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RESEARCH ARTICLE

Phytochemical Analysis and Bioactivity Evaluation of Root Extract of *Selinum tenuifolium* Wall. from Nepal

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Abstract

Since ancient time, the herbal medicinal plants have been widely used in the treatment of various ailments due to fewer or no side effects, cheap, and easy availability. This study aimed to evaluate the phytochemical screening, antioxidant, antibacterial, and anticancer activity of *Selinum tenuifolium* root methanol extract (STRM). Based on our information, this is the first study evaluating its anticancer activity. The antioxidant, antibacterial, and anticancer activities were determined by DPPH, agar plate well diffusion method, and MTT assay, respectively. The preliminary phytochemical screening of STRM revealed the presence of various phytochemicals like alkaloids, glycosides, terpenoids, quinone, and carbohydrate. STRM showed dose-dependent moderate antioxidant activity (102.18 µg/mL IC_{50} value) ($P < 0.05$). STRM showed dose-dependent active antibacterial activity against *S. aureus* and no antibacterial activity against *S. flexneri, E. coli, P. aeruginosa,* and *K. pneumoniae* at 50 mg/mL concentration. The plant extract (STRM) showed dose-dependent active antibacterial activity against *S. aureus* with 9 mm ZOI at 25 mg/mL to 18 mm ZOI at 200 mg/mL concentration. The anticancer activity of STRM was dose-dependent moderate against HeLa (188.01 µg/mL IC_{50} value) cancer cells ($P < 0.05$). Overall, STRM showed dose-dependent moderate antioxidant, active antibacterial, and moderate anticancer activity due to the synergistic effect of different phytochemicals present in the plant extract (STRM).

Keywords: *Selinum tenuifolium,* Phytochemicals, Antioxidant, Antibacterial, Anticancer

Introduction

Traditional medicinal system uses the medicinal plants, their parts, and products as the primary source of medicine. In traditional medicinal system, an estimated 35,000 - 70,000 plant species are used worldwide. Of these, about 6,500 species in Asia, and at least 1,600 - 1,900 species in Nepal are commonly used. Herbs are most commonly used in traditional medicinal system, followed by shrubs, trees, and climbers [1]. Many medicinal plants are still unexplored and proper scientific research on these medicinal plants could help in the search of new compounds having potential medicinal value. *Selinum tenuifolium* Wall. Ex C. B. Clarke (synonyms: *Selinum wallichianum* (DC.) Raizada & H. O. Saxena and *Ligusticopsis wallichiana* (DC) Pimenov & Kljuykov) belongs to the Apiaceae family (Umbelliferae), commonly known as 'Bhutkesh' in Nepal [2 - 5]. It is perennial aromatic herb and widely distributed in temperate and alpine zone at the elevation range of 2600 to 4800 meter of the Himalaya region from Kashmir to Nepal and Bhutan, North East India, China etc [2 - 4]. It is a perennial, endemic, high altitude, hairy, rhizomatous, endangered, primitive, and therapeutic herb [5]. A decoction of *Selinum tenuifolium* root is febrifuge and analgesic [2]. *S. wallichianum* (DC) whole plant is used as an incense and therapeutics in common ailments like cold, cough, fever, wounds, stomachache and toothache etc [6]. Traditionally, *Selinum*

tenuifolium is used for various diseases such as epilepsy, seizures, hysteria etc. Roots and leaves are the main plant parts used in treatment of various ailments such as diarrhea, cuts, wounds, swelling, vomiting etc [5]. Oil derived from *S. wallichianum* roots has leucodermal, hypotensive, incense, analgesic, and sedative properties. The whole plant or roots are used as a nervine sedative. [7]. *S. tenuifolium* Wall. root is used in hypotension and sedative [8]. *S. tenuifolium* Wall. root is used in cuts, wound, stomachache, and vomiting. The mixture of root powder and mustard oil is used to cure swelling which develops after delivery in women [9]. *S. tenuifolium* root decoction is useful in diarrhea, cuts, wounds and vomiting. The mixture of root powder with mustard oil is used to cure swelling [10]. *S. tenuifolium* roots is used to treat body pain, fever, cough and cold [11]. The smoke produced from *S. tenuifolium* roots is used for killing or repelling insects [12].

The present research study was accompanied for the purpose of phytochemical screening and to evaluate antioxidant, antibacterial, and anticancer activities of *S. tenuifolium* root methanol extract (STRM). To the best of our knowledge, this is the first study evaluating the anticancer activity of *S. tenuifolium* root methanol extract (STRM).

