Phytochemical analysis of an anti-venom traditional herbal preparation for snake-bite

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ABSTRACT

Objective: Snake-bite is one of the important public health problems of tropical countries including Sri Lanka. The risk of snake-bites is higher in rural areas of the country and people mainly rely on herbal medicines. Antiserum is the only therapeutic agent in Western medicine available throughout the world. A major drawback of serum therapy is its higher cost and also serum sickness is a possible side effect of serum therapy that results in inflammation of tissues and other symptoms. In the present study, an attempt was taken to prepare a traditional herbal paste which used to treat snake-bites and carry out chemical analysis. Methods: Chemical analysis carried out by investigation of its (a) phytochemical constituents (b) total phenol and flavonoid contents and (c) development of Thin Layer Chromatography (TLC) fingerprints. **Results:** Results revealed that phenols, flavonoids, tannins and saponins were abundant in the herbal paste whereas terpenoids and alkaloids were absent. Further high amounts of total phenols (120.30 ± 0.83 mg gallic acid equivalents /g) and flavonoids (69.76 ± 1.62 quercetin equivalents /g) were present in the herbal paste. TLC fingerprints were able to developed for the herbal pate and its mixture of ingredients. **Conclusion:** Present study showed the phytochemicals such as phenols, flavonoids, tannins and saponins present in the traditional herbal paste.

Key words: chemical constituents, snake -bites, Sri Lankan traditional medicine

1. INTRODUCTION

Animal bite is a serious public health problem in many tropical and sub-tropical countries which mainly affects in poorest countries in the world. According to the World Health Organization (WHO) about 5.4 million snake bites occur in each year resulting in 1.8 to 2.7 million cases of envenoming. There are between 81,410 and 137,880 deaths and around three times as many amputations and other permanent disabilities in each year [1 – 3]. Sri Lanka is well popular for its rich snake diversity consisting of land snake species which have been clustered into different categories according to their level of toxicity including highly venomous, moderately venomous and non venomous. According to Sri Lankan Epidemiology Unit, Ministry of Health, reported snake-bite numbers increased from 12,175 per year in 1991 to peak at 37,244 in 2002 and 36,861 in 2005 [4]. There are few reliable data on snakebite which make difficult to estimate the true disease burden. Hospital statistics under-estimates numbers of snake bite because a significant proportion of victims in tropical countries seek traditional treatment. Island wide community based survey showed that more than 80,000 bites, 30,000 envenoming and 400 deaths reported per year which is much more than claimed by official statistics [5]. Sri Lanka has its own indigenous medical system of Traditional Medicine. This system has been practiced for many centuries in the island nations. Sri Lanka developed its own traditional system based on a series of prescriptions handed down from generation to generation over a period of 3000 years. This valuable medical system encompasses varieties of health practices which is indigenous to this country. Antiserum is the only therapeutic agent in western medicine available throughout the world. A major drawback of serum therapy is its higher cost and also serum sickness is a possible side effect of serum therapy that results in inflammation of tissues and other symptoms [6]. In the present study, an attempt was taken to prepare a traditional herbal paste which used to

treat snake-bites and investigate its phytochemical constituents. It consists of four medicinal plants and salt crystals (Table 1).

2. MATERIALS AND METHODS

2.1. Plant ingredients

Traditional herbal recipe selected from a traditional snake-bite treatment manuscript in the library, Institute of Indigenous Medicine. All the plant ingredients needed for the traditional herbal paste were collected from Western Province of Sri Lanka during November – December 2018, identified and authenticated by a Senior Lecturer, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka.

2.2. Preparation of traditional herbal paste

In brief, all the necessary plant parts (Table 1) were washed thoroughly and air dried. Then they were separately pulverized into a coarse powder using a blender (Kenwood, model: BL440, made in China), then added to a stainless-steel vessel and mixed well. Finally, salt crystals (Sodium Chloride) were added and mixed again.

2.3. Phytochemicals of traditional herbal paste

Phytochemical screening was carried out using water extract of herbal paste as described by Yadav and Agarwala [7] and Karunakaran and co-workers [8] with slight modifications. In brief, 50 g of the herbal paste was added to a beaker containing water (100 ml), stirred for 1h and filtered. The filtrate was subjected for screening of phytochemical such as phenolic compounds, tannins, flavonoids, coumarins, saponins, alkaloids and steroid glycosides

2.4. Total phenol and total flavonoid contents of traditional herbal paste

Poly herbal paste (50 g) was added to a beaker containing water (100 ml), stirred for 1h and filtered. The filtrate was concentrated and freeze dried. Total polyphenol content of the herbal paste was determined using the Folin-Ciocalteu reagent [9] in 96-well micro-plates and results were expressed as mg gallic-acid equivalents per gram of extract on a dry weight basis. Total flavonoid content of the herbal paste was determined using the aluminium chloride [10] in 96-well micro-plates and results were expressed as mg quercetin equivalents per gram of extract on a dry weight basis.

