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# Correlative study of heavy metal content with biological importance of *Solanum virginianum* leaf extract

Biswash Guragain, Khem Raj Pant, Saugat Bhattarai and Narendra Kumar Chaudhary\*

## Abstract

**Background:** Rapid urbanization and industrialization have greatly impacted the inherent soil composition. Heavy metals disposed in the environment by anthropogenic activities toxicate flora and ultimately affect the phytochemical profile of medicinal plants. We report here such an investigation of the heavy metal concentrations in the leaf extract of *Solanum virginianum* (*S. virginianum*). This work has been extended to observe the phytochemical constituents and antibacterial significance of leaf extracts in methanol and aqueous medium.

**Methods:** The metal concentration was analysed on ICE 3000 series atomic absorption spectrometer. The antibacterial assessment was carried by disc diffusion technique against three gram-negative (*Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*) and one gram-positive (*Staphylococcus aureus*) bacteria.

**Results:** The content of Iron (Fe), Manganese (Mn), Zinc (Zn), and Lead (Pb) were 2.04, 0.47, 0.41, and 0.10 mg/L, respectively. Saponin and coumarin were present in both extracts. Various other phytochemicals like steroids, terpenoid, and flavonoid, were present only in the water extract, while tannin was present only on methanol extract. The methanol and aqueous extracts exhibited their highest inhibition on *S. aureus* with zones of inhibition of 12 mm and 14 mm, respectively.

**Conclusion:** The aqueous extract possessed more phytochemicals than the methanol extract, and the aqueous extract exhibited better antibacterial activity. The high Fe content in the leaf extract may suggest its use as an anaemic medicine. Other metal contents are under the WHO range.

**Keywords:** *S. virginianum*, Plant extract, Heavy metals, Phytochemicals, Antibacterial evaluation

## Background

Nepal is a beautiful country nestled in the lap of the Himalayas, known for its diversity in mountains, hills, low land, greenery, rituals, tradition, ethnicity, climate, and many more. An untapped source of rich forest products with miraculous plants is its natural gift. The people here largely rely on the traditional use of flora as medicine and have very old practices in so [1, 2]. However with the considerable enhancement in the food processing techniques and the sedentary lifestyle, chronic and

degenerative non-communicable diseases are increasing. Since four decades, there has been a global increase in the number of outbreaks in casual diseases [3]. For such poor and developing countries, allopathic medicines are very expensive, and the relatively small group of people in the world have an optimal share with modern medicines [4]. The necessary impetus here is to explore our natural resources and generate the portfolio of the plants with aided scientific studies complementing the traditional uses and promoting the well-being of the people. The direction of herbal drugs is attractive for modern medicine [5]. With modern scientific tools and research designs, the isolation and study of metabolites has become possible and rapid. In treatment of almost every

\* Correspondence: [chem\\_narendra@yahoo.com](mailto:chem_narendra@yahoo.com)

Department of Chemistry, Bio-inorganic and Materials Chemistry Research Laboratory, Mahendra Morang Adarsh Multiple Campus, Tribhuvan University, Biratnagar 56613, Nepal

ailment, the share of drugs via natural products is substantial. Even in complicated ailments such as cancer, natural products have a good contribution. In a review of approved drugs for cancer between 1940 and 2014, 175 small molecules were approved, of which 75% were synthetic and 49% were other natural products or their directly derived products [6]. The effectiveness of the medicinal herbs lies in the phytochemicals present, which exhibit specific as well as diverse physiological actions. Phytochemicals are synthesized by plants to protect them against bacterial, fungal, viral, and free-radical damage [7]. Such secondary metabolites perform various functions in living systems, most of which are conducive to healthy living. Flavonoids, phenolic acids, and tannins are polyphenolic compounds offering significant advantages, including anti-aging, anti-inflammatory, and antioxidant, and even prevent the development of long-term diabetes complications. Alkaloids, terpenoids, and polyphenols are known for their antibacterial activities [8–10].