Materials and Methods

The schematic outline of this research study is shown in **Figure 1**.

Plant Collection and Identification

The plant sample was collected from Tinjure Danda, Tehrathum District, Nepal in May 2019. The plant sample was identified as *Selinum tenuifolium* Wall. by National Herbarium and Plant Laboratories (KATH), Godawari-3, Lalitpur, Nepal, on September 16, 2019 AD as a plant herbarium specimen no. 8.

Plant Material and Extraction

The plant extraction was performed using methanol solvent by following the standard protocol of the references [13 - 14]. The plant root was first cleaned, shade dried, and the dried plant root was ground into fine powder. The plant extraction was done using methanol solvent by cold maceration process in a closed vessel for three days at room temperature. Then, the extract was collected. This process was repeated three times consecutively for the same plant material. The extract was filtered using Whatman filter paper No. 1. The filtered extract was concentrated below 40°C using a rotary evaporator (BIOBASE RE-2000B Rotary Evaporator, China) and then, dried using freeze dryer. Then, the extract was stored in a closed vial at 4 ℃ for further analysis.

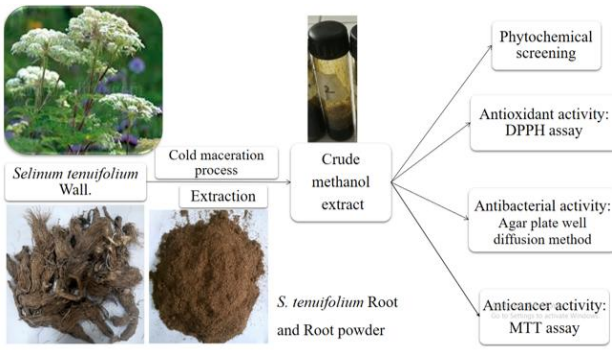

Figure 1: Schematic outline of the research study

Phytochemical screening

The preliminary phytochemical screening of the plant methanol extract (STRM) was carried out by following the standard protocol described in the references [14 - 20].

Antioxidant Assay

The antioxidant activity of plant methanol extract (STRM) was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay using 96-well plates [14, 20 - 23]. The experiment was done in triplicate.

Firstly, 100 µL of STRM of different concentrations (10, 15, 20, 30, 40, 50, 100, and 200 µg/mL) were taken in all triplicate wells, and then 100 µL of DPPH solution (39.4 µg/mL) was added in each well. The mixture was placed in the dark for 30 mins at room temperature. Then, an absorbance reading was taken at the 517 nm wavelength in the Biotek epoch micro plate reader. Ascorbic acid (7.5, 5, 3.75, 2.5,

1.875, 1.25, and 0.9375 µg/mL) was used as the positive control and methanol as the negative control.

The antioxidant activity result was recorded as an IC_{50} value (sample concentration that decreases the initial DPPH concentration by 50%). The DPPH free radical scavenging activity was calculated using the following formula.

$$\%\ \text{radical scavenging} = \left(\frac{A_o - A_t}{A_o}\right) \times 100\ \%$$

Where, A_0 = Absorbance of Methanol with DPPH solution

A_t = Absorbance of Test samples or Positive Control

Antibacterial Activity: Agar Plate Well Diffusion Method

The antibacterial potency of the plant extract (STRM) was evaluated against five bacterial strains. They were provided by Department of Biotechnology, Kathmandu University, Nepal. Namely, one bacterial strain of Gram positive: *Staphylococus aureus* (ATCC: 12600) and four Gram negative: *Escherichia coli* (ATCC: 8739), *Shigella flexneri* (ATCC: 12022), *Klebsiella pneumoniae* (ATCC: 13883), and *Pseudomonas aeruginosa* (ATCC: 10145) were taken. Standard antibiotic namely Tetracycline (30 µg/disc) was used as a positive control and DMSO (0.1 %) as a negative control.

The agar plate well diffusion method was performed following the standard protocol of references [14, 20, 23 - 26]. The Mueller Hinton agar (MHA) was dissolved and autoclaved at 121°C for 15 min and cooled up to 45°C. Then, 40 - 50 mL media was poured in a sterile Petri plate, allowed to solidify, and kept at room temperature.

