CHAPTER I

INTRODUCTION

1.1 BACKGROUND

Maggots or fly larvae have been known for centuries to help to heal chronically infected and necrotic human wounds (Baer 1931; Pechter and Sherman 1983; Sherman *et al.* 2000, Jaklic 2008) including leg, diabetic and necrotic ulcers as well as pressure sores, infected surgical wounds, trauma injuries (Nigam *et al.* 2006a), osteomyelitis, abscesses, burns and sub-acute mastoiditis (Thomas *et al.* 1996). However, by the mid-1940s, maggot therapy was supplanted by the introduction of antibiotics and improved surgical techniques, until the emergence of bacterial strains with multiple antibiotic resistance (McKellar 1998; Sherman *et al.* 2000) and recognition of the limits of conventional medical and surgical treatment (Sherman *et al.* 2007b), after which it has experienced a resurgence of interest (Bunkis *et al.* 1985; Church 1996).

However, not all fly larvae are suitable for maggot therapy. Species of the obligate agents of myiasis, such as the flesh fly *Wohlfahrtia magnifica* and the screwworm species, *Chrysomya bezziana* or *Cochliomyia hominivorax* are not used as

they feed aggressively on underlying living tissues (Hall and Wall 1995) in order to complete their lifecycles (Erzinclioglu 1996) whilst the larvae of facultative agents of myiasis, particularly *Lucilia sericata* are the most suitable and commonly used species in maggot therapy since they feed relatively superficially (Jones and Wall 2008) and are recognized to digest and remove only necrotic or dead tissues and therefore will not eat living flesh. Hence, the larva of *L. sericata* has been widely used as the candidate for maggot therapy in treating necrotic wounds, particularly chronic wounds where conventional treatments have failed (Stoddard *et al.* 1995; Kerridge *et al.* 2005). In the last decade, thousands of patients with chronic wounds around the world have undergone *L. sericata* larval therapy and it is now well and truly recognized by many clinicians as an important adjunct to conventional medicines (Nigam *et al.* 2006b).

The therapeutic effects of medical grade maggot can be attributed to the synergistic actions of three core activities: enzymatic degradation of necrotic tissue (Hobson 1931), destruction of ingested bacteria in larval digestive tract (Lerch *et al.* 2003; Mumcuoglu *et al*. 2001) and disinfection of bacteria-loaded wounds via the potent antibacterial activity of larval excreta/secreta (Robinson and Norwood 1933; Simmons 1935; Bexfield *et al.* 2004; Daeschlein *et al.* 2007) as well as stimulation of granulation tissue formation (Robinson 1935; Prete 1997; Horobin *et al.* 2003).

Hence, owing to the encouraging and beneficial effects of maggot therapy in wound healing, much research have been conducted to investigate the basic healing principles of maggot, especially on the antibacterial activity of maggot excretions/secretions (Jaklic *et al.* 2008). Although many *in vitro* studies of the antibacterial activities of excretions/secretions (ES) from *L. sericata* larvae have revealed that they are potent against many species of pathogenic bacteria (Vistnes *et al.*

1981; Bexfield *et al.* 2004; Kerridge *et al.* 2005; Daeschlein *et al.* 2007; Huberman *et al.* 2007; van der Plas *et al.* 2007; Bexfield *et al.* 2008; Jaklic *et al.* 2008), the effects of the antibacterial activity of the local strain of blowfly larva, *L. cuprina* larval extract against pathogenic bacteria that can infect chronic wounds have never been determined. The present study aims to develop a standard production procedure of *L. cuprina* larval extract and investigate the antibacterial activity of *L. cuprina* larval extract against a range of selected pathogenic bacteria that are frequently found in chronically infected wounds as well as to determine the physicochemical properties of the extract.

1.2 RESEARCH QUESTION

Theoretically, the extracted product from the whole body of *L. cuprina* larva contains the same antibacterial compounds as those in the excretions/secretions (ES) which included the salivary gland secretions and faecal waste products. This suggested that the larval extract would possess antibacterial activity as exhibited by the larval ES of *L. sericata*.

1.3 GENERAL OBJECTIVE

To study the *in vitro* antibacterial activity of *L. cuprina* larval extract against seven selected pathogenic wound bacteria: *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* as well as to investigate its physicochemical properties

1.4 SPECIFIC OBJECTIVES

1. To develop a standard procedure of producing sterile *L. cuprina* larval extract

2. To examine the *in vitro* antibacterial activity and properties (bactericidal or bacteriostatic) of *L. cuprina* larval extract against the seven selected pathogenic wound bacteria

3. To determine the minimum inhibitory concentrations (MICs) of *L. cuprina* larval extract for the seven selected pathogenic wound bacteria

4. To investigate the robustness and thermal stability of *L. cuprina* larval extract in inhibiting the seven selected pathogenic wound bacteria

1.5 HYPOTHESES

1. *Lucilia cuprina* larval extract possesses antibacterial activity against the seven selected pathogenic wound bacteria

2. *Lucilia cuprina* larval extract possesses heat-resistant antibacterial activity