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Chemical Analysis of a Traditional Herbal Decoction use in Sri Lanka for Snake Bite Treatments

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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

Snake bite is considered as a major occupational health problem in Sri Lanka. A traditional decoction consists of nine medicinal plants clinically proven its efficacy for snake bites. In the present study, an attempt was done to carry out chemical analysis of the decoction. Chemical analyses were carried out for the decoction in terms of (a) phytochemical screening (b) quantification of total phenols and total flavonoids and (c) *in vitro* antioxidant activities. Present study, revealed the presence of alkaloids, flavonoids, phenols, saponins, steroids and tannins in the decoction. Moreover, total polyphenol and total flavonoid contents of the decoction were 66.03 ± 2.74 mg gallic acid equivalents/g of extract and 18.93 ± 0.90 mg quercetin acid equivalents/g of extract respectively. ORAC value was 3.51 ± 0.23 mg trolox equivalents/g of extract and dose dependent (R^2 = 0.9788) DPPH radical scavenging ability was observed. IC_{50} of the decoction for the DPPH assay was 4.58±0.12 µg/ml. In conclusion**,** traditional decoction which used to treat snake bites consists of many important phytochemical classes and exhibit potent *in vitro* antioxidant activity.

Keywords: Antioxidant potential; phytochemicals; snake bites; traditional decoction.

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1. INTRODUCTION

Snake bite is regarded as a serious occupational health issue. Various active young people, especially those involved in farming and plantation work, have died or become disabled as a result of it in many regions of the world, particularly in the Southeast Asian region [1]. Sri Lanka has a lengthy history of dealing with snake bites. History books state that even the king "Dutugemunu" has been bitten by snakes and had been cured. Management of snake bites is mostly done by traditional physicians. These physicians usually have a family history of snake bite management. Methods of snake bite treatment 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. Traditional snake bite treatment practitioners in Sri Lanka have accurate antidotes for dangerous bites. Different plant components, such as leaves, fruits, stem bark, tubers, and roots, have been used as antidotes in the form of paste, powder, juice, infusion, decoction, and crude form. Other ingredients, such as goat milk, butter milk, lime juice, and ghee, are sometimes added to these plant parts [2].

Even today, most of the people living in rural areas in Sri Lanka believe traditional treatment for snake envenomation. Snake bites are treated with medicinal plants in indigenous medicine systems. There is a vast repository of plants that have been reported to have anti-snake venom activity [3, 4]. Investigation of therapeutic potential of plants used for snake bites shows the presence of different phytochemicals such as phenols, flavonoids, tannins and saponins, etc [5,6]. Globally, traditional snake bite treatment practitioners are practicing herbal medicine to cure snake bites. Few investigations have been conducted to evaluate the pharmacologically active ingredients against snake bites [7]. Therefore, present study was aimed to analyze a traditional decoction consists of nine medicinal plants (Table 1) which is given by Ayurvedic physicians for snake bites as a promising treatment.

2. MATERIAL AND METHODS

2.1 Plant Materials

All the plant materials were collected from Western Province, Sri Lanka during August 2021 to September 2021 and authenticated by a Senior Lecturer, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka. Voucher specimen of each plant material (Table 1) was deposited at Institute of Indigenous Medicine.

2.2 Preparation of the Decoction

All the plant parts were cleaned, washed and dried at 40 ^{o}C in an oven. Then equal amounts (approx. 7 g) were taken from each plant and pulverized into a coarse powder using a blender (Kenwood, model: BL440, made in China). Then 60 g of the herbal mixture was boiled in water (1920 ml) until the final volume reduced up to 240 ml. Finally, decoction was filtered and concentrated under reduced pressure using a rotary evaporator (yield 6.5% w/w).

2.3 Phyto-chemical Screening

Phyto-chemical screening was carried out as described by Karunakaran et al. [8] and Dahanayake at al. [9] Phytochemical screening was carried out base on the presence of color, precipitate or interface.

