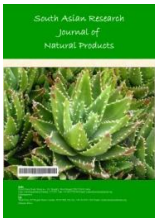

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# Chemical Analysis of Anti-Venom Herbal Paste Use in Sri Lanka

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the manuscript.*

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## ABSTRACT

**Background:** Sri Lanka is one of the Asian countries mainly rely on herbal medicines for snake bites. A herbal paste consist of nine medicinal plants have been using for snake bites and clinically proven its efficacy. In the present study, an attempt was done to carry out chemical analysis of the paste.
**Methods:** Chemical analysis were carried out for the herbal paste in terms of (a) phytochemical screening (b) development of Thin Layer Chromatography fingerprint (c) antioxidant activities.
**Results:** Phenols, tannins, steroids, saponins and cardiac glycosides were present whereas both flavonoids and alkaloids were absent in the paste. In addition, 14 spots were observed under 254 nm and 366 nm whereas 17 spots were observed after spraying vanillin sulphate in the Thin Layer Chromatography fingerprint. Total polyphenols in the herbal paste was 94.15 ± 5.32 mg gallic acid equivalents/g of extract. Moreover, $IC_{50}$ value was 628.4 ± 6.5 µg/ml for DPPH assay whereas $IC_{50}$ value was 180.9 ± 2.3 µg/ml for ABTS assay.
**Conclusion:** Herbal paste was rich in chemical constituents and showed potent in vitro antioxidant activity.

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*Keywords: Antioxidants; chemical constituents; herbal paste; snake bites.*

## 1. INTRODUCTION

Sri Lanka being an oriental country inheriting a great indigenous system of medicine which has promising herbal medicines to treat snake bites. According to the recent archaeological excavations unearthed certain artifacts clearly shows the unreported historic era through which the traditional medical systems existed and prevailed in this country [1]. These traditional medical practices used for treating venomous snake bites which are hand by documented in literature but are used by traditional practitioners for thousand years. These physicians usually have a family history of snake bite management. Methods of management are gifted from one generation to another and those are practiced and protected very seriously by the members of each generation. Each family has an identical set of management methods which are different from the others [2].

In Sri Lankan indigenous snake-venom treatment system, there are many basic treatment modalities that are used by the traditional physicians in order to prevent the spread of the venom. When a snake bitten patient is presented, the physician uses specially prepared anti-venom herbal powder as basic treatment [3]. However, a very few snakebite treatment related studies have done in Sri Lanka. Among them a prospective study done in Anuradhapura District of Sri Lanka, which aimed to describe socio-demographics, behavioral responses, treatment, and pre-hospital interventions of snakebite victims presenting to a tertiary care facility. Many victims had recommended first aid interventions, a considerable proportion of snakebite victims in this area still seek traditional first aid and treatments after snakebite [4]. However, some traditional physicians not like to disclose these valuable medicines and they named it as *Rahas Beheth* (hidden treatment). The aim of this study was to conduct a chemical analysis in a selected herbal paste which is used as a hidden treatment for snake bites by traditional practitioners.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

All the plant materials were collected in dry form except *C. aurantiifolia* from Western Province, Sri Lanka during January 2020 to February 2020 and authenticated by a Senior Lecturer, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka. Voucher specimen of each plant material was deposited at Institute of Indigenous Medicine.

### 2.2 Preparation of Traditional Herbal Paste

All the plant parts except *C. aurantiifolia* fruit (Table 1) cleaned, washed and dried at 40$^{o}$C in an oven. Then equal amounts (25 g) were taken from each plant and pulverized into a coarse powder using a blender (Kenwood, model: BL440, made in China). Finally, the mixture was added to a vessel and mixed well with *C. aurantiifolia* juice.

### 2.3 Chemical Investigations for the Traditional Herbal Paste

Herbal paste was subjected to investigate (a) phytochemical classes (b) Thin Layer Chromatography (TLC) fingerprint profile (c) *in vitro* antioxidant activity.