Preparation of Plant Extract Solutions

The plant extract (STRM) solutions were prepared in DMSO for antibacterial test. The volume 30 µL of 50 mg/mL plant extract (STRM) was taken for the preliminary test. The bacteria which were susceptible to plant extract (STRM) were taken for antibacterial susceptibility test. The different concentrations of the plant extract (STRM) in DMSO such as 25 mg/mL, 50 mg/mL, 100 mg/mL, and 200 mg/mL were prepared for antibacterial test.

Preparation of Inoculums

The bacterial strains were sub-cultured overnight at 37°C in Nutrient Broth medium. The bacterial growth was harvested and diluted to attain a viable cell count of 0.5 McFarland (1.5×10^8 CFU/mL) for bacteria using a spectrophotometer.

Inoculation

For inoculation, swabbing was done with the help of sterile cotton. The volume 30 µL of the plant extract (STRM) solution with different concentrations and DMSO (0.1%) were added to the wells of the plate; incubated at 37 °C for 24 h. After incubation, the diameter of the Zone of inhibition (ZOI) was measured.

Anticancer Activity

The anticancer activity of plant extract (STRM) against human cervical cancer cells (HeLa) was evaluated by MTT colorimetric assay in 96-well plates following the standard protocol of references [13, 14, 19, 20, 22, 27, 28].

Cell Culture

Department of Biotechnology, Kathmandu University, Nepal, provided human cervical cancer cells (HeLa) (ECACC: 93021013), which was acquired from Shikhar Biotech Pvt. Ltd., Khumaltar Height, Ward No. 15, Lalitpur, Nepal. The HeLa cancer cells were cultivated in DMEM medium (Dulbecco's Modified Eagle Medium) (Gibco, USA) supplemented with an 10% fetal bovine serum (FBS) (HIMEDIA) and 1% antibiotics (penicillin & streptomycin) (HIMEDIA) at 37°C in a humidified 5% CO_2 incubator until 80% confluence. The confluency was observed under an inverted microscope and subcultured at three to four days interval.

***In Vitro* Cell Viability Assay: MTT Assay**

After 80% confluence, media was discarded, and the cells were washed with phosphate

buffer saline (PBS). The cells were trypsinized and suspended in new media. Then, the cell counting was done using a hemocytometer. 1×10^4 cells were seeded in 96-well plates and incubated for 24 hrs. After 24 hrs, media from the well was removed and treated by different concentrations (25 - 400 µg/mL) of STRM in media. The negative control was DMSO (0.1 %). Then, the cells were incubated for 48 hrs. After 48 hrs, media were discarded, and 100 µL MTT (0.5 mg/mL) in media was added and incubated for 4 hrs. After 4 hrs incubation, MTT dye will be converted to formazan, which is water insoluble. Then, the media was removed and replaced with 100 µL DMSO in each well to dissolve formazan. The absorbance reading was taken in a microplate reader (Thermo Scientific MULTISKAN GO) at 570 nm wavelength. The experiment was done in triplicate.

The cytotoxicity results were recorded as IC_{50} value (concentration causing 50% growth inhibition for the cell lines). The cell viability was calculated by the following formula,

$$\%\ \text{Cell viability} = \left(\frac{A_t}{A_o}\right)\times 100\,\%$$

Where,

A_o = Absorbance of the cell line without plant extract treatment

A_t = Absorbance of the cell line with plant extract treatment

Statistical Analysis

The experiments were performed in triplicate. The results were analyzed by Microsoft Excel and Origin 7.5 and expressed as mean ± standard deviation of mean determined. The statistical significance of differences between test groups was analyzed by one-way analysis of variance (ANOVA: Single Factor). The differences were considered statistically significant where *P* values < 0.05.

Results

Phytochemical screening

The qualitative phytochemical screening of *Selinum tenuifolium* root methanol extract (STRM) revealed the presence of various phytochemicals like alkaloids, glycosides, terpenoids, quinone, and carbohydrate but absence of tannins, flavonoids, polyphenols, saponins, steroids, and Resin. The results are shown in (**Table 1**).

