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Evaluation of Phytochemicals and Antibacterial Activity of Indigenous Medicinal plants: *Amomum subulatum* Roxb. (F.), *Astilbe rivularis* Buch.-Ham. ex D.Don (R.) and *Swertia chirayita* (Roxb.) H. Karst (L.) of Dhankuta, Eastern Nepal

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ABSTRACT

This research evaluated the phytochemical composition and antibacterial activity of commonly available indigenous medicinal plant species of Dhankuta, Eastern Nepal. Different plant parts: fruit of *Amomum subulatum*, rhizome of *Astilbe rivularis*, and leaves *Swertia Chirayita* were used to prepare methanol extracts and the total phenolic and flavonoid content was determined by the Folin-Ciocalteu reagent and Aluminium Chloride method respectively. The antibacterial activity was determined by agar well diffusion assay using bacterial strains, i.e. two gram-negative: *Escherichia coli* and *Salmonella typhi*; two gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*. Qualitative phytochemical screening revealed that all three plants contain phenols, flavonoids, tannins, and saponins compounds widely recognized for their antibacterial properties. Steroids were detected in both *A. subulatum* and *A. rivularis*, while coumarins were present in *A. subulatum* and *S. chirayita*. Terpenoids were identified in *A. rivularis* and *S. chirayita*, but absent in *A. subulatum*. Notably, *S. chirayita* exhibited the greatest phytochemical diversity, with the presence of alkaloids and glycosides, which were not found in the other two species. The methanolic plant extracts are reported to have 51.11 ± 8.53, 243.70 ± 7.40, 898.02 ± 11.31 (mg GAE/g of extract) of total phenolic content and 157.21 ± 0.99, 590.71 ± 1.51, 869.32 ± 1.14 (mg QE/g of extract) of total flavonoid content of *Amomum subulatum* (F.), *Astilbe rivularis* (R.), and *Swertia chirayita* (L.) respectively. The zone of inhibition ranged from 7 mm to 13.33 mm. Among the three plant extracts *S. chirayita* showed the highest, *A. rivularis* showed the lowest, and *A. subulatum* showed an intermediate zone of inhibition compared to others in all concentrations (50, 100, and 200mg/ml). The plant species showed potential antibacterial properties due to the higher availability of the phytochemicals.

1. INTRODUCTION

Medicinal plants, often referred to as medicinal herbs, have been recognized and utilized in traditional medicine for centuries. These plants produce a wide range of chemical compounds to serve various functions, such as protecting themselves from insects, fungi, diseases, and herbivores (Gershenzon & Ullah, 2022). The medicinal plants that have been used medicinally for thousands of years are therapeutically beneficial (Dewick, 1996; Phillipson & Wright, 1996). Plant extracts have been developed and suggested for use as antibacterial compounds (Del Campo et al., 2000). While synthetic antimicrobial medicines are widely approved in many countries, researchers are increasingly exploring the use of natural chemicals obtained from medicinal plants (Moloney, 2016).

Phytochemicals (derived from the Greek word phyto, which means plant) are naturally occurring chemical substances found in plants that provide health benefits to humans (Hasler & Blumberg, 1999). Phytochemicals can be broadly categorized into two groups: primary metabolites and secondary metabolites. Primary metabolites are amino acids, carbohydrates, nucleotides, fatty acids, and polymers derived from (polysaccharides, lipids, proteins, DNA, RNA, etc.), whereas secondary metabolites are phenols, flavonoids, alkaloids, saponins, tannins, terpenes, steroids, coumarins, glycosides, anthocyanins, anthraquinones, etc. Secondary metabolites are a diverse range of chemical compounds produced by plant cells through metabolic pathways derived from primary metabolic pathways. These compounds, which are not essential nutrients but are commonly present in the human diet, can have various health effects, either beneficial or harmful (Benzie & Wachtel-Galor, 2011; Grigg, 2001). Phytochemicals are bioactive secondary metabolites found in plant tissues such as leaves, bark, roots, and seeds (Dike et al., 2012; Samatha et al., 2012). Several research studies show active components of plant extracts have been utilized for centuries to cure a wide range of diseases and exhibit enhanced antioxidative, antibacterial, and antiviral properties (Altıok, 2010).