### 2.4 Preparation of Hot Water Extract

Herbal paste (50 g) was added to a round bottom containing 500 ml of distilled water and refluxed for 4h. Then filtered and filtrate was concentrated and freeze dried.

### 2.5 Phyto-chemical Screening

Freeze dried powder (5 g) was dissolved in 20 ml of distilled water and subjected for phyto-chemical screening as described by Karunakaran et al. [5] and Dahanayake et al. [6] Phytochemical screening was carried out base on the presence of color, precipitate or interface.

### 2.6 Development of Thin Layer Chromatography (TLC) Fingerprint

Herbal paste (10 g) was refluxed with distilled water (50 ml) for 1h and filtered. The filtrate was added to a separatory funnel containing dichloromethane (100 ml) and mixed well. Then dichloromethane fraction was collected. This was repeated thrice, pooled the dichloromethane fractions and concentrated. As the mobile phase hexane and ethyl acetate in a ratio of 7:3 v/v was used. Once the TLC fingerprint developed

observations were carried out (a) under UV light (at 254 nm and 366 nm) and (b) after spraying vanillin sulphate. Finally, $R_f$ values were calculated for the spots observed under UV light and after spraying vanillin sulphate [7].

## 2.7 Antioxidant Activities of Herbal Paste

Different concentrations were made out by dissolving freeze dried powder (1 mg) of herbal paste in 1 ml of distilled water and subjected for quantification of (a) total phenolic content and scavenging ability of (b) DPPH (1,1–diphenyl-2-picryl hydrazyl) radical (c) ABTS [2.2-azino-bis (3ethylbenzothiazoline-6-sulfonicacid) diammonium] radical.

### 2.7.1 Total phenolic content

Total phenolic content was determined as Singleton and co-workers [8] and gallic acid used as the reference compound.

### 2.7.2 DPPH (1,1–diphenyl-2-picryl hydrazyl) assay

DPPH assay was performed in 96-well micro-plates according to the method described by Blois [9] with some modifications.

### 2.7.3 ABTS (2.2-azino-bis [3ethylbenzothiazoline-6-sulfonicacid] diammonium) assay

ABTS assay was performed in 96-well micro-plates according to the method described by Re [10] with some modifications.

## 2.8 Statistical Analysis

Statistical analysis was performed using statistical software origin pro 8. All data were expressed as Mean ± SEM. All statistical comparison compared through one-way analysis of variance (ANOVA), using Tukey's HSD post hoc test ($p \leq 0.05$).

