

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 coumarins, 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 develop for the traditional herbal formulation and its mixture of ingredients. **Conclusion:** Present study revealed the presence of phytochemicals such as phenols, flavonoids, tannins and saponins in the traditional herbal preparation.

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

1. INTRODUCTION

Snake 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, snake-bite envenoming is reported to cause death of up to 138 000 people annually. Moreover; there are many amputations and other permanent disabilities due to snake-bites [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 [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. Anti-serum 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 an anti-venom traditional herbal preparation for snake-bites and investigate its phytochemical constituents. It consists of four medicinal plants (Table 1) and salt crystals (Sodium Chloride).

2. MATERIALS AND METHODS

2.1. Plant ingredients

Traditional herbal recipe was selected from a traditional snake-bite treatment manuscript in the library, Institute of Indigenous Medicine, Sri Lanka. All the plant ingredients need for the traditional herbal preparation 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 preparation

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 preparation

2.3.1. Extraction procedure

In brief, 50 g of the herbal preparation was added to a beaker containing water (100 ml), stirred for 1h and filtered. The filtrate was subjected for screening of phytochemical such as phenols tannins, flavonoids, coumarins, saponins, alkaloids and terpenoids.

2.3.2. Phytochemical screening

Phytochemical screening was carried out using water extract of herbal preparation as described by Yadav and Agarwala [7] and Karunakaran and co-workers [8] with slight modifications.

2.3.2.1. Test for Phenols

Few drops of FeCl_3 were added to a few drops of aqueous extract and mixed well. The presences of water soluble phenolics are indicated by green or blue colour.

2.3.2.2. Test for Tannins

Few drops of concentrated HCl and 10% vanillin in ethanol were added to a few drops of aqueous extract. Red colour is the indication for tannins.

2.3.2.3. Test for Flavonoids

To dilute ammonia solution (5 ml), 3 ml of aqueous extract was added and mixed well. Then concentrated H_2SO_4 was added drop wise. If yellow colour is formed and then disappears on standing is an indication for flavonoids.

2.3.2.4. Test for Coumarins

Coumarins form a yellow color with 1% KOH in absolute ethanol. Few drops of 1% KOH in absolute ethanol was added to aqueous extract (1 mL) and mixed well.

2.3.2.5. Test for Saponins

To aqueous extract (5 ml), water (5 ml) was added and shaken vigorously. Froth persistency for at least 10 minutes is an indication for saponins. With 3 drops of olive oil, the froth was mixed and shaken vigorously. The emulsion formation is an indication for saponins.

2.3.2.6. Test for Alkaloids

To aqueous extract (2 ml), 6 drops of Mayer's reagent and 1% HCl were added. Orange or cream brown red precipitate is an indication for alkaloids.

2.3.2.7. Tests for terpenoids

Chloroform (2 ml) was mixed with 5 ml of aqueous extract. Then, 3 ml of concentrated H₂SO₄ was added along the sides for the formation of a layer. The presence of terpenoids is indicated by a reddish brown colour.

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

Poly herbal preparation (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 preparation 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 preparation and its plant mixture

2.5.1. Extraction of poly herbal preparation

Approximately 50 g from the herbal preparation 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.

2.5.2. Extraction of plant ingredients

Approximately 5 g from each plant of herbal preparation 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 preparation (10 µl) and 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 anti-snake 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 preparation where as coumarins, 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 preparation.

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 the presence of high amounts tannins, saponins and flavonoids in the herbal preparation. This suggests that herbal preparation has anti-snake venom activities since 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 seiperine, bebeerines, cissampellin, atropine [17, 18] which act against snake venom. Similarly, terpenoids also have shown anti-venom activity [19]. However, both alkaloids and steroids were absent in this traditional herbal preparation.