Table 1: Results of phytochemical screening of *Selinum tenuifolium* (STRM)

Phytochemicals	*Selinum tenuifolium*
Alkaloids	+
Steroids	-
Glycosides	+
Polyphenols	-
Tannins	-
Flavonoids	-
Terpenoids	+
Quinone	+
Saponins	-
Resin	-
Carbohydrate	+

Antioxidant Activity

The percentage scavenging activity and IC_{50} values of *Selinum tenuifolium* (STRM) as a sample and ascorbic acid as a standard were evaluated by plotting the regression line curve using Origin 7.5 and Microsoft Excel. The antioxidant IC_{50} value 102.18 µg/mL of STRM was calculated from the standard calibration curve (R^2 = 0.99931) shown in **Figure 2**.

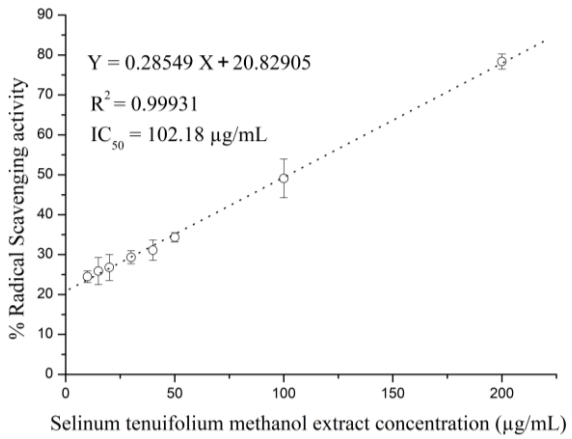

Figure 2: Determination of antioxidant activity (IC_{50} value) in STRM using standard calibration curve by DPPH free radical scavenging assay (*Significant differences are indicated by P < 0.05 as compared with control.)

Table 2: Antioxidant and anticancer activity (IC_{50} value) of *Selinum tenuifolium* (STRM)

Activity	IC_{50} value (µg/mL)	
	STRM	Ascorbic acid
Antioxidant activity	102.18	4.76
Anticancer activity	188.01	

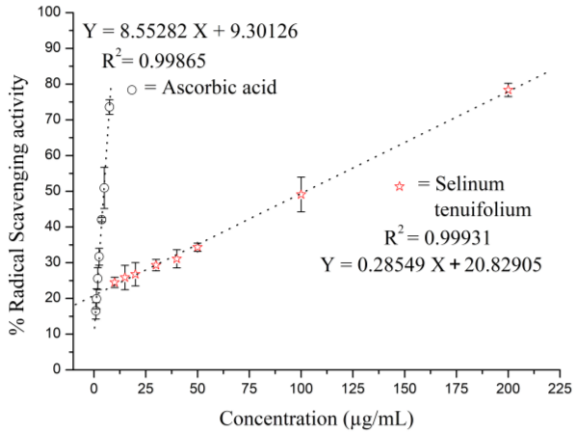

Figure 3: Comparison of percentage radical scavenging activity between ascorbic acid and STRM (*Significant differences are indicated by P < 0.05 as compared with control.)

The sample *Selinum tenuifolium* (STRM) exhibited concentration dependent scavenging activity of 24.48 ± 1.44% at 10 µg/mL and 78.37 ± 1.87% at 200 µg/mL with 102.18 µg/mL IC_{50} value ($P < 0.05$) shown in **Table 2** and **Figure 2**. The standard ascorbic acid showed concentration dependent scavenging activity of 16.51 ± 2.27% at 0.9375 µg/mL and 73.58 ± 2.06% at 7.5 µg/mL with 4.76 µg/mL IC_{50} value ($P < 0.05$). The sample *Selinum tenuifolium* (STRM) exhibited moderate antioxidant activity.

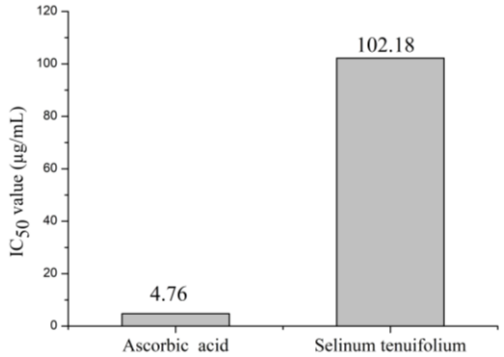

Figure 4I The comparison of antioxidant activity (IC $_{50}$ value) of ascorbic acid and STRM

Figure 3 shows the comparison of percentage radical scavenging activity of STRM with a standard ascorbic acid. **Figure 4** shows the comparison of IC_{50} value for antioxidant activity of STRM with ascorbic acid. The antioxidant activity of the plant extract is considered as very strong (IC_{50} < 50 µg/mL), strong (IC_{50}: 50 – 100 µg/mL), moderate (IC_{50}: 101 – 250 µg/mL), weak (IC_{50}: 250 – 500 µg/mL) and inactive (IC_{50} > 500 µg/mL) [29].