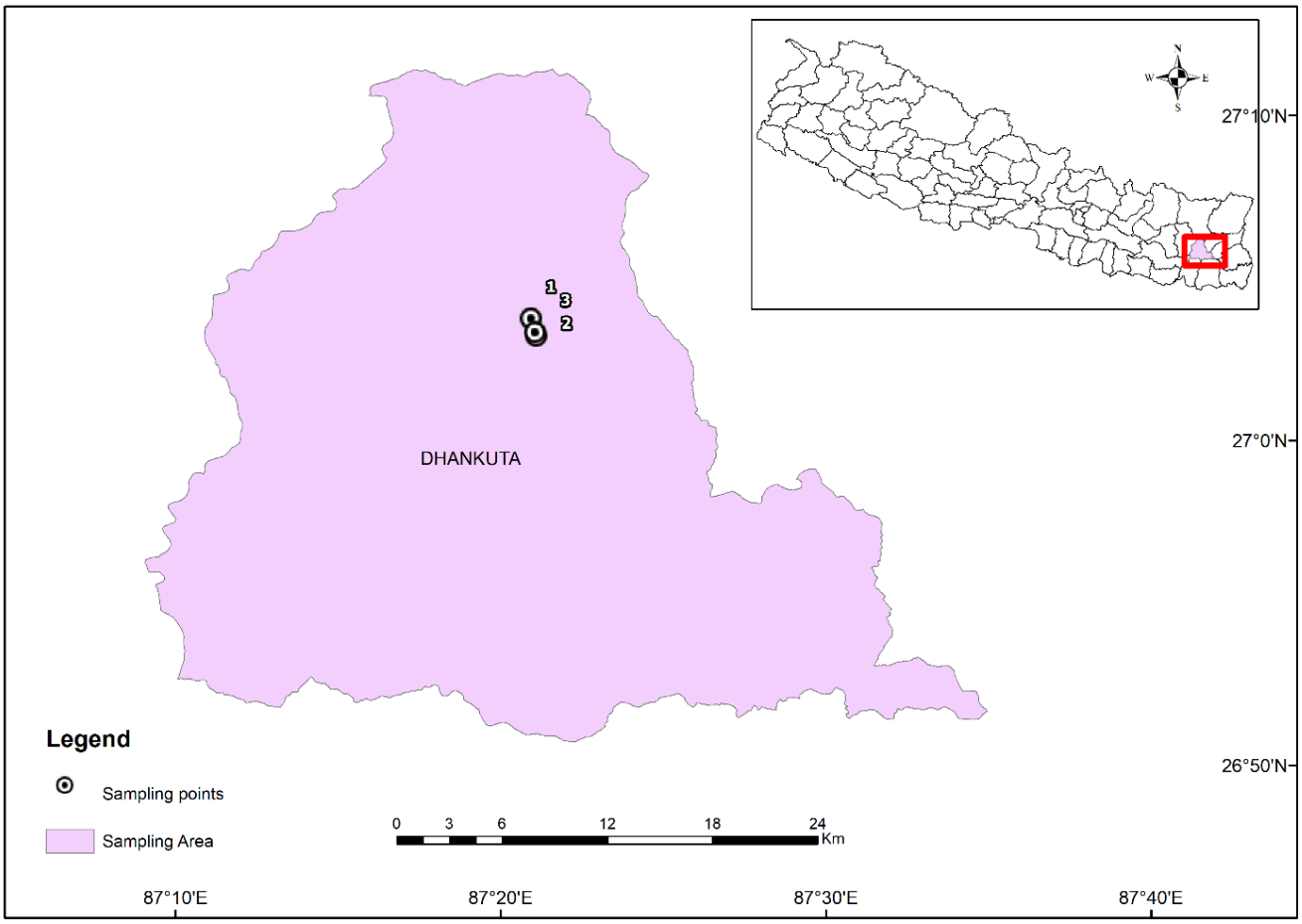

Figure 1
Map of the study area.