Most pharmacological procedures and treatments are dependent on antibiotics. The unradical use and inadequate synthesis of the new class of antibiotics have put entire chemotherapy at risk due to the rapid growth of superbugs. From development to approval of new antibiotics, only through synthesis is a very lengthy and daunting task. Plants are very attractive alternatives. To date, more than 1000 plant extracts have shown promising activity against several pathogens. Several studies have demonstrated their increased potency when coupled with antibiotics, reducing the uptake of antibiotics [11, 12]. Therefore, investigation of the antibacterial power of every plant is an urgent need. Contamination of drugs with heavy metals can cause adverse effects [13, 14]. Metals like K, Na, Fe, Mn, Mg, and Zn etc. play vital roles in several biochemical processes, and Hg, Cd, Pb, and As, etc. even at trace amounts pose deleterious effects on living systems. Rapid industrialization as well as geologic and anthropogenic activities, often increases the concentration of heavy metals in the soil. Some plants have the power to absorb metals from soil and water, and are efficient at reducing pollution. This technique is being accepted and praised for phytoremediation. Consumption of such plants can inadvertently transfer accumulated metals to body. Although a normal balance of biometals is desired, a higher concentration of essential metals also harms [15–17]. Based on the ADI values, the presence of Pb, Cd, and As in dried plant powders less than 10, 0.3, and 1 mg/kg are accepted in herbal medicines [18, 19].

In Nepal, *Solanum virginianum* L. (family *Solanaceae*) is waste-land vegetation (Fig. 1). The plant has long been used to treat coughs, asthma, toothache, hair loss, skin diseases, and respiratory diseases [20–22]. Secondary

metabolites like diosgenin and  $\beta$ -sistosterol were isolated in 1968 [23], Carpesterol in 1971 [24], and 11 more in 1973 [25]. Further, B2-Solamargine, Solamargine, Solasonine, solasodine, Caffeic acid, oleanolic acid are also reported [26–28]. Studies have shown antimicrobial, antiradical, and insecticidal properties of the plant [29]. Steroidal constituents present in the plant have shown potential for tumour cell death. A detailed study on powder plant extracts showed positive results for the treatment of bronchial asthma in hospitalized patients [30]. Furthermore, the fruit has shown hepatoprotective action against  $\text{CCl}_4$  induced [31] and antitubercular drug-induced [32] liver toxicity in rodents. The leaf extract exhibits good antidiabetic activity in alloxan-induced diabetic rats [33]. The phytochemical evaluation of the whole plant [34] and roots [35] revealed various therapeutically important secondary metabolites to a good extent. Since most of the remedial measures regarding the folklore and traditional use of this plant are supported by various scientific studies, it seems a useful plant.

*S. virginianum* upon cultivation is found to efficiently reduces the half-life of carbofuran residues from the soil, thereby do an efficient phytoremediation [36]. According to other studies at due, the heavy metal concentrations determination in plants is urgent. As known that the same plants at different geography could have different compositions of phytochemicals in them. Almost every other study on this plant has been conducted elsewhere. Further, overall studies of this plant have much been focused either only on the fruit part or on the whole plant. Only little studies are available on the roots and leaves. Therefore, the present study is to assess phytochemical screening, heavy metal concentration determination, and antibacterial assessment of the leaf part of the plant.

## Methods

### Plant collection

The reported plant was collected from the Sundarharai-municipality of Morang district in province 1, Nepal ( $26^\circ 40' 5.53''$  N,  $87^\circ 23' 7.12''$  E) during April 2019 (Fig. 2). It was authenticated from the Department of Botany, Mahendra Morang Adarsh Multiple Campus (Tribhuvan University), Biratnagar. The voucher specimen was deposited in the same department as the herbarium specimen. Healthy and mature plant leaves were selected for this study. The plant leaves were first washed thoroughly with tap water and then with distilled water to remove dust and dirt. The leaves were left to dry in shade under dust free environment to free them from dust and impurities and also to reduce contaminants.



**Fig. 1** *Solanum virginianum* plant displaying Leaves and Fruits

### Extraction

Extraction for phytochemical screening was carried out via the maceration technique [37]. The leaves were mechanically grinded into a grinder and obtained as powder. 10 g powder plant material in 100 mL triple distilled water was left for about 48 h with frequent stirring and finally centrifuged to obtain the crude extract. Similarly, 10 g powder plant material in 100 mL methanol was stirred overnight, and crude solutions were filtered using Whatman No.1 filter paper and stored in a sterile container at 5 °C for further use.