Antibacterial Activity

For antibacterial activity study, five pathogenic bacterial strains namely *Staphylococcus aureus, Shigella flexneri, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae* were taken. *Selinum tenuifolium* methanol extract (STRM) concentration of 50 mg/mL was tested against these bacterial strains for preliminary antibacterial screening. It inhibited the growth of *S. aureus,* but did not inhibit other bacteria shown in **Table 3**. Then, the concentrations of *Selinum tenuifolium* methanol extract, STRM (25, 50, 100, and 200 mg/mL) were taken for the antibacterial test against *S. aureus*. The plant extract (STRM) showed dose-dependent active antibacterial activity against *S. aureus* with 9 mm ZOI at 25 mg/mL to 18 mm ZOI at 200 mg/mL concentration shown in **Table 3 and Figure 5**.

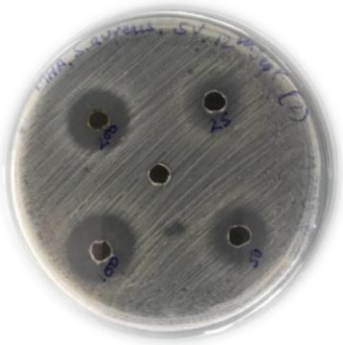

Figure 5: Antibacterial activity test of *Selinum tenuifolium* root methanol extract (STRM) against *S. aureus* at different concentrations (25, 50, 100, and 200 mg/mL) by agar plate well diffusion method

The standard tetracycline (30 µg/disc) showed the highest effect against *S. aureus*

with 30 mm ZOI, followed against *S. flexneri* (27 mm ZOI), *E. coli* (25 mm ZOI), K. *pneumoniae* (20 mm ZOI), and *P. aeruginosa* (14 mm ZOI) shown in **Table 3**. The results of the antimicrobial activity in terms of ZOI were expressed as sample is inactive (ZOI < 9 mm), partially active (ZOI = 9 – 12 mm), active (ZOI = 13 – 18 mm), and very active (ZOI > 18 mm) [26, 30].

Anticancer activity

The percentage cell viability and IC_{50} values of anticancer activity of *Selinum tenuifolium* (STRM) were evaluated by plotting the regression line curve using Origin 7.5 and Microsoft Excel. The anticancer IC_{50} value 188.01 µg/mL of STRM was calculated from the standard calibration curve (R^2 = 0.99094) shown in **Figure 6**.

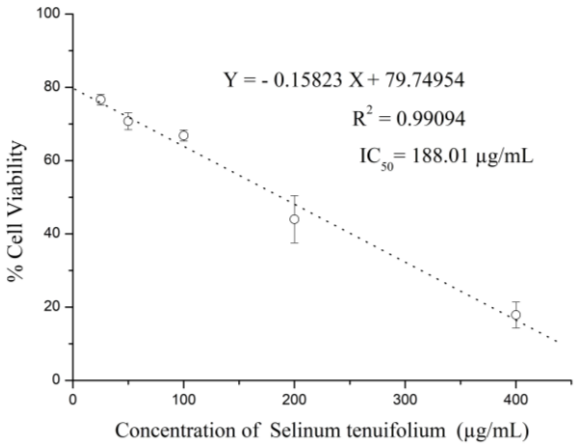

Figure 6: Determination of anticancer activity (IC_{50} value) in STRM using standard calibration curve by MTT colorimetric assay (*Significant differences are indicated by P < 0.05 as compared with control.)

Table 3: Antibacterial activity of *Selinum tenuifolium* methanol extract (STRM)

Bacteria	Preliminary antibacterial screening of STRM	Zone of Inhibition (ZOI)				
		Tetracycline (30 µg/disc)	STRM concentrations (mg/mL)			
			25	50	100	200
S. aureus	+	30 mm	9 mm	14 mm	17 mm	18 mm
S. flexneri	-	27 mm				
P. aeruginosa	-	14 mm				
E. coli	-	25 mm				
K. pneumoniae	-	20 mm				