Swertia chirayita belongs to the Gentianaceae family and contains a variety of bioactive substances, including xanthones, flavonoids, terpenoids, iridoids, and secoiridoid glycosides, that are responsible for its medicinal properties (Pant et al., 2000). *Amomum subulatum* belongs to the botanical family called Zengiberaceae, known as 'black gold' or 'black cardamom' and locally called alainchi in Nepal. It is an evergreen, large perennial, herbaceous plant Indigenous to Himalaya regions, Roxburgh first described this species in 'Plants of the Coast of Coromandel' and 'Flora Indica'. *Astilbe rivularis* belongs to the family Saxifragaceae and is distributed in Nepal, Bhutan, China, India, Thailand, Laos, Cambodia, and Vietnam at elevations ranging from 2000 to 3600 meters. *A. rivularis* rhizomes, also known as 'Thulo Okhati'. Although many studies have explored medicinal plants in Nepal, there is still limited scientific information on the phytochemical content and antibacterial properties of *Amomum subulatum*, *Astilbe rivularis*, and *Swertia chirayita*, especially from the Dhankuta region of eastern Nepal. Most previous research has either focused on other parts of the country or used different plant species altogether. This creates a gap in our

understanding of how these specific plants, which are traditionally used in local medicine, actually work at the chemical and biological levels. By studying these plants using simple, affordable extraction and testing methods, this research helps fill that gap. It provides useful data for both scientific study and traditional knowledge, which can be helpful for future drug discovery, conservation, and sustainable use of local medicinal resources. Thus, the rationale of the study is excavating different phytochemicals compounds of Amomum subulatum (F.), Astilbe rivularis (R.), and Swertia chirayita (L.), and screening the antibacterial potential of the plants against different bacterial strains.

2. MATERIALS AND METHODS

2.1 Study Area

Dhankuta is a mid-hill district in the Koshi Province of Eastern Nepal (Figure 1). It spans an area of 888.7 square kilometers and is situated between latitudes 26°53' to 27°19' North and longitudes 87°8' to 88°33' East. The district's elevation varies from 243 meters to 629 meters above sea level, with the altitude ranging between 300 meters and 2500 meters. A wide variety of plants and wildlife can be found in lush hilly forests and river basins. Dhankuta's climate is classified as warm and temperate. having an average temperature of around 19.3°C with annual precipitation of nearly 2603mm.

2.2 Collection of plants samples

Plant samples were collected from specified sites, including altitude and GPS position as indicated in Table 1. Leaves of *Swertia chirayita* and the rhizomes of *Astilbe rivularis* were collected during January. Fruits of *Amomum subulatum* were collected during October. Fresh plant materials were cut into little pieces and allowed to air dry for two to three weeks undercover in a newspaper. From time to time the newspaper was changed between 2-3 days. The plant material was ground into a fine powder by using an electric grinder once it had dried completely.

Table 1
Collection of plant samples

S.N.	Plant		Collection area	Altitude (m)	GPS (Latitude, Longitude)
	Botanical name	Common name (Nepali)			
1.	*Amomum subulatum*	Alainchi	Chhathar-Jorpati Rural Municipality, Dhankuta	1400-1600	27.063359 N, 87.3493844 E
2.	*Astilbe rivularis*	Thulo Okhati		1800-2000	27.0544813 N, 87.3518986 E
3.	*Swertia chirayita*	Chirrato		1700-2000	27.0562961 N, 87.3514266 E

2.3 Extraction

The extraction of powdered samples was carried out by atmospheric liquid extraction using the Soxhlet apparatus (Soxhlet, 1879). Methanol was utilized as the solvent in the extraction procedure. 10g and 15g (25g in total) of powdered samples were put evenly into a thimble and extracted with 180ml and 360ml (540ml in total) of methanol in two Soxhlet apparatus respectively. The extraction procedure was carried out until the solvent in the siphon tube of the Soxhlet equipment became transparent. The extracts were concentrated with a rotatory evaporator (Nkere & Iroegbu, 2005) and then transferred to a beaker and sealed. The dried plant extracts were stored in the refrigerator at 4ºC for further use.

