



Standardization of *Nagaraja Guliya*; A Sri Lankan Traditional Formula Used in Poisons of Animal Origin (Jangama Visha)

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Authors' contributions

This work was carried out in collaboration among all authors. Author RDHK designed the concept of the study. Author JHKE performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RDHK and EDTPG managed the analyses managed the literature searches and finalized the draft. All authors read and approved the final manuscript.

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ABSTRACT

Sri Lanka has unique heritage of own medical system handed down from generation to another over a period of 3,000 years. Native medical practitioners in different disciplines are inherited within a family tradition and their treatments are still effective and accepted by the Sri Lankan community. *Nagaraja Guliya* is one of traditional preparations prescribed as internal or external medicaments by the traditional physicians in the southern province in Sri Lanka since ten decades for poisons of animal origin. The study has been focused to standardize the herbo mineral formula in respect of quality, safety and analyze the potential of *Nagaraja Guliya*. The formulation consists of the eight herbo mineral ingredients; *Aconitum ferox*, *Zingiber officinale*, *Myristica fragrans*, *Syzygium aromaticum*, Mercury, Arsenic trisulfide, Copper sulphate and *Luffa cylindrica*. Authentication of the ingredients was carried out at Bandaranayaka Memorial Ayurveda Research Institute, Nawinna, Sri Lanka. Data has been gathered from Sri Lankan traditional manuscripts, Ayurveda authentic texts and different scientific journals. The results revealed that the *Nagaraja Guliya* contains secondary plant metabolites like Alkaloids, Tannins, Saponin and Phenols. The pH

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of the formulation was found to be 5.28 and is in acceptable range for oral administration and external application. Further, heavy metal contents of Mercury and Arsenic in the preparation was not reached to the harmful level to the human body. In Ayurveda view point; pharmacodynamic properties of the formula showed that maximum number of ingredients consist of *Katu Rasa* (pungent taste) *Laghu Guna* (Light property), *Ushna Veerya* (Hot in potency) and *Katu vipaka* with properties of pacification of *Kapha and Vata dosha*. Kushtagna, *Vedanastapana*, *Raktashodaka*, *Shotahara* and *Vishahara*. Hence, future studies should be planned to evaluate the existing data on traditional use of *Nagaraja Guliya*, along with experimental and clinical trials.

Keywords: *Jangama Visha*; *Nagaraja Guliya*; *standardization*.

1. INTRODUCTION

Sri Lankan traditional medicine has its own historical evidence of contemporary medical service to the country over a period of 3,000 years [1] and it was based on series of prescriptions tendered down to the generations to another. *Nagaraja Guliya* is a herbo mineral medicinal preparation in pill form prescribed by “*Ekanayake tradition*” in southern province in Sri Lanka which was indicated for venom poison since ten decades. Still *Nagaraja Guliya* has been used for internal administration or external application and not recommended orally for centipede’s bites.

In Ayurveda perspective; “*Visha*” is a substance which after entering into the body may vitiate the healthy *Dhatu* leading to death of the person. Among the two types of *Visha* (Poisons), described in Ayurveda, *Jangama visha* is a type of poison including snake venom, poisons of scorpion, sting, rodent, dog, leech and insects. Currently, these poisons are identified as occupational health hazards in agricultural

communities in rural areas of Sri Lanka, and it has the highest snake bite incidence in the world [2]. Snake bites lead to life-threatening complications such as local necrosis, hemorrhage, renal and respiratory failure [3]. Antivenom is the only therapeutic agent in modern medicine available throughout the globe but major drawbacks are high cost and adverse effects including anaphylaxis. Even today most of the victims initially approach traditional physicians for the native treatment rather than seeking modern medical management due to their faith and past experiences on the effectiveness of traditional medicine. Therefore, it is envisaged to experiment with other conventional therapy in exploring antidotes. Hence, the study has been carried out to standardize the herbo - mineral formula according to the WHO standards.

1.1 Ingredients of *Nagaraja Guliya* (A Herbo - Mineral Preparation)

A Sri Lankan traditional formula being prescribed by the ancestors of *Ekanayake* family.