2.5. Thin Layer Chromatography (TLC) Fingerprint profile of traditional herbal paste and its plant mixture

2.5.1. Extraction of poly herbal paste

Approximately 50 g from the herbal paste was added to a round bottom containing 100 ml of dichloromethane and refluxed for 1 h. Then filtered and filtrated was evaporated to dryness and re-dissolved in 5 ml of dichloromethane.

2.5.2. Extraction of plant ingredients

Approximately 5 g from each plant of herbal paste was added to a round bottom containing 100 ml of dichloromethane and refluxed for 1 h. Then filtered and filtrate was evaporated to dryness and re-dissolved in 5 ml of dichloromethane.

2.5.3. Development of Thin Layer Chromatography (TLC) Fingerprint profiles

Both herbal paste (10 µl) its plant ingredients (10 µl) were spotted on a TLC plate and fingerprints were developed using cyclohexane, dichloromethane, ethyl acetate and methanol (in a ratio of 4: 3: 1:1 v/v) as the mobile phase.

3. RESULTS AND DISCUSSION

Snake bite is an important global health issue and it constitutes an occupational hazard mainly in the field of agriculture [11]. High mortality is reported due to snake bites because of transportation delays, poor health services and also delays in the antisnake venom administration [12]. In the present study, traditional herbal formulation which consists of four plant ingredients was evaluated in terms of (a) qualitative phytochemical analysis (b) quantitative analysis of total phenol and flavonoid contents and (d) development of TLC fingerprints.

Phytochemical such as phenols, flavonoids, tannins and saponins were abundant in the herbal paste whereas terpenoids and alkaloids were absent. Therefore, amounts of phenol and flavonoid contents were quantified using colorimetric methods. Results revealed that high amounts of total phenols (120.30 ± 0.83 mg gallic acid equivalents /g) and flavonoids (69.76 ± 1.62 quercetin equivalents /g) were present in the herbal paste.

Enenebeaku and co-workers [13] scientifically proved the ability of neutralizing the lethal effects of venom by tannins, saponins and flavonoids using *in vivo* assays. Present study also revealed presence of high amounts tannins, saponins and flavonoids in the herbal paste. This suggests that herbal paste has anti-snake venom activities since polyphenols such as tannins, saponins, phenols and flavonoids possess protein - binding and enzyme inhibiting properties which also inhibit

snake venom phospholipase A2 activities which present in cobra venom [14,15]. Therapeutic effect of alkaloids as antidotes for snake venom was well reported [16]. Examples include alkaloids such as seeperine, bebeerines, cissampellin, atropine [17, 18] which act against snake venom. Similarly, saponins also have shown anti-venom activity [19]. However, both alkaloids and saponins were absent in this traditional herbal paste.

TLC fingerprinting is one of the simple and cheap technique of standardization of herbal drugs. Further R_f values of a TLC profile denote the position of a chemical compound/s and Table 2 is demonstrated the R_f values of the traditional herbal paste with its mixture of plant ingredients. Similar studies were carried out for other herbal drugs such as Sarasvatha Choorna [20], Mustadi Taila [21], Palakalyana Ghrita [22], etc.

4. CONCLUSION

Present study showed the phytochemicals such as phenols, flavonoids, tannins and saponins present in the traditional herbal paste and further elaborative work is necessary for the better understanding of the mechanism of venom inhibition.

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COMPETING INTERESTS

Authors have declared that no competing interest exist.

Table 1. Plant ingredients of traditional herbal paste for snake bites

Botanical name	Family	Used part	Amount
Vernonia zeylanica (L.) Less	Asteraceae	Leaves	25 g
Hibiscus furcatus Willd	Malvaceae	Leaves	25 g
Alstonia scholaris	Apocynaceae	Bark	25 g
Curcuma longa Linn	Zingiberaceae	Rhizome	10 g

Table 2. Retention factors (R_f) of Thin Layer Chromatography (TLC) Fingerprint profile of traditional herbal paste and its plant mixture

R_f values and colors of the herbal paste provided by the manufacturer			R_f values and colors of plant ingredients of the herbal paste provided by the manufacturer		
Before Spraying 254 nm / 366 nm	After spraying		Before Spraying 254 nm / 366 nm	After spraying	
0.05	0.05	Light Brown	0.05	0.05	Light Brown
0.07	0.11	Pink	0.07	0.11	Pink
0.10	0.14	Light Yellow	0.10	0.14	Light Yellow
0.11	0.18	Light Purple	0.11	0.18	Light Purple
0.14	0.24	Purple	0.14	0.24	Purple
0.18	0.51	Light Pink	0.18	0.51	Light Pink
0.23	0.58	Pink	0.23	0.58	Pink
0.31	0.70	Pink	0.31	0.70	Pink
0.35	0.79	Light Pink	0.35	0.79	Light Pink
0.37	0.88	Purple	0.37	0.88	Purple
0.42	0.94	Light Purple	0.42	0.94	Light Purple
0.49			0.49		
0.51			0.51		
0.62			0.62		
0.67			0.67		
0.69			0.69		
0.75			0.75		
0.77			0.77		
0.86			0.86		

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