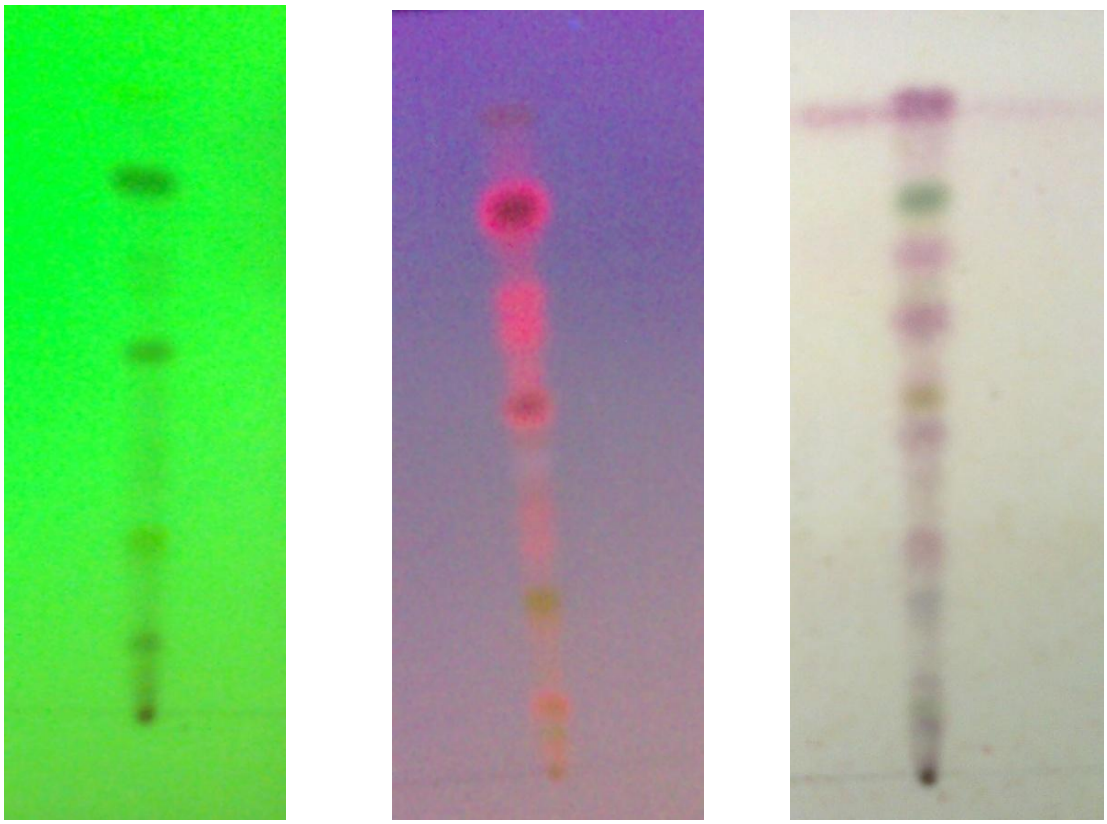
## 3. RESULTS AND DISCUSSION

Herbal paste consists of nine medicinal plants (Table 1) and among the tested phyto-chemical classes phenols, tannins, steroids, saponins and cardiac glycosides were present whereas both flavonoids and alkaloids were absent. In the present study, a TLC fingerprint was developed for the herbal paste and observed under UV light and visualized the colors of the spots after spraying vanillin sulphate (Fig. 1). Further, $R_f$ values of the each spot (before and after spraying vanillin sulphate) was calculated (Table 2). Development of TLC fingerprints for herbal drugs/extracts [11,12] or plants is a common practice to check batch wise consistency [13] because it is a simple, cheap and quick method. Polyphenols in the herbal paste react with Folin-Ciocalteu reagent to form blue complex [14]. Moreover, total polyphenols in the herbal paste was 94.15 ± 5.32 mg gallic acid equivalents/g of extract. Phenols exhibit (a) detoxification ability against mycotoxins [15] and many (b) bioactivities [16,17] such as wound healing. Therefore, presence of high content of phenols in the herbal paste may play an important role in curing snake bites.

Water extract of the herbal paste showed dose dependent DPPH radical scavenging (Fig. 2) and ABTS radical scavenging (Fig. 3) activities. DPPH radical can accept an electron or a hydrogen radical to become a stable and diamagnetic molecule. DPPH appears as deep violet in methanol and shows a strong absorption band at 517 nm due to its odd electron. Once an electron or hydrogen radical is paired with the DPPH radical deep violet color will be vanished [18]. ABTS is oxidized by potassium persulfate or manganese dioxide and formed the ABTS radical by losing an electron from the nitrogen atom of ABTS. It gives a bluish-green color at 743 nm. In the presence of hydrogen donating antioxidant, the nitrogen atom quenched the hydrogen atom. As a result the solution becomes decolorization and the absorbance is taken at 743 nm [19]. Moreover, $IC_{50}$ value was 628.4 ± 6.5 µg/ml for DPPH assay whereas $IC_{50}$ value was 180.9 ± 2.3 µg/ml for ABTS assay. The capabilities of scavenging ABTS radicals were more prominent by the antioxidant compound/s present in the herbal paste than that of DPPH radicals. This may due to capability of measuring both hydrophilic and lipophilic antioxidants in ABTS assay whereas only hydrophilic antioxidants measure in DPPH assay [10]. Similar results were observed with other research studies also [20–22].