### Heavy metal concentration test

1.0 g dry and powder sample was taken in a 250 mL conical flask. 10 mL conc.HNO<sub>3</sub> was added to it and the mixture was evaporated on a hot plate until the brown fumes disappeared. The digested sample was dissolved in water, and the residue was rejected after filtration. It was then poured into a 100 mL of volumetric flask. The volume was made 100 mL by adding triple distilled water [38]. The quantification of metals in plant extracts was done by flame AAS technique outfitted in ICE 3000 series atomic absorption spectrometer at Nepal Batawaraniya Sewa Kendra Biratnagar.

### Preliminary phytochemical screening

Preliminary phytochemical screening of plant extracts was carried out according to standard procedures [39]. Bioactive compounds were analyzed by chemical tests to detect and validate their presence. Detailed analytical procedures are given in Table 1.

### Antibacterial assessment

The antibacterial evaluation of extracts was tested against four clinical strains of bacteria such as *E. coli*, *S. aureus*, *S. typhi*, and *P. aeruginosa* using the standard Kirby-Bauer paper disc diffusion method with

Muller-Hinton's agar media for bacterial growth [40]. The bacterial pathogens were collected in 2 mL tryptone soya broth and incubated for 2 h at 37 °C for better and complete growth. The incubated broth was swabbed over agar media in sterile Petri plates. Sterilized paper discs made of Whatman Paper No. 1 with 5 mm diameter size were first stuck over the media and plant extracts at different concentrations prepared with DMSO were loaded. A plain DMSO disk was used as a negative control and amikacin (30 µg/disc) was used as the standard reference. The Petri plates were incubated at 37 °C for 24 h and the diameter of the zone of inhibition was measured using the antibiogram zone measuring scale [41].

### Statistical analysis

The statistical analysis of antibacterial data was analysed using Origin 2017 version software program. The statistical results are expressed as mean ± SD ( $n = 3$ ). We ran one way ANOVA to test level of significance and differences between means were determined by running Tukey's test.  $P$  values < 0.05 were regarded as significant.