Table 1. Ingredients of *Nagaraja Guliya* (A herbo - mineral preparation)

	Sanskrit/Sinhala Name	Scientific / English name	Botanical name	Used part
1	<i>Parada/ Gendagam</i>	Mercury (Hg)	-	
2	<i>Vassanabha/Wachchanavi</i>	Aconite	<i>Aconitum ferox</i>	Root
3	<i>Harithala /Hiriyal</i>	Arsenic trisulphide (As ₂ S ₃)	-	
4	<i>Shunti / Vijali Iguru</i>	Ginger	<i>Zingiber officinale</i> ,	Rhizome
5	<i>Tutta /Palmanikkam</i>	Copper sulfate	-	
6	<i>Jaiphal /Sadikka</i>	Nutmeg	<i>Myristica fragrans</i>	Seed
7	<i>Lavanga/Karabunati</i>	Clove	<i>Syzygium aromaticum</i>	Flower buds
8	<i>Jaiphal /Wasavasi</i>	Mace	<i>Myristica fragrans</i>	Mace
9	<i>Kosaphala/ Tikta vatakolu</i>	Sponge gourd	<i>Luffa Cylindrica</i>	Leaves

1.2 Objectives of the Study

- To standardize the *Nagaraja Guliya*; Sri Lankan traditional herbo - mineral preparation
- To study the pharmacodynamic and pharmacokinetic parameters of ingredients of *Nagaraja Guliya*

2. METHODOLOGY

2.1 Collection of Data

Data has been collected from Sri Lankan Traditional medicinal texts, authentic Ayurveda texts, dictionaries, journals and other related scientific articles from library of Institute of indigenous medicine and published research articles via internet.

2.2 Collection of the Ingredients

Arsenic trisulphide (Haritala) was collected from the pharmacy at department of Dravya Guna Vignana, Institute of Indigenous Medicine, university of Colombo and rest of the ingredients were collected from local market in Colombo, Sri Lanka.

2.2.1 Authentication of the ingredients of *Nagaraja Guliya* (A herbo - mineral preparation)

All the herbo - mineral ingredients were authenticated by a senior scientist,

Bandaranayaka memorial Ayurvedic research institute, Nawinna, Sri Lanka.

2.2.2 Purification procedures of mineral compounds in *Nagaraja Guliya*

Prior to the preparation of *Nagaraja Guliya*; Mercury (Hg), Arsenic trisulphide (As_2S_3) and Copper sulfate ($CuSO_4$) were purified according to the Ayurveda textual references.

2.2.3 Purification procedure of Mercury (Hg)

Equal amount (10 g) of slaked lime [$Ca(OH)_2$] was mixed with 10 g of mercury and triturated properly for 72 hours. Washed the mixture with pure water and filtered through fourfold cloth. Equal amount of *Allium sativum* (garlic-10 g), *Syzygium aromaticum* (cloves-10 g) along with rock salt (2.5 g) were added to the mixture and triturated till it turned to black in color. Finally, washed it with pure water and filtered through a clean cotton cloth.



Fig. 1. Mercury



Fig. 2. Copper sulfate



Fig 3. Arsenic trisulfide



Fig. 4. Aconitum



Fig. 5. *Myristica fragrans*



Fig. 6. *Syzygium aromaticum*

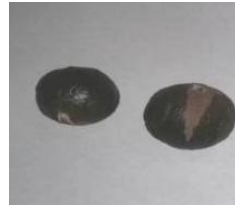


Fig. 7. *Myristica fragrans*



Fig. 8. *Zingiber officinale*



Fig. 9. Before the purification of Mercury (Hg)



Fig. 10. Triturate Mercury with adding slaked



Fig. 11. Mixture of mercury with Garlic, Cloves, Rock salt



Fig. 12. Purified Mercury(Hg)



Fig. 13.
Purification procedure of Arsenic trisulfide



Fig. 14.
Purified Arsenic trisulfide (Harithala)