Table 1. Plant ingredients of the traditional decoction use for snake bite treatment

Botanical name	Voucher number	Family	Part of the plant
Terminalia chebula Retz	TC_K-1	Combretaceae	Fruit
Terminalia bellirica (Gaertn.) roxb	TB_K-2	Combretaceae	Fruit
Phyllanthus emblica Linn	PE_K-3	Phyllanthaceae	Fruit
Azadirachta indica A. Juss	AI-K-4	Meliaceae	Bark
Rubia cordifolia Linn	RC_K-5	Rubiaceae	Whole Plant
Acorus calamus Linn	AC_K-6	Araceae	Rhizome
Picrorrhiza kurroa Royle ex. Benth	BK_K-7	Plantaginaceae	Stem
Coscinium fenestratum (Goetgh.) Colebr	CS_K-8	Menispermaceae	Stem
Tinospora cordifolia (Thunb.) Meiers	TC_K-9	Menispermaceae	Stem

2.3.1 Test for alkaloids

(a) Picric acid test: Picric acid (1 ml) was added to 1 ml of decoction. Yellow crystalline precipitate indicates the presence of alkaloids.
(b) Mayer's test: Few drops of Mayer's reagent were added to 1 ml of decoction. Yellow precipitate indicates the presence of alkaloids.
(c) Tannic acid test: Tannic acid (1 ml) was added to 1 ml of decoction. Yellow crystalline precipitate indicates the presence of alkaloids.

2.3.2.Test for flavonoids

(a) Conc. H_2SO_4 and ammonia solution: Ammonia solution (1 ml) was added to the 2 ml of decoction. Finally, few drops of conc. H_2SO_4 were added to the solution via the test tube. Yellow coloration indicates the presence of flavonoids.
(b) Aluminum chloride test: A few drops of Aluminum chloride were added to 1 ml of decoction. Yellow color indicates the presence of flavonoids.

2.3.3 Test for phenols

(a) Folin reagent test: A few drops of Folin reagent were added to 1 ml of decoction. Blue color indicates the presence of phenols.
(b) Ferric chloride test: A few drops of Ferric chloride were added to 1ml of decoction. Blue color indicates the presence of phenols.
(c) Lead acetate test: Lead acetate (1 ml from 10% solution) was added to 1 ml of decoction. Yellow precipitate indicates the presence of phenols.

2.3.4 Test for saponins

(a) Frothing test: Water (5 ml) was added to the decoction (2.5 ml) and shaken vigorously. Persistence of stable froth indicates the presence of saponins

2.3.5. Test for steroids

(a) Acetic Anhydride (1 ml) was added to decoction (2 ml). Then conc. H_2SO_4 (1 ml) was added to the mixture. A color change from violate to blue or green indicates the presence of steroids

2.3.6 Test for tannins

(a) Lead acetate test: Lead acetate (1 ml from 10% solution) was added to 1 ml of decoction. Yellow precipitate indicates the presence of tannins.
(b) Ferric chloride Test: A few drops of Ferric chloride were added to 1ml of decoction. Blackish blue color indicates the presence of tannins.
(c) Vanillin test: Few drops of 10% Vanillin in Ethyl alcohol and conc. HCl were added to 2 ml of decoction. Red color indicated the presence of tannins.

2.3.7 Test for Sesquiterpenes

(a) Test for Sesquiterpenes: Decoction (1 ml) was mixed with conc. H_2SO_4 (0.5 ml). A brown green, red or blue color indicates the presence of sesquiterpenes.

2.4 *In vitro* Antioxidant Activities

Different concentrations were made out by dissolving water extract in a mixture of water and methanol (1:1 w/w) 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.

2.4.1 Total phenolic content

Total phenolic content was determined as Singleton and co-workers [10] and gallic acid used as the reference compound.

2.4.2 Total flavonoid content

Total phenolic content was determined as Siddhuraju and Becker [11] and quercetin used as the reference compound.

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

DPPH assay was performed in 96-well micro-plates according to the method described by Blois [12] using the water extract of the drug. Inhibition activity percentage (I%) was calculated as follows:

$$I\% = \frac{100 \times (A0 - A1)}{A0}$$

Where A_0 is the absorbance of the control sample and $\mathbf{A_1}$ is the absorbance of the test extract. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration.

2.4.4 Oxygen radical absorbance capacity (ORAC) assay

The ORAC radical scavenging assay was performed in 96-well microplates according to the method described by Ou and co-workers [13] using the water extract of the drug.

2.5 Statistical Analysis

Statistical analysis was performed using statistical software origin pro 8. All data were expressed as Mean ± SEM.

3. RESULTS AND DISCUSSION

Many farmer communities in Asian and African countries using plant base treatments (eg. decoctions, pates, juices) to minimize the venom effects such as haemorrhage and edema [14,15]. The toxic compounds of the venom can be neutralized by active compound/s in the snake venom via several mechanisms including (a) precipitation or inactivation of proteins [16], inactivation or enzyme inhibition [17,18], antioxidant activity [19] or combination of one or more activities. Present study, revealed the presence of alkaloids, flavonoids, phenols, saponins, steroids and tannins (Table 2) in the decoction. More than one screening tests were performed to detect the phytochemicals in the decoction. Presence of these active compound/s may responsible for one or multiple mechanisms that neutralize the snake venom.

