjakani*). *Q. infectoria* or widely known as *Manjakani* is a small tree native to Greece, Asia Minor and Iran (Basri and Fan, 2005), which is also popular as oak tree. The galls arise on young branches of this tree as a result of the attack by the gall-wasp *Adleriagallae-tinctoria*. It has been used since ancient time to treat the inflammatory disease in oriental traditional medicines. Furthermore, pharmacology tests have demonstrated that the galls of *Q. infectoria* possess astringent, antidiabetic (Dar *et al.*, 1976), antitremorine, local anesthetic (Hussein *et al.*, 2000), antiviral (Fatima *et al.*, 2001), antibacterial (Digraki *et al.*, 1999), antifungal (Redwane *et al.*, 2002) and larvicidal (Kaur *et al.*, 2004).



(a)

(b)

Figure 1.1: Galls of *Q.infectoria* (a) before harvest (b) after harvest

Q. infectoria is actually a round-shaped abnormal growth and can be found easily in Asian countries. On the other hand, *Q. infectoria* is the result of ordinary chemical reaction among the bark of the crop and contamination from different insects that consumed parts of the tree to place its eggs. The alteration progression that takes place on the hatched eggs of the insects generates enzymes that stimulate food supply on the cell leaves, and a hard round ball is formed (Umachigi *et al.*, 2008).

Q. infectoria is determined as a natural caustic which has antibacterial and antioxidants properties as well as containing several important bioactive compounds such as tannic acid, flavonoids, gallic acid, ellagic acid and others. All of these bioactive constituents are scientifically demonstrated to give many benefits to human kind, especially in terms of pharmacological studies. Hence, due to this reason, a series of research has been conducted to recognize its beneficial effects in pharmaceuticals area (Umachigi *et al.*, 2008).

1.2 Problem Statement

Currently, there are various *Q. infectoria*-based products that can be obtained from the local market including beverage products, personal care products and also wound healing gel products. However, the scientific study related to its wound healing activity is very rare and to date the wound healing potential of *Q. infectoria* from Malaysia is not well established. In addition, even it is proven can tend the wounded area, most of the traditional healers apply the extracts with little understanding toward the exact dosage that should be applied. Furthermore, the research on the extraction of *Q. infectoria* galls using various solvent with different polarities also not much reported in the literature.

Therefore, through these findings, information on the appropriate technique for the use of *Q. infectoria* to be applied as topical wound healing product can be provided. For this reason, *Q. infectoria* was chosen in this present study and its potential to heal the wound was examined using *in vitro* assay, since there is no *in vitro* study related to its wound healing properties reported in the literatures.

1.3 Objective of Study

The objective of this study is to evaluate the wound healing properties of *Quercus infectoria* (Manjakani) galls crude extract using relevant antioxidant, antimicrobial and cell migration *in vitro* assay for application in topical wound management

1.4 Scopes of Study

To achieve the objective, the following scopes were carried out:

1. Study on the effect of using different types of solvents towards the extraction yield of *Q. infectoria* galls.
2. Determination and characterization of *Q. infectoria* extracts in terms of bioactive compounds, total phenolics content and antioxidant activity.
3. Utilization of antibacterial assay to study the effect of *Q. infectoria* extracts on commonly found bacteria in wound infection.
4. Evaluation on the potential of *Q. infectoria* extracts to stimulate the proliferation and migration of human skin fibroblast (HSF 1184) cells.

1.5 Significance of Study

Q. infectoria is widely known among Asian community as one of the traditional plants that can enhance wound healing process. However, the scientific evidence to support its therapeutic potential in pharmaceuticals area is limited. Hence, the findings from this present study support the traditional claims on the wound healing activity. The scientific data collected in this study also explained its wound healing activity from the scientific point of view. Furthermore, the results presented in this study can give valuable knowledge or important information that can contribute for further research, especially in understanding a complex mechanism of *Q. infectoria* in aiding wound healing activity.

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