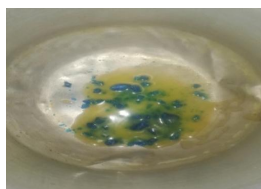


Fig. 15. CuSO_4 & Ghee heating under low fire



Fig. 16. Purified CuSO_4



Fig. 17.
Powder of
Zingiber officinale



Fig. 18.
Seed powder of
Myristica fragrans



Fig. 19.
Mace powder of
Myristica fragrans



Fig. 20.
Powder of
Aconitum ferox



Fig. 21.
Arsenic trisulfide



Fig. 22.
Purified CuSO_4

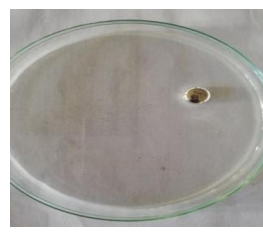


Fig. 23. Mercury



Fig. 24.
Syzygium Aromaticum



Fig. 25.
Sponge gourd



Fig. 26.
Juice of sponge
gourd leaf



Fig. 27.
Mixture of ingredients
with juice of Sponge
gourd



Fig. 28.
Final product

2.2.4 Purification procedure of Arsenic trisulfide (As_2S_3)

Crushed Arsenic trisulphide (5 g) was covered with cotton cloth and made a ball. It was hanged with wooden stick and dipped the bolus in the clay pot which was filled (Dola yantra) with juice of *Benincasa hispida* (Kushmanda swarasa). Then it was heated under moderate fire for 24 hours. The ball was taken out from the Kushmanda Swarasa and removed the cotton cloth and washed the sample with hot water. Kept the purified Arsenic trisulphide sample under shade for drying.

2.2.5 Purification procedure of Copper sulfate ($CuSO_4$)

5 g of Copper sulfate was mixed with 5 ml of ghee and heated under moderate fire until 5 minutes.

2.2.6 Preparation of Nagaraja Guliya (herbo - mineral preparation)

All the herbal ingredient was pulverized separately into fine powder using a grinder (Singer, model: Elite, made in India). Then equal quantity of (2.5 g) of Mercury, *Aconitum ferox*, Arsenic trisulfide, *Zingiber officinale*, Copper sulphate, *Syzygium aromaticum*, seeds and mace of *Myristica fragrans* were added to a porcelain vessel and mixed well. *Syzygium Aromaticum* (17.5 g) was taken equal to the total weight of all the above ingredients and added to the same vessel and mixed with juice of leaves of *Luffa Cylindrica* (Sponge gourd) to prepare Nagaraja Guliya in pill form weighing 150 mg similar to the seeds of *Gossypium arboretum* (cotton seed).

2.3 Evaluation Parameters

2.3.1 Organoleptic evaluation of Nagaraja Guliya (herbo - mineral preparation)

The formulation was evaluated by its organoleptic properties of color, Taste, odor and texture

Table 2. Screening of phytochemicals in Nagaraja Guliya (herbo - mineral preparation)

	Phytochemicals	Method
1	Alkaloids	Few drops of Wagner's reagent were added to 2ml of extract
2	Saponins	Added 5ml of water to 2ml of extract and shaken vigorously
3	Tannin	0.1% $FeCl_3$ solution was added to 2ml of extract
4	Phenols	few drops of Lead acetate were added to 2ml of extract

2.4 Physical Evaluation

2.4.1 Determination of pH value of Nagaraja Guliya (herbo - mineral preparation)

pH value was determined at room temperature using pH meter. Initially the sample was macerated with distilled water for 2 hours and filtered it. Then pH meter was calibrated from the standard pH buffer available at the laboratory. Then the pH of solution was measured. Triplicates were taken.

2.4.2 Analysis of phytochemicals in Nagaraja Guliya (herbo - mineral preparation)

Phytochemical analysis was done for identification of the medicinally active substances in the drug. 2 mg of powder of the drug was mixed with 20 ml of water and macerated for 24 hours. Tests for Alkaloids, Saponin, Tannins and Phenols were conducted with the extract.

2.4.3 Analysis of heavy metal in Nagaraja Guliya (herbo - mineral preparation)

2.4.3.1 Microwave digestion by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Heavy metal trace test was carried out at Industrial Technology Institute, Malabe Colombo.

Microwave digestion is a common technique used by elemental scientist to dissolve heavy metals in the presence of organic molecules prior to analysis by inductively couple plasma, atomic absorption or atomic emission measurements.

2.4.4 Procedure

Concentrated HNO_3 (10 ml) was added to 0.5 g of sample and placed in the microwave digestion system. The digested sample was filtered through the 5.42 filter paper and mixture was poured in to the volume metric flask and made up with distilled water. The prepared sample was checked by ICP-MS (Inductively Coupled plasma Mass Spectrometry) to defect the concentration of heavy metals in mg/l.