**Table 1. Plant ingredients of the herbal paste**

| Botanical Name | Family | Part of the plant | Amount (g) |
|---|---|---|---|
| *Brassica nigra* L. | Brassicaceae | Seeds | 25 |
| *Carum carvi* L. | Umbelifereae | Seeds | 25 |
| *Zingiber officinale* L. | Zingiberaceae | Rhizome | 25 |
| *Trachyspermum involucratum* Roxb. | Apiaceae | Seeds | 25 |
| *Acorus calamus* L. | Acoraceae | Rhizome | 25 |
| *Allium sativum* L. | Amarythdaceae | Rhizome | 25 |
| *Piper nigrum* L. | Piperaceae | Seeds | 25 |
| *Piper longum* L. | Piperaceae | Fruit | 25 |
| *Citrus aurantiifolia* L. | Rutaceae | Fruit (juice) | 25 |



(a) At 254 nm (b) At 366 (c) After spraying vanillin sulphate

**Fig. 1. Thin Layer Chromatography fingerprint profiles of the herbal paste**

**Table 2. $R_f$ values of the herbal paste**

| $R_f$ values of the herbal paste | |
|---|---|
| **Before spraying** | **After spraying** |
| **λ 254 nm & λ 366 nm** | |
| 0.03 | 0.05 (Brown) |
| 0.05 | 0.08 (Purple) |
| 0.09 | 0.13 (Orange) |
| 0.17 | 0.16 (Grey) |
| 0.26 | 0.22 (Purple) |
| 0.31 | 0.28 (Orange) |
| 0.43 | 0.31 (Purple) |
| 0.52 | 0.38 (Pink) |
| 0.60 | 0.44 (Purple) |
| 0.66 | 0.53 (Magenta) |
| 0.69 | 0.59 (Purple) |
| 0.74 | 0.64 (Purple) |
| 0.83 | 0.75 (Brown) |
| 0.91 | 0.78 (Purple) |
| | 0.86 (Purple) |
| | 0.94 (Brown) |
| | 0.98 (Orange) |

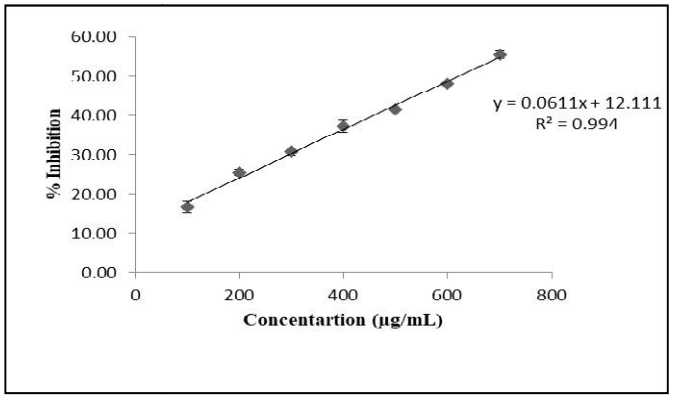



**Fig. 2. Dose response relationship of DPPH free radical scavenging activity for water extract of the herbal paste**

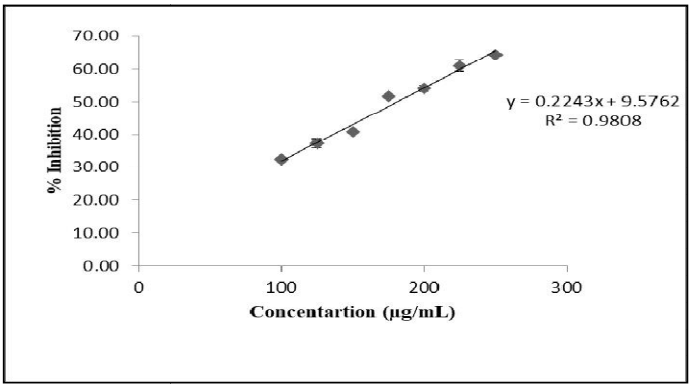



**Fig. 3. Dose response relationship for ABTS free radical scavenging activity of water extract of the herbal paste**

## 4. CONCLUSION

Herbal paste which consists of nine medicinal plants was rich in chemical constituents and showed potent in vitro antioxidant activity

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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