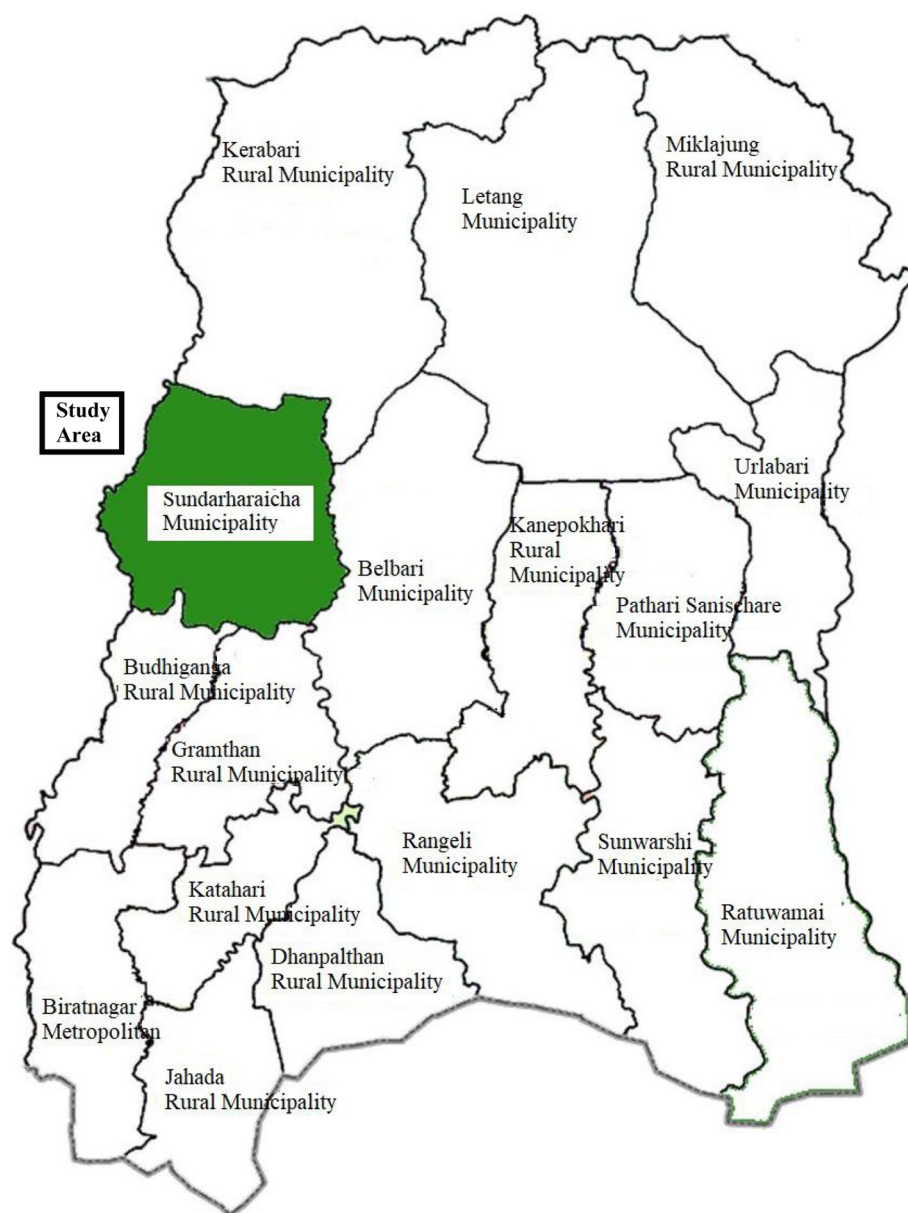
## Results

### Heavy metal concentration test

The metal concentration in the leaf extract of *S. virginianum* was determined, and the obtained values are illustrated in Table 2. It shows the presence of high Fe concentration (2.04 mg/L). The concentrations of other metals are Mn (0.47 mg/L), Zn (0.41 mg/L), and Pb (0.10 mg/L). Data reported here are from a single experiment. Metals like Cr, Co, Ni, and Cd were found at very low concentrations (< 0.05 mg/L).

### Phytochemical screening

Phytochemical screening revealed the presence of steroids, terpenoid, flavonoid, glycosides, saponins, coumarin,



**Fig. 2** Map of Morang district province 1, Nepal, showing study area (green shaded)

anthocyanins, and polyphenols in the water extract. Each test was conducted in triplicates for confirmation. Tannin was only observed in methanol extract, while saponin was found in both methanol and aqueous extracts. Alkaloids, carbohydrates, proteins, emodols, and glucosides were absent in both extracts. The phytochemical results are illustrated in Table 3.

#### Antibacterial assessment

The values of the diameter of the zone of inhibition of the tested bacterial pathogens at two different concentrations of extracts (100 µg/µL and 50 µg/µL) are tabulated in Table 4, and the graphical interpretation of the

data is presented in Fig. 3-4. The growth inhibition zone values of both extracts were quite similar and low, revealing the considerable antibacterial potency of plant leaves for the tested organisms. Moreover, growth inhibition was found greater for *P. aeruginosa* ( $p < 0.05$ ) comparing with growth inhibition zone of *E. coli* as standard. The means comparison plot of antibacterial data performed by Tuckey's test for different concentrations of extracts is presented in Fig. 5a-d.

#### Discussion

The concentrations of metals exhibited patterns as  $\text{Fe} > \text{Mn} > \text{Zn} > \text{Pb} > \text{Cr}$ , Co, Ni, and Cd. The values indicate