Fig. 29.
Alkaloid test-
Brown precipitate
present at the
bottom of the test
tube

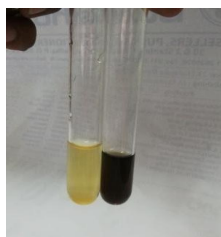


Fig. 30.
Tannin test -
Solution became
bluish black color



Fig. 31.
Phenols test- Yellow
color precipitate
present at the bottom
of the test tube

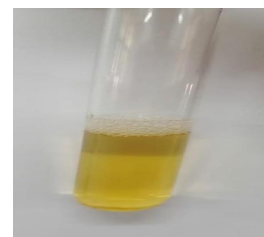


Fig. 32.
Saponin test-
Persistence of froth

3. RESULTS

3.1 Organoleptic Evaluation

Organoleptic evaluation of *Nagaraja Guliya* was greenish brown in color with Aromatic odour of mixture of all herbo - minerals, solid consistency in texture and astringent taste.

Organoleptic evaluation of *Nagaraja Guliya* was greenish brown in color with Aromatic odour of mixture of all herbo - minerals,

solid consistency in texture and astringent taste.

3.1.1 Phytochemical analysis of *Nagaraja Guliya*

Phytochemical screening evaluated in different solvent extraction showed the presences of secondary metabolites like alkaloid, Tannin, Saponins and phenol which are helpful in predicting their therapeutic properties.

Table 3. Organoleptic parameters of *Nagaraja Guliya*

Sr. No	Organoleptic parameters	Observation
1.	Appearance	Solid
2.	Colour	Greenish brown
3.	Taste	Astringent
4.	Odour	Aromatic

Table 4. Phytochemical analysis of *Nagaraja Guliya*

Sr. No	Phytochemicals	Observation	Result
1.	Alkaloid	Brown precipitate present at the bottom of the test tube	Positive
2.	Tannin	Solution became bluish black color	Positive
3.	Saponins	Persistence of froth	Positive
4.	Phenol	Yellow colored precipitate present at the bottom of the test tube	Positive

3.1.2 Physiochemical study of *Nagaraja Guliya*

The pH value of *Nagaraja Guliya* was acidic.