Selinum tenuifolium (STRM) showed dose-dependent anticancer activity against human cervical cancer cells (HeLa) analyzed by *in vitro* MTT assay and *in vitro* IC_{50} value was shown in **Table 2**. *Selinum tenuifolium* (STRM) showed the cell viability 76.67 ± 1.41% at 25 µg/mL and 17.87 ± 3.53% at 400 µg/mL against HeLa human cervical cancer cells shown in **Figure 7** with 188.01 µg/mL IC_{50} value as shown in **Figure 6**. *Selinum tenuifolium* (STRM) showed moderate anticancer activity against HeLa. The anticancer activity of the plant extract is considered as potential (IC_{50} < 100 µg/mL), moderate (100 µg/mL < IC_{50} < 1000 µg/mL), and nontoxic (IC_{50} > 1000 µg/mL) [31].

Discussion

Selinum tenuifolium root methanol extract showed the presence of polyphenols, glycosides, quinines, anthracyanosides, flavonic glycosides, and coumarins [32]. However, the presence of polyphenol and flavonoid was not observed in this study. Among the phenolic compounds from the roots of *Ligusticopsis wallichiana*, 3, 5-di-*O*Caffeoyl-quinic acid, chlorogenic acid, ferulic acid, and 5-*O*-*p*-coumaroyl-quinic acid showed potent antioxidant activity with IC_{50} values being 39, 89, 127, and 189 µM, respectively [4].

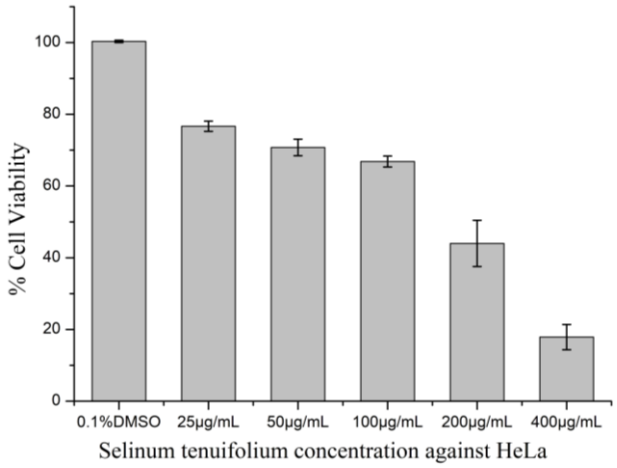

Figure 7: Comparison of percent cell viability shown by STRM against HeLa cancer cells (ECACC-93021013) with 0.1% DMSO (as negative control) analyzed by *in vitro* MTT assay (*Significant differences are indicated by P < 0.05 as compared with control

Selinum tenuifolium root methanol extract showed no antibacterial activity against all tested bacteria namely *B. subtilis*, *S. aureus*, *E. coli*, *S. typhi*, *S. paratyphi, P. aeruginosa*, *E. faecalis*, *S. species*, *A. species*, *P. mirabilis*, and *K. pneumoniae*. It showed partially active antifungal activity against all tested fungal strains namely *F. oxysporum*, *F. moniliformie*, *F. proliferatum*, *E. turticum*, and *S. rolfsii* [32]. *S. tenuifolium* Wall. root showed anti-bacterial properties [12]. *Selinum tenuifolium* root oil has antibacterial property [33]. The earlier study reported the antibacterial activity of *S. wallichianum* root ethanol extract and its aerial parts essential oil showed potent antimicrobial activity [6]. The earlier studies reported the different phytochemical compounds in *Selinum tenuifolium* root. The essential oil from *Selinum tenuifolium* root exhibits five major polyacetylene compounds namely nona-3,5-diyne, nona-3,5-diyn-2-one, nona-4,6-diyn-3-one, nona-3,5-diyn-2-ol, and nona-4,6-diyn-3-ol [12]. *Selinum tenuifolium* root oil exhibits the presence of limonene, elemol, terpineol, geraniol, and eudesmol as the major constituents and small amounts of *α*-pinene, 5-nonen-3-yne, and l-nonen-3,5-diyne [33]. Three furocoumarms namely bergapten, heraclenin, and heraclenol were isolated from light petrol and benzene extract of *Selinum tenuifolium* root [34]. Two new diacetylene glycosides: bhutkesoside A and bhutkesoside B, along with 10 known compounds, i.e. falcarindiol, chlorogenic acid, 5-*O*-*p*-coumaroyl-quinic acid, 3,5-di-*O*-caffeoyl-quinic acid, 4-hydroxy-7-methoxy-phenylethanol, ferulic acid, dehydrodiconiferyl alcohol-4-*O*-*β*-d-glucopyranoside, 5,7-dihydroxy-methyl-chromone-7-*O*-rutinoside, schumannio-fioside B, and marmesinin were isolated from the roots of *Ligusticopsis wallichiana* (DC) Pimenov & Kljuykov [4].