TLC fingerprinting is one of the simple and cheap technique of standardization of herbal drugs. 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 preparation with its mixture of plant ingredients. Moreover, Figure 1 illustrated the TLC fingerprint profiles of traditional herbal preparation with its mixture of plant ingredients. TLC fingerprints have been developed for other herbal drugs such as SarasvathaChoorna [20], MustadiTaila [21] and PalakalyanaGhrita [22].

4. CONCLUSION

Present study showed the phytochemicals such as phenols, flavonoids, tannins and saponins were present in the traditional herbal preparation 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 preparation for snake bites

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

Table 2. Retention factors (R_f) of Thin Layer Chromatography (TLC) Fingerprint profile of traditional herbal preparation 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		

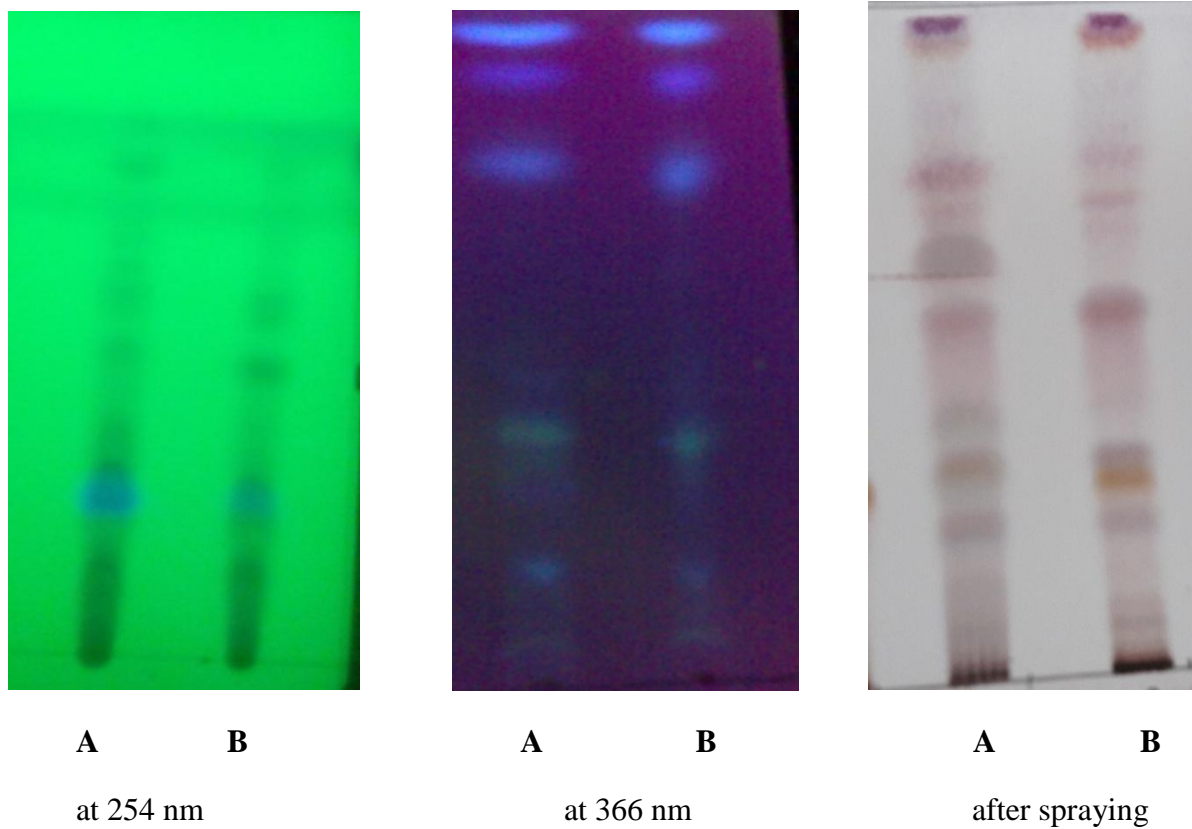


Figure 1. Thin Layer Chromatogram fingerprint profiles of traditional herbal preparation (A) and its plant mixture (B)

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