**Table 1** Preliminary phytochemical tests for plant extracts

S.N.	Metabolites	Test	Validation
1	Alkaloid	2 ml of extract + 2 ml of 2 N HCl + 2 drops of Wagner's reagent 2 ml of extract + 2 ml of 2 N HCl + 2 drops of Mayer's reagent	Reddish-brown precipitation validates the presence of alkaloid Gelatinous white precipitation validates the presence of alkaloid
2	Tanins and phenol	2 ml of extract + 2 ml water + 2–3 drops of 5% FeCl <sub>3</sub>	Green precipitate validates the presence of tannin. Purple solution validates presence of phenol.
3	Flavonoid	3 ml of extract + evaporated to dryness. Residue + 1–2 ml 50% CH <sub>3</sub> CH <sub>2</sub> OH, heat + 4–5 drops HCl + Mg ribbon	Red or orange color validates the presence of flavonoid.
4	Steroid (Salkowski Test)	2 ml extract + 2 ml CHCl <sub>3</sub> + 3 ml conc. H <sub>2</sub> SO <sub>4</sub>	• The reddish brown precipitate validates the presence of steroid.
5	Glycoside	2 ml extract + 2 ml CHCl <sub>3</sub> + 1–2 ml CH <sub>3</sub> COOH	Formation of violet or blue to green coloration validates presence of glycoside.
6	Saponin	2 ml extract + evaporated to dryness. Residue + 1 ml water + shaken vigorously.	The persistent foam (1 cm in the test tube) validates the presence of saponin.
7	Emodol	2 ml extract + 1–2 ml 25% NH <sub>3</sub> + Shaken.	A cherries red color indicates the presence of emodol
8	Coumarin	2 ml extract + 3 ml (10%) NaOH	Yellow coloration validates presence of coumarin.
9	Anthocyanin	2 ml extract + 2 ml (2 N) HCl + 1 ml NH <sub>3</sub>	Formation of pinkish red to bluish violet coloration validates presence of the anthocyanin.
10	Protein	2 ml extract + 1 ml (40%) NaOH + 1–2 drops 1% CuSO <sub>4</sub> solution.	Formation of violet color validates presence of protein.
11	Carbohydrate	<b>Fehling's test:</b> 1 ml extract + 2 ml water + 1 ml Fehling solution (1 and 2) + heated in a water bath <b>Molisch's test:</b> 1 ml extract + 5 ml distilled water + 2 drops alcoholic $\alpha$ -naphthol	A brick red precipitate validates presence of reducing sugar. violet ring at the junction validate the presence of carbohydrates
12	Terpenoids	2 ml extract + 2 ml CHCl <sub>3</sub> + 5 ml conc. H <sub>2</sub> SO <sub>4</sub>	Reddish-brown coloration validates the presence of terpenoids
13	Glucoside	2 ml extract + few drops conc. H <sub>2</sub> SO <sub>4</sub>	black coloration validates the presence of glucoside

that all metal concentrations are below the threshold marked by the WHO. The standard value of Fe content in the plant has not been established; however, the concentration of Fe (2.04 mg/L) is far more than the concentration of Cd. It has been found that high concentrations of Cd in plants cause a decrease in Fe concentrations [42]. The comparative data of Fe suggests good plant metabolism. Similarly Mn, another essential element, is useful in various metabolic processes like photosynthesis, respiration, and nitrogen

assimilation, etc. The value of Mn (0.47 mg/L) suggests that it has accumulated in plant parts by the use of acid-facilitating fertilizers [43]. The presence of Zn in plant parts reveals nothing extraordinary as it is an essential constituent of plant useful in metabolic and enzymatic processes [44]. The concentration of lead (0.10 mg/L) is relatively low in comparison with the threshold value provided by WHO standards (10.0), but it must be taken under consideration that the air expellant system of several industries, fuel combustion of vehicles expelled lead

**Table 2** Concentration of different heavy metal (mg/L) in a plant sample with WHO Standards

Metal	Concentration (mg/L)	mg/g sample	WHO Standard (mg/L)
Iron (Fe)	2.04	0.204	–
Manganese (Mn)	0.47	0.047	–
Zinc (Zn)	0.41	0.041	50.0
Lead (Pb)	0.10	0.01	10.0
Chromium (Cr)	< 0.05	< 0.005	0.02
Cobalt (Co)	< 0.05	< 0.005	–
Nickel (Ni)	< 0.05	< 0.005	1.63
Cadmium (Cd)	< 0.003	< 0.003	0.3