The previous studies reported the different pharmacological properties of phytochemical compounds namely geraniol, terpineol, limonene, eudesmol, bergapten, falcarindiol, ferulic acid, and chlorogenic acid. Ferulic acid present in *Selinum tenuifolium* root. Geraniol, an acyclic isoprenoid monoterpene, has various biological activities such as anticancer, anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, cardioprotective, neuro-protective effects etc [35]. α-Terpineol, a monocyclic monoterpenoid tertiary alcohol with lilac-like aroma, has various biological activities such as antioxidant, anti-inflammatory, antimicrobial, anticancer, analgesic, gastroprotective, cardioprotective, neuroprotective, antidiarrheal effects etc [36]. Limonene, a monoterpene and the main component of citrus essential oil, has many pharmacological effects such as antibacterial, anticancer, analgesic, immune regulation, neuroprotection, antioxidant, anti-inflammatory properties, the treatment of metabolic diseases etc [37]. β- Eudesmol, a sesquiterpenoid alcohol, has potential antitumor and antiangiogenic activities [38]. Bergapten (5-methoxypsoralen), a natural furocoumarin compound, has neuroprotection, organ protection, anticancer, anti-inflammatory, antimicrobial, antidiabetes

effects etc [39]. Falcarindiol, a polyacetylene widely distributed within the Apiaceae family, has antimicrobial and cytotoxic activity [40]. Chlorogenic acid, a polyphenol compound, has different pharmacological functions such as neuroprotection, anti-inflammation, anti-oxidation, anti-pathogens, antitumor activities, mitigation of cardiovascular disorders, skin diseases, diabetes mellitus, liver and kidney injuries, etc [41]. Ferulic acid, a phenolic compound, has different biological activities such as antioxidant, anti-inflammatory, antimicrobial, anticancer properties etc [42].

The medicinal plants have different phytochemicals, which have various pharmacological properties such as analgesic, antimicrobial, anti-inflammatory, anticancer, antioxidant activities etc [43, 44]. The major phytochemicals responsible for anticancer activity are alkaloids, terpenoids, saponins, tannins, and polyphenols [45]. The plant alkaloids have antibacterial, antiviral, antifungal, anticancer, antioxidant activities etc [44]. The plant terpenoids have many pharmacological properties like analgesic, antibacterial, antifungal, anti-inflammatory, antineoplastic activities etc [46]. The difference in antioxidant, antibacterial, and anticancer activities of same plant is due to the variation in the phytochemical composition of plant extracts, which depends on different factors such as altitude, seasons, age of plants, different plant parts, environmental conditions during growth, extraction methods, polarity of solvents used, storage conditions, etc [14, 47 - 50].

Conclusion

The preliminary phytochemical screening of STRM revealed the presence of various phytochemicals like alkaloids, glycosides, terpenoids, quinone, and carbohydrate. STRM showed dose-dependent moderate antioxidant activity. STRM showed dose-dependent active antibacterial activity against *S. aureus* and no antibacterial activity against *S. flexneri, E. coli, P. aeruginosa,* and *K. pneumoniae*. The anticancer activity of STRM was dose-dependent moderate against HeLa cancer cells. Overall, *Selinum tenuifolium* root methanol extract (STRM) showed dose-dependent moderate antioxidant, active antibacterial, and moderate anticancer activity due to the synergistic effect of different phytochemicals present in the plant extract (STRM). The isolation of active bioactive compounds and their *in vitro* and *in vivo* biological tests could be a future research.

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Author's Contribution Statement

R. K. Shrestha: Plant collection, Methodology, Data analysis, Statistical analysis, and Writing: original manuscript, Writing: review & editing, **P. S. Maharjan:** Plant collection, Methodology, and Writing: review & editing. **B.G. Shrestha**: Conceptualization, Supervision, and Writing: review & editing.

Conflict of Interest

The authors do not have any conflict of interest throughout this research work.

Data Availability Statement

The data supporting this study's findings are available from the corresponding authors upon reasonable request.

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