The yield percentage was calculated as:

$$\text{Yield (\%)} = \frac{\text{Weight of Extract}}{\text{Dry Weight of Plant Material}} \times 100$$

2.4 Phytochemical Tests

The plants' extracts were subjected to qualitative phytochemical tests to detect the major phytochemicals present in them. The methanol extracts were screened out to identify the major phytochemicals (phenols, flavonoids, alkaloids, tannins, saponins, terpenoids, glycosides, coumarin, and steroids) using the standard procedures (Jaradat et al., 2015). To ensure accuracy, each sample was analyzed three times. During each trial, measurements were taken in three consistent directions, and the mean values were documented.

2.5 Estimation of Total Phenol Content (TPC)

To measure the total phenol content, the Folin-Ciocalteu method was used as described by (Singleton & Rossi, 1965), with slight modification. 0.02 ml of a 10 mg/ml plant extract was mixed with 1.58 ml of distilled water. Then, 0.1 ml of a diluted Folin-Ciocalteau reagent was added to the mixture. After letting it treat for 3-7 minutes at room temperature, 0.3 ml of 7.5% Na_2CO_3 solution was added. The mixture was then kept in the dark for 30 minutes. The absorbance was measured at 765 nm using a UV-spectrophotometer. A standard curve was created using gallic acid at concentrations of 1, 2.5, 5, 7.5, and 10 mg/ml. The total phenolic content was measured in triplicate and expressed as gallic acid equivalents (mg GAE/g dry extract).

2.6 Estimation of Total Flavonoid Content (TFC)

To measure the total flavonoid content, an Aluminum chloride ($AlCl_3$) colorimetric assay was used as described by (Dowd, 1959), with slight modification. 0.1 ml of 5mg/ml of plant extract was added to a mixture of 0.3 ml distilled water and 0.03 ml of 5% NaNO2. This reaction was allowed to incubate for 5 minutes at 25°C. Next, 0.03 ml of 10% AlCl3 was added, and after an additional 5 minutes, 0.2 ml of 1 mM NaOH was added. The final volume of the reaction mixture was adjusted to 1 ml with distilled water. Then the absorbance of the mixture was measured at 510 nm using a UV spectrophotometer. To quantify the flavonoid content, a

standard curve was created with quercetin dihydrate at concentrations of 1, 2, 3, 4, and 5 mg/ml. The total flavonoid content of the plant extracts was measured in triplicate and expressed as quercetin equivalents (mg QE/g dry extract).

2.7 Antibacterial Assay

The antibacterial activity of plant extracts was tested using the agar well diffusion method on Mueller Hinton Agar (MHA) plates (Hudzicki, 2009). The test organisms were first cultured in Nutrient Broth (NB) overnight at 37°C. The bacterial culture was then adjusted to match the turbidity of 0.5 McFarland standard, which gives a final concentration of $1.5 \times 10^{\wedge 8}$ CFU/ml. Mueller Hinton Agar (MHA) plates were then lawn-cultured with this standardized bacterial suspension. Plant extracts at concentrations of 50, 100, and 200 mg/ml were prepared using 1% Dimethyl Sulfoxide (DMSO). Four wells, each 6 mm in diameter, were made in the inoculated agar using a sterile cork borer. Each well was then filled with 50 µl of the different plant extracts. Norfloxacin (10 µg) was used as a positive control for bacteria, while DMSO was used as a negative control. The plates were left at room temperature for 30 minutes to allow the extracts to diffuse and then incubated at 37°C for 18-24 hours. After incubation, the clear zones around the wells, indicating antibacterial activity, were observed and measured in mm (Barry et al., 1979).

3. RESULT AND DISCUSSION

3.1 Plants Extraction

The results of the percentage of crude extract obtained from the plants are shown in Table 2. The yield crude extracts of *Amomum subulatum* (F.) was (2.039gm) 8.372%, *Astilbe rivularis* (R.) was (2.307gm) 9.228%, and *Swertia chirayita* (L.) was (5.346gm) 21.384% from 25gm powdered samples. The highest amount of methanolic extract was obtained from *Swertia chirayita (L.)*, which was more than double the yield of the other two plants. All the extracts had a semisolid texture and felt sticky and oily to the touch.

