Chemical analysis of a traditional herbal decoction use in Sri Lanka for snake bite treatments

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

Key words: antioxidant potential, phytochemicals, snake bites, traditional decoction

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1. Introduction

Snake bite is considered as a major occupational health problem. It has been a perennial cause of death or chronic disability to many active young people, especially those who are involved in farming and plantation work in many parts of the world especially in the South East Asian Region [1]. Sri Lanka has a very long history for snake bite management. 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. Sri Lankan traditional snake bite treatment practitioners have authentic information about antidotes for poisonous bites. They have been using different plant parts like leaves, fruits, stem bark, tubers and roots as antidotes in the form of paste, powder, juice, infusion, decoction, and in crude form. These plant parts are sometimes mixed with other additives like goat milk, butter milk, lime juice and ghee [2].

Even today, most of the people living in rural areas in Sri Lanka believe traditional treatment for snake envenomation. The indigenous systems of medicine use medicinal plants for the treatment of snake bites. There is a huge repository of plants reported to possess 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. The numbers of studies evaluating the pharmacologically active principles against snake bites are few[7]. Therefore, present study was aimed to analyze a traditional

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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 was deposited at Institute of Indigenous Medicine.

Comment [G1]: You should give the number of identification of you various plants

2.2. Preparation of the decoction

All the plant parts 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.

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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] with some modifications.

Comment [G2]: Which modifications have you done on the method?

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] with some modification.

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2.5. Statistical analysis

Comment [G3]: Which logiciel have you used for your various analyses?

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

Comment [G4]: You should presente and discus each resuld. It should be separate considering that you have done many tests, so each test should conduct on a resuld which should be discussed normally

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Manny farmer communities in Asian and African countries using plant base treatments (eg. decoctions, pates, juices) to minimize the venom effects such as hemorrhage 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. It is well documented that phenols and flavonoids act against snake venom [5, 6, 20, 21]. 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 [20,21]. In the present study, *in vitro* antioxidant potential of the traditional decoction was investigated via ORAC and DPPH assays. 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

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(Figure 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 color at 517 nm and measures only the hydrophilic antioxidants [22] 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 [23].

4. Conclusion

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

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

Botanical name	Family	Part of the plant
Terminalia chebula Retz	Combretaceae	Fruit
Terminalia bellirica (Gaertn.) roxb	Combretaceae	Fruit
Phyllanthus emblica Linn	Phyllanthaceae	Fruit
Azadirachta indica A. Juss	Meliaceae	Bark
Rubia cordifolia Linn	Rubiaceae	Whole Plant
Acorus calamus Linn	Araceae	Rhizome
Picrorrhiza kurroa Royle ex. Benth	Plantaginaceae	Stem
Coscinium fenestratum (Goetgh.) Colebr	Menispermaceae	Stem
Tinospora cordifolia (Thunb.) Meiers	Menispermaceae	Stem

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Table 2. Phytochemical screening of the traditional decoction uses 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	Positive
Phenolics	Folin Reagent Test	Positive
	Ferric chloride Test	Positive
	Lead Acetate Test	Positive
Saponins	Frothing Test	Positive
Steroids	Acetic anhydride	Positive
	Liebermann Burchard Test	Negative
Tannins	Lead acetate Test	Positive
	0.1% $FeCl_3$	Positive
	Vanillin Test	Negative
Sesquiterpenes	ConcH_2SO_4	Positive

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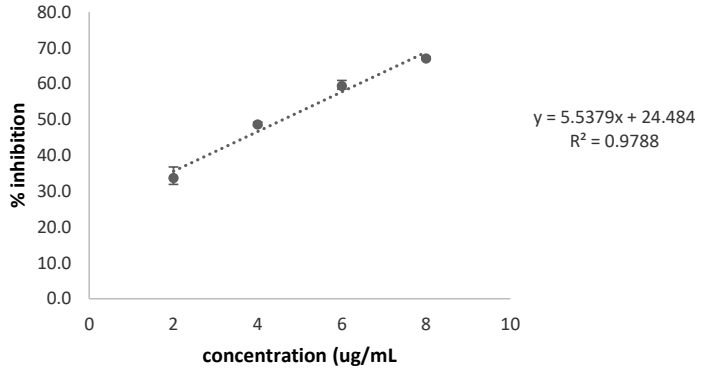

Figure 1. Dose response relationship of DPPH free radical scavenging activity of traditional decoction

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