**Table 3** Phytochemical results of *S. virginianum* in methanol and aqueous extract

S.N.	Phytochemical tests	Methanol extract	Aqueous extract
1.	Steroid test	–	++
2.	Terpenoids test	–	+
3.	Alkaloid test	–	–
	Wagner's test	–	–
	Mayer's test	–	–
4.	Flavonoid test	–	+
5.	Tannin test	+	–
6.	Glycoside test	–	+
7.	Carbohydrate test		
	Molisch's test	–	–
	Fehling's test	–	–
8.	Protein test	–	–
9.	Saponin test	+	++
10.	Coumarin test	+	++
11.	Emodol test	–	–
12.	Anthocyanin test	–	+
13.	Phenolic test	–	+
14.	Glucoside test	–	–

++ Present, – Absent

in air, which may be deposited on the leaf. A similar study in the same plant collected from different places in Pakistan reported a comparatively higher content of Zn, Cd, Pb, and Fe compared to those metals reported in this study [34]. Co, Ni, and Cr are all trace metals found low in concentration than their relative standards; however, the concentration might vary from site to site. The accumulation of pollutants from various sources through air, soil, water, etc. plays an important role in the buildup of metals in the plant.

The phytochemical screening results showed a significant extraction of chemicals by water (~ 13% yield) over methanol (~ 8% yield). It is interesting to report six chemicals, viz. steroids, terpenoid, flavonoid, glycosides, anthocyanins and phenols that were detected only on the aqueous extract of leaf. Glucoside, carbohydrate, emodol, protein, and alkaloids were absent in both extracts of the

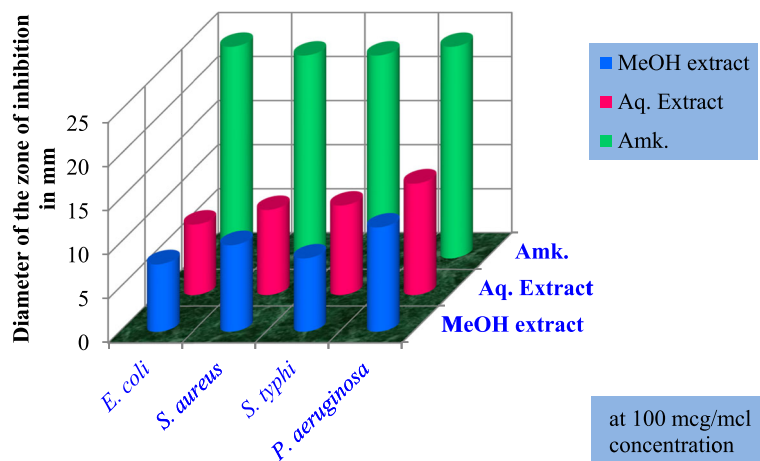
leaf part of *S. virginianum*, as shown in Table 3. In the root extract of the same plant, alkaloids and flavonoids were found in both extracts, while glycosides were not reported at all. In a whole plant study conducted in Pakistan, alkaloids, saponins, and flavonoids were found to a good extent [35]. But with plants collected from three different places of Pakistan, crude alkaloids present are 9.4%, 7.6%, and 2.4% [34]. This variance shows the effect of environmental conditions on the yield of phytochemicals. According to Lin et al. (2016), phenolic compounds are usually associated with defence responses in plants. Therefore, we can say that plants produce phytochemicals as per their need to respond to stimuli imposed by the environment [9]. These phytochemicals are very important because of their usefulness in a number of complex systems. With no claim of completeness, we report here the uses of phytochemicals present in the leaves of *S. virginianum*. Tannin has a tendency to play an important role as a natural corrosion inhibitor [45] and antifungal agents [46]. Saponin is widely used as an antifungal agent, insecticidal, antihelminthic, and anti-inflammatory agents [47]. Terpenoids bearing constituents are used as antibacterial, antimicrobial, antitumor, anti-inflammatory [48], and natural products for anti-cancer therapy [49]. Coumarin has been found to play a significant role as an antioxidant agent in mammalian cells [50, 51]. Phenol-bearing compounds have efficient antipyretic and analgesic activities [52]. Anthocyanin and its metabolites have a broad use as therapeutic agents [53]. All the phytochemical components suggest that *S. virginianum* would be fruitful for humans as different agents under proper conditions if applied. No adverse effects were reported in patients with mild to moderate asthmatic symptoms during a study administering 300 mg of powdered areal part of *S. virginianum* per day, but patients greatly improved after the 3rd day [30].