Based on earlier phytochemical studies yield was found 10.48% (Khanal et al., 2015). While Joshi & Dhawan, (2005) reported methanolic yields up to 12.6%. The higher yield percentage obtained in this study is likely attributable to differences in extraction conditions, particularly the plant part used. It is well-documented that leaves often yield a higher percentage of crude extract compared to whole plants or other plant part**s**, such as stems and roots. This is because the leaves of *Swertia chirayita* contain the highest concentrations of pharmacologically active secondary metabolites, including xanthones and glycosides**,** which are readily extractable with polar solvents like methanol (Joshi & Dhawan, 2005). Rhizomes typically contain moderate to high levels of secondary metabolites such as flavonoids, phenolics, terpenoids, and saponins, which are efficiently extracted by methanol (Sasidharan et al., 2011). Rai et al., (2019) analyzed rhizomes from the Darjeeling Himalayas using Soxhlet extraction with methanol and surprisingly reported a high 39.75% yield which may be caused by differences in solvent ratio and moisture content.Fruits of *Amomum* species are known to contain essential oils, fixed oils, resins, flavonoids, and other polar secondary metabolites that are soluble in methanol (Dhakal et al., 2023). Similar studies on *Amomum* species have such as Kumar et al. (2012) reported a 12% methanolic extract yield from *Amomum subulatum* fruit when using Soxhlet extraction over 48 hours and Ma'rifah et al. (2020) investigated phenolic extraction from *Amomum compactum* fruit using 100% water obtained 10.52% yield.

Table 2
Physical reference (color) and percentage yield of plants' crude extracts.

Plant Botanical name	Plant's parts	Solvent	Reference (color)	Dry weight taken (gm)	Weight of extract (gm)	Yield percentage (%)
Amomum	Fruit		Dark brown		2.093	8.372%
Astilbe rivularis	Rhizome	Methanol	Dark yellow	25	2.307	9.228%
Swertia chirayita	Leaves		Dark green		5.346	21.384%

Table 3
Results of the phytochemical screening of methanol extract of *Amomum subulatum* (F.), *Astilbe rivularis* (R.), and *Swertia chirayita* (L.).

Detection	Tests	Reference (Color)	Results		
			***Amomum subulatum* (F.)**	***Astilbe rivularis* (R.)**	***Swertia chirayita* (L.)**
Phenols	Ferric chloride test	Dark green/bluish black	+ + +	+ + +	+ + +
Flavonoids	Lead acetate test	Yellow precipitate	+ + +	+ − +	+ + +
Alkaloids	Mayer's test	Creamy white/yellow precipitate	− − −	− − −	+ + +
Tannins	NaOH test	Formation of emulsion	+ + +	+ + +	+ + +
Saponins	Foam test		− + +	+ + +	− + +
Terpenoids	H2SO4 test	Grey/reddish-brown color	− − −	− + +	+ + +
Glycosides	Ferric chloride test	Blue color at the junction of two liquids	− − −	− − −	+ + +
Coumarin	NaOH test		− + +	− − −	+ + +
Steroids	H2SO4 test	Upper layer red/sulphuric acid layer is yellow color	+ + +	+ + +	− − −

3.2 Qualitative Phytochemical Test

The findings of the phytochemical screening are shown in Table 3. From the qualitative tests of different parts of selected three Indigenous medicinal plants, the result shows that all plant samples include phenols, flavonoids, tannins, and saponins. Alkaloids and glycosides are only present in *S. chirayita* but absent in *A. subulatum* and *A. rivularis*. Terpenoids are present in *A. rivularis* and *S. chirayita* but absent in *A. subulatum*, whereas coumarin is only absent in *A. rivularis* but present in others. Lastly, steroids are absent in *S. chirayita* but present in *A. subulatum* and *A. rivularis*.

These results are consistent and strongly similar to the phytochemical profile described by (Subedi & Karki, 2018) who reported that the methanolic extract of *S. chirayita* contains phytochemicals like alkaloids, glycosides, flavonoids, steroids, coumarins, quinones, and terpenoids, (Tijjani et al., 2012) who investigated the presence of carbohydrates, cardioactive glycosides, terpenes, flavonoids, alkaloids, tannins, and saponins in methanolic seed extracts of *Amomum subulatum* and (Rai et al., 2019) who identified phenols, flavonoids, reducing sugars, saponins, alkaloids, terpenoids, and tannins in the rhizome extract.