Total polyphenol and total flavonoid contents of the decoction were 66.03 ± 2.74 mg gallic acid equivalents/g of extract and 18.93 ± 0.90 mg quercetin acid equivalents/g of extract respectively. Polyphenols in the water extract react with Folin-Ciocalteu reagent to form a blue complex that can be quantified by visible-light spectrophotometry at 765 nm [20]. A blue chromophore is formed by the reaction, which is made up of a phosphotungstic-phosphomolybdenum complex [20, 21]. The alkaline solution and the concentration of phenolic compounds [20] in the extract determine the maximum absorption of the chromophores. One of the widely followed methods for the determination of TFC in plant extracts is the aluminum chloride colorimetric assay, where Al(III) is utilized as a complexing agent. The method is based on the formation of chelates of Al(III)-flavonoids due to their many oxo and hydroxyl groups. Furthermore, flavonoids bind metal ions such as Al(III) with a high affinity,

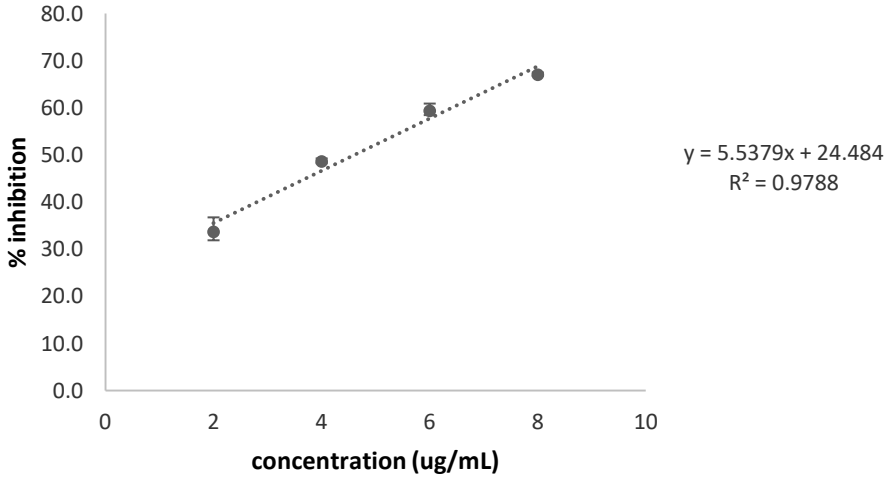

Fig. 1. Dose response relationship of DPPH free radical scavenging activity of the water extract I (%) = 100 × ($A0$ − $A1$) $A0$, where $A0$ is the absorbance of the control sample and $A1$ is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotting inhibition percentage against extract concentration.

Table 2. Phytochemical screening of the traditional decoction use for snake bite treatment

Phytochenicals	Test/s	Results
Alkaloids	Picric acid Test	Positive
	Mayer's Test	Positive
	Tannic acid Test	Negative
Flavonoids	$NH_3 + H_2SO_4$	Positive
	1% Aluminum chloride	Positive
Phenols	Folin Reagent Test	Positive
	Ferric chloride Test	Positive
	Lead Acetate Test	Positive
Saponins	Frothing Test	Positive
Steroids	Acetic anhydride	Positive
Tannins	Lead acetate Test	Positive
	0.1% $FeCl_3$	Positive
	Vanillin Test	Negative
Sesquiterpenes	Conc.H_2SO_4	Positive

usually at a 1:1 ratio, depending on experimental conditions such as pH [22]. It is well documented that phenols and flavonoids act against snake venom [5, 6, 23, 24]. This may be one of the reasons that the present traditional decoction has promising effects for snake bites. Moreover, plant extracts which have potent antioxidant potential also exhibit anti- venom effects [23,24]. In the present study, *in vitro* antioxidant potential of the traditional decoction was investigated via ORAC and DPPH assays. ORAC value was 3.51 $\pm$ 0.23 mg trolox equivalents/g of extract and dose dependent (R^2 = 0.9788) DPPH radical scavenging ability was observed (Fig. 1) along with a IC_{50} value of 4.58±0.12 µg/ml. When DPPH radical react with an antioxidant, its purple colour is disappeared and gives pale yellow colour at 517 nm and measures only the hydrophilic antioxidants [25] while the ORAC test measures the splitting ability of the radical chain reaction by antioxidants through monitoring the inhibition of the oxidation of the peroxyl radicals [26].

4. CONCLUSION

Traditional decoction which used to treat snake bites consists of many important phytochemicals and exhibit potent in vitro antioxidant activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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