The in vitro antibacterial sensitivity against three clinical strains of gram-negative bacterias (*E. coli*, *S. typhi*, *P. aeruginosa*) and one-gram positive bacteria (*S. aureus*) were studied at two different concentrations (100 and 50 µg/µL). The diameter of the zone of inhibition in Figs. 2 and 3 suggested a lower antibacterial activity than that of the control drug. These inhibition data exhibited the

**Table 4** Antibacterial growth inhibition data

Plant Extract	The diameter of the zone of Inhibition in mm							
	<i>E. coli</i>		<i>S. aureus</i>		<i>S. typhi</i>		<i>P. aeruginosa</i>	
Conc <sup>n</sup> (µg/µL)	100	50	100	50	100	50	100	50
Methanol	7.66 ± 0.57	6.16 ± 0.23	9.83 ± 0.76*	8.33 ± 0.57**	8.33 ± 0.57	7.5 ± 0.5	11.83 ± 0.76**	9.3 ± 0.57*
Aqueous	8 ± 0	6.66 ± 0.57	9.66 ± 0.577**	8.5 ± 0.86	10.16 ± 0.76	8.16 ± 0.288	12.66 ± 0.577*	9.66 ± 0.577**
Amikacin	24		23		23		24	

Numbers connote mean ± SD (n = 3) \*p &lt; 0.01, \*\*p &lt; 0.05



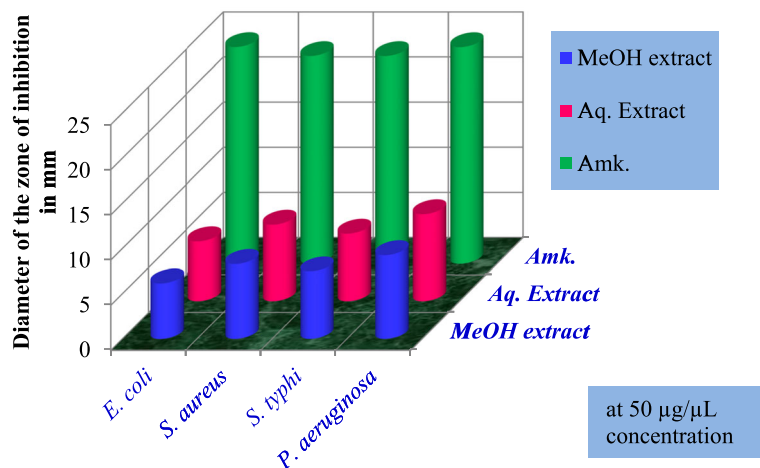
**Fig. 3** Bar graph of antibacterial sensitivity of leaf extract at 100  $\mu\text{g}/\mu\text{L}$  concentration

justifiable efficiency of the extracts against the pathogens [11]. The non-involvement of the solvent was evidenced by the nil inhibition data of DMSO. The extracts in two different media exposed significant variation of antibacterial properties with the greater in vitro effect of the aqueous extract, and as such, the presence of phytochemicals differed. The phytochemicals are organic compounds which have several donor atoms to provide binding site for cell components of organisms and show their antibacterial activity. The phytochemicals also have capacity to form temporary complex when metal ions approach to it. With this phenomenon, the lipophilicity of complex for target nuclear materials (RNA) of organisms goes enhanced which may be the cause of enhanced antibacterial sensitivity [54]. The aqueous extracts possess most of the phytochemicals and metals in good ratio, because of which we observed increased antibacterial sensitivity relative to methanol extract.