Table 5. pH value of *Nagaraja Guliya*

Sr No.	Physical parameters	Formula
1.	pH (5% w/v aqueous solution)	5.28

3.1.3 Pharmacodynamic properties of the ingredients of Nagaraja Guliya

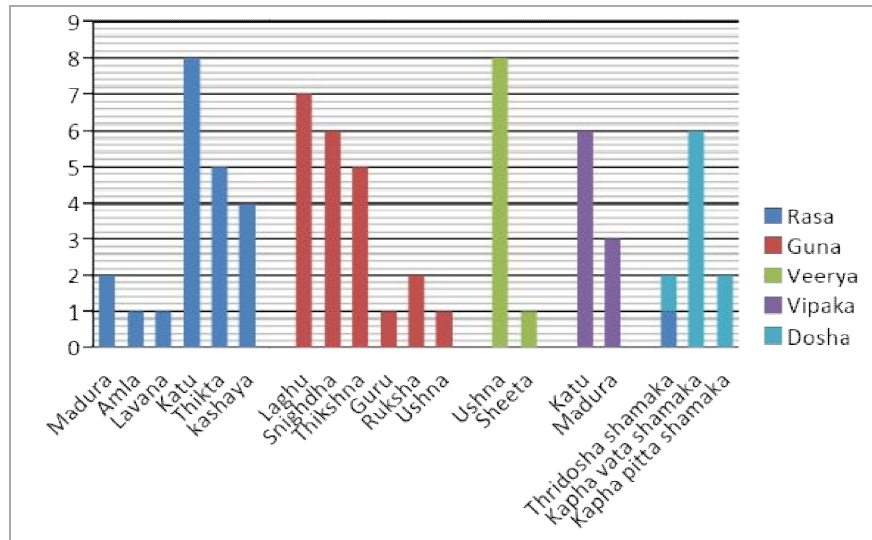


Fig. 33. Distribution of Pharmacodynamic properties of the Nagaraja Guliya

3.1.4 Pharmacokinetic properties of the ingredients of Nagaraja Guliya

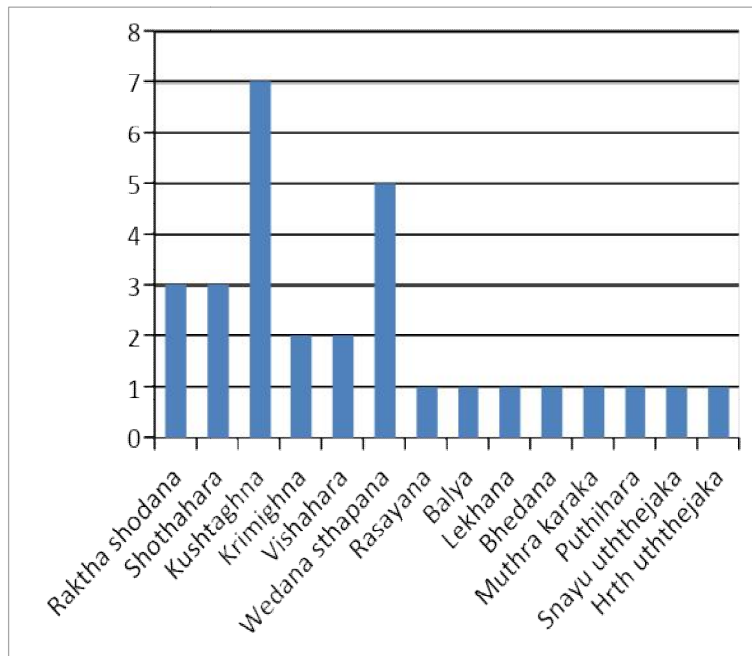


Fig. 34. Distribution of pharmacokinetic properties of the Nagaraja Guliya

3.1.5 Heavy metal content test result (Hg, As)

Arsenic (As) and Mercury (Hg) content of *Nagaraja Guliya* found 2.9 and 4.8 percent by mass respectively.

Table 6. Pharmacodynamic properties of the ingredients of the formula [3-8]

Ingredients of Nagaraja Guliya	Rasa						Guna			Veerya			Vipaka				
	Madhura	Amla	Lvana	Katu	Thiktha	Kashaya	Laghu	Snigdha	Theekshna	Guru	Ruksha	Ushna	Shitha	Katu	Madhura	VPK	KV
1 Mercury (Hg)	+	+	+	+	+	+	+	+	+	+	+			+	+		
2 <i>Aconitum ferox</i>	+						+		+		+			+			+
3 Arsenic trisulphide (As ₂ S ₃)				+				+			+			+			+
4 <i>Zingiber officinale</i> ,				+			+	+			+			+			+
5 Copper sulphate				+		+	+				+			+			+
6 <i>Myristica fragrans</i>				+	+	+	+	+	+		+			+			+
7 <i>Lavanga/Karabunati</i>				+	+		+	+	+		+		+	+			+
8 <i>Jaiphal /Wasavasi</i>				+	+	+	+	+	+		+			+		+	
9 Kosaphala /Tikta vatakolu				+	+		+		+		+			+			+

Table 7. Pharmacokinetic properties of the ingredients of the formula [3-9]

Ingredients		Pharmacokinetic properties
<i>Parada</i>	Murcury (Hg)	Putihara, Vedana stapana, Rakta shodhaka, Kushtaghna
<i>Vassanabha</i>	<i>Aconitum ferox</i>	Hrit Uttejaka, Shotahara, Kushtaghna, Vedanastapana
<i>Harithala</i>	Arsenic tri sulphide (As ₂ S ₃)	Vishahara, Raktashodhaka, krmighna, Kushtaghna, Rasayana
<i>Shunti</i>	<i>Zingiber officinale</i> ,	Vedana sthapana, Shotahara, Snayu utthethjaka
<i>Tutta</i>	Copper sulfate	Vishahara, Kushtaghna, Krmighna, Lekhana, Bhedana
<i>Jaiphal</i>	<i>Myristica fragrans</i> - Mace	Vedana stapana, Kushtaghna
<i>Jaiphal</i>	<i>Myristica fragrans</i> - Nutmeg	Vedana stapana, Kushtaghna
<i>Lavanga</i>	<i>Syzygium aromaticum</i>	Mutra karaka, Ruchi Vardhaka
<i>Thiththa wetakolu</i>	<i>Luffa Cylindrica</i>	Rakta shodhaka, Shotahara, Kushtaghna

Table 8. ICP -MS analysis of heavy metal content of *Nagaraja Guliya*

Test	Unit	Result
1. Arsanic (As)	Percent by mass	2.9
2. Mercury (Hg)	Percent by mass	4.8

4. DISCUSSION

Snake bite is a major occupational health hazard and its burden is mostly confined to the poor communities in agricultural sector in Sri Lanka [10]. Antivenom is the only therapeutic agent in modern medicine available throughout the world for venom poison, but high cost does not make it readily accessible to the poorer community. Indigenous practice in Sri Lanka has been using powerful herbo -mineral recipes for treating venom poison since thousand years. Currently, it is an emerging trend by the researches to explore possible antidote for the venom from eastern traditional system of medicine.

