

CHAPTER 1

INTRODUCTION

Snake envenomation is an important yet a neglected disease in many tropical and subtropical countries including Asia, Africa, Oceania and Latin America (Gutiérrez *et al.*, 2006) and has a very significant negative impact on the poor populations (World Health Organization, 2007b). The actual burdens of snake envenomation in the affected countries remained poorly understood, and one of the underlying reasons is lacking of mandatory and systematic epidemiology reports. Estimates of mortality and permanent morbidity due to snakebite are largely hospital based which lead to underestimation of the problem (Fox *et al.*, 2006). Statistics show at least 421,000 of cases of envenomation and 20,000 mortalities per year, with the highest numbers of cases reported in South Asia followed by Southeast Asia (Kasturiratne *et al.*, 2008). Approximately 15% of all the known 3000 snake species are considered venomous (Gold *et al.*, 2002).

South Asia has the highest estimated numbers of snake bite due to its high rural population that largely involved in agricultural activities (Alirol *et al.*, 2010). Cobras (*Naja* sp.) and kraits (*Bungarus* sp.) are the two most common species that lead to severe envenomation and high mortality rate in Asia (Warrell, 1995). In Malaysia, snake envenomation is attributed mostly to *Naja* sp. (Jamaiah *et al.*, 2004; Jamaiah *et al.*, 2006 and Chew *et al.*, 2011) and *Calloselasma rhodostoma* (Muthusamy, 1988 and Tan *et al.*, 1990).

The systematics of Asiatic cobra (formerly known as *Naja naja*) has been recently revised and many subspecies have been elevated to the status of full species (Wüster, 1996). Thus, a number of the cobras have been appropriately re-named. The spitting cobras of Southeast Asia, once collectively known as *Naja sputatrix* (formerly known as Malayan cobra) actually consist of three separate species: *Naja siamensis* (Indochinese spitting cobra) in Thailand, *Naja sumatrana* (Equatorial spitting cobra) in Peninsula

Malaysia and Sumatra, and *Naja sputatrix* (Javan spitting cobra) in Java and southern Indonesia. Despite some early reports on the toxinological properties of the spitting cobra in Malaysia, it is not known whether the venom samples used for the investigations, labeled as *N. sputatrix*, were from *N. sumatrana* or *N. sputatrix* (Tan, 1991). This was due to the insufficient understanding of the systematic of Southeast Asian spitting cobra prior to the 1990's and it was assumed at that time that *N. sputatrix* refers to the Malayan spitting cobra. It is thus difficult to interpret the results of the earlier investigations on spitting cobras in Southeast Asia.

World Health Organization has categorized the Equatorial spitting cobra (*Naja sumatrana*) as one of the category I medically important snakes in Southeast Asia. The Equatorial spitting cobra (*N. sumatrana*) is widely distributed in the equatorial parts: southern Peninsula of Thailand, Peninsula Malaysia, and the Sumatra (Wüster & Thorpe, 1991). They are highly variable populations depending on different geographical origin. In southern Peninsula Malaysia, Singapore and Sumatra, they are usually black in color (Figure 1). Juveniles exhibit one or several pairs of median, lateral spots at throat area and in adults, black pigments are usually obscure most of the throat area. In northern Peninsula Malaysia, they appear uniformly yellowish or light brown with no hood marking. Adult *N. sumatrana* can reach up to a total length of 1.48 meter (Chanhomea *et al.*, 2011). The cobra is capable of 'spraying' venom toward the eyes of victims that can cause ocular envenoming known as venom ophthalmic (Chu *et al.*, 2010). This spitting behavior is due to the reduction of discharge orifice size of the front-fangs (Wüster & Thorpe, 1991). Generally, envenomation by cobra is characterized by local necrosis, respiratory paralysis and cardiovascular effects, and in some cases secondary wound infections are also observed (Chen *et al.*, 2011; Reid, 1964 and Warrell, 1999).



Figure 1: Equatorial spitting cobra (*Naja sumatrana*).

1.1 Objectives

The objectives of the present study are:

1. To investigate the biochemical and toxinological properties of *N. sumatrana* venom and comparison with other Southeast Asia cobra venoms.
2. To investigate the proteome of *N. sumatrana* venom using multidimensional chromatographic approach and mass spectrometry.
3. To study the pharmacokinetics of *N. sumatrana* venom and its major venom toxins, following intravenous (*i.v.*) and intramuscular (*i.m.*) administration into experimentally envenomed animals.
4. To study the pharmacokinetics of *N. sputatrix* (Javan spitting cobra) venom following intravenous (*i.v.*) and intramuscular (*i.m.*) administration into experimentally envenomed animals, and to compare the results with pharmacokinetics of *N. sumatrana* venom.