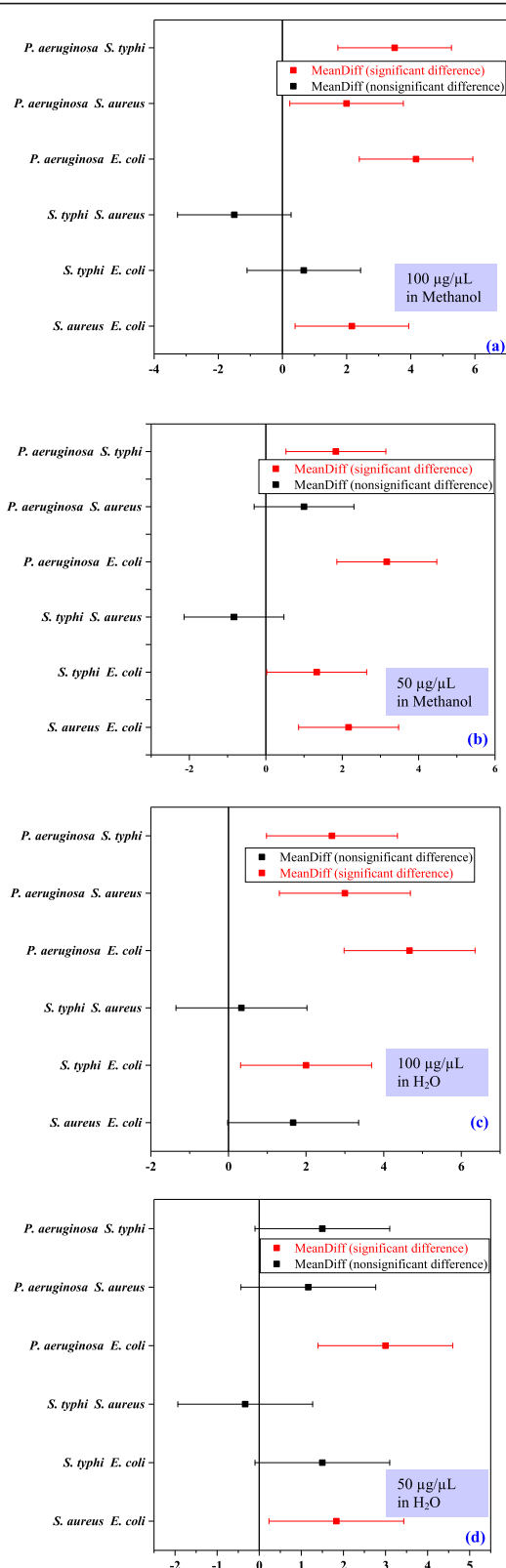
From this data, we can surely claim that the antibacterial activity of plant extracts is due to the combined effect of different phytochemicals rather than a single unit. And that the studies involving various solvent media have their significance, and should be planned with significant differences in polarity.

### Conclusion

The key point of this study is the metal concentration determination, and the study plays an imperative role in forming an image by comparing similar statistics with other research. Nepal is a developing country that cannot afford a high and facilitated extent of research due to various restrictions, and we are far off the development of FDA-approvable drugs comprising extensive studies. Therefore, the documentation of our traditional medicines with the exploration of unexamined natural products is the current necessity. Phytochemical



**Fig. 4** Bar graph of antibacterial sensitivity of leaf extract at 50  $\mu\text{g}/\mu\text{L}$  concentration



**Fig. 5** Mean comparison plot of antibacterial data by Tukey's test for (a) 100 µg/µL in Methanol (b) 50 µg/µL in Methanol (c) 100 µg/µL in H<sub>2</sub>O (d) 50 µg/µL in H<sub>2</sub>O

examination showed that *S. virginianum* leaf extract contained a blend of phytochemicals like steroids, terpenoid, flavonoid, glycosides, coumarin, and anthocyanins etc., and possessed satisfactory antibacterial activity. The study of metal concentration revealed the present level of metals below the alarming range. It is an important pollution indicator and needs frequent inquiry.

#### Abbreviations

WHO: World Health Organization; AAS: Atomic Absorption Spectroscopy; ADI: Acceptable daily intake; FDA: Food and Drug Administration; DMSO: Dimethyl Sulphoxide

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#### Authors' contributions

BG and KM performed the study. BG and SB prepared manuscript. NKC supervised the work and finalize manuscript. All authors have read and approved the manuscript.

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#### Availability of data and materials

All the data generated and analyzed during the study are included in the manuscript and are available for the readers.

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that there are no competing interests regarding the publication of this paper.

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