Nagaraja Guliya is one of traditional herbo - mineral preparations [Table 1] for venom poisons prescribed by the “*Ekanayaka*” traditional physicians in southern province in Sri Lanka since ten decades. It is in pill form weighing 150 mg similar to the seeds of *Gossypium arboretum* (cotton seed). It is a multi-ingredient product containing four herbal ingredients such as *Aconitum ferox*, *Zingiber officinale*, *Myristica fragrans*, and *Syzygium aromaticum* [11] along with three inorganic compounds including Mercury, Arsenic sulphide and Copper sulfate, which are not in plant origin. Percentage of Mercury (*Parada*) and Arsanic Trisulfid (*Haritala*) in weight of 150 mg pill contains 7.2

mg of Mercury (Hg) and 4.35 mg of Arsenic trisulphide (As₂S₃). It was calculated that Mercury and Arsenic trisulphide contained in the *Nagaraja Guliya* was 4.8% and 2.9% respectively [Table:8]. The percentage of minerals appears in the *Nagaraja Guliya* is less than the acute minimal lethal dose of Mercury and Arsenic for the person of 1 mg/kg/day pH of the *Nagaraja Guliya* is 5.28 [Table: 5] [12]. It is in the acceptable pH range for oral administration (pH 5-8) [16] as well as external application (pH 4.5 – 5.5). Secondary plant metabolites such as Alkaloids, tannins, Saponins, phenols were present in the *Nagaraja Guliya* [Table 4]. Alkaloids have cytotoxic, hemolytic, antibacterial and insecticidal properties [13]. Furthermore, Saponins also have haemolytic, antimicrobial, insecticidal, molluscicidal anti-inflammatory and anti-allergic activities.

Tannin are plant poly phenolic compound which has antiseptics, anti-carcinogenic and anti-inflammatory properties [14] and it may serve as treatment of venom poisoning either as first aid or as supplements for the traditional antivenoms on the inhibition of local tissue necrosis (Ownby et al., 1984; Gene et al., 1985). [15] Polyphenolic compounds have shown to inhibit toxic effects by snake venom proteins [16]. Pharmacodynamic properties of the ingredients of *Nagaraja Guliya* showed that *Katu Rasa* (Pungent

taste), *Laghu Guna* (Quality of lightness), *Ushna Veerya* (Hot in potency), *Katu Vipaka* (Pungent) and *Vata kapha shamaka* (Pacify Vata and Kapha dosha) [Table 6]. *Katu Rasa* cleaning the micro circulatory channels and stimulate the *Agni* (Digestive fire) which leads to enhance digestion and absorption.

Moreover, the vitiation of *dosha* by snake bite is different for different species of snakes. Cobra and Krait vitiate *Vata dosha*, Viper vitiate *Pitta dosha* and Cat snake vitiate *Kapha dosha*. In addition, Pharmacokinetic potentials showed. The ingredients of formula have *Kushtaghna*, *Vedanasthapana*, *Rakthashodhna*, *Shothahara*, *Vishahara*, and *Krimighna* properties [Fig. 34]. while, pharmacodynamics actions revealed that *Nagaraja Guliya* is having *Thridosha shamaka* properties.

5. CONCLUSION

Based on the current findings it is evident that Alkaloids, Saponins, Tannis and Phenols are reported in the *Nagaraja Guliya* (herbo mineral preparation) and it may serve as a rich source for treating venom poisoning either first aid or supplement of traditional antivenom. The ingredients of formula have *Kushtaghna*, *Vedanasthapana*, *Rakthashodhna*, *Shothahara*, *Vishahara*, and *Krimighna* properties. Further, Mercury and Arsenic content is not reached to the lethal dose of the humans. pH value of the formula is also within the normal range and acceptable to use the pill externally and internally.

6. RECOMMENDATIONS AND SUGGESTIONS

Further experimental, cytotoxic studies and clinical trials should be performed in order to prove the safety and efficacy of *Nagaraja Guliya* against snake bite.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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