3.3 Total Phenolic Content

The total phenolic content of all the extracts was measured as Gallic Acid Equivalent (mg QE/g dry extract) illustrated in Table 4, using the calibration curve of gallic acid (y = 0.0135x + 0.1621, R^2 = 0.9771).

The total phenolic content of extracts of Amomum subulatum (F.), Astilbe rivularis (R.), and Swertia chirayita (L.) were calculated by using a formula $C = \frac{cV}{m}$.

The result shows that the methanolic extract of *Swertia chirayita* (leaves) contains the highest amount of phenols (898.025 ± 11.315) mg GAE/g dry wt. of extract when compared to others. *Amomum subulatum* fruit extract had the lowest phenolic content (51.1111 ± 8.5533) mg GAE/g dry wt. of extract. However, *Astilbe rivularis* (rhizome) extract contained moderate phenolic content (243.704 ± 7.4074) mg GAE/g dry wt. of extract.

Previous studies on the methanolic extract of *Swertia chirayita* showed the total phenol content was determined to be 87.44 ± 0.30 mg of GAE/g of the extract (Bhandari et al., 2019) and 26.16 ± 0.25 mg of GAE/g of the extract (Khanal et al., 2015), the methanolic extract of *Amomum subulatum* showed the total phenol content of 35 ± 0.8 mg of GAE/g of the extract (Belew, 2023) and 20.94 ± 0.21 mg of GAE/g of the extract (Kanthlal et al., 2021), and the methanolic extract of *Astilbe rivularis* showed the total phenol content of 23 ± 1.0 mg of GAE/mg of the extract (Mitra et al., 2017) and 183.11±0.50 mg of GAE/g of the extract (L. Subedi et al., 2014); which supports our findings.

The high phenolic content of *Swertia chirayita* suggests a strong antioxidant potential and supports its traditional use in ethnomedicine. These results provide a valuable foundation for future pharmacological studies and the potential development of plant-derived therapeutic agents.

Table 4
The Total Phenolic Content (TPC) in mg GAE/g dry weight of extract.

Plant extracts	Quantity (mg/ml)	Mean absorbance (nm)	TPC as GCE = $\frac{c \times v}{m}$ mg/g
Amomum subulatum (F.)		0.167	51.1111 ± 8.5533
Astilbe rivularis (R.)	10	0.195	243.704 ± 7.4074
Swertia chirayita (L.)		0.283	898.025 ± 11.315

Table 5
The Total Flavonoid Content (TFC) in mg QE/g dry weight of extract.

Plant extracts	Quantity (mg/ml)	Mean absorbance (nm)	TPC as QE = $\frac{c \times v}{m}$ mg/g
Amomum subulatum (F.)		0.755	157.2139 ± 0.995
Astilbe rivularis (R.)	10	0.039	590.7131 ± 1.5199
Swertia chirayita (L.)		0.474	869.3201 ± 1.1489

3.4 Total Flavonoids Content

The total flavonoid content of all the extracts was measured as Quercetin Equivalent (mg QE/g dry extract) illustrated in Table 5, using the quercetin dehydrate calibration curve (y = 0.201x − 0.119, R^2 = 0.9987).

The results show that the *Swertia chirayita* leaves exhibit the highest flavonoid concentration at 869.3201 ± 1.1489 mg QE/g dry weight of extract compared to the other samples. *Amomum subulatum* fruit extract had the lowest flavonoid content (157.2139 ± 0.995) mg QE/g dry wt. of extract. However, *Astilbe rivularis* (rhizome) extract contained moderate flavonoid content (590.7131 ± 1.5199) mg QE/g dry wt. of extract.

Previous studies on the methanolic extract of *Swertia chirayita* showed the total flavonoid content was determined to be 25.09 ± 0.31 mg of QE/g of the extract (Bhandari et al., 2019) and 67.49 ± 0.50 mg of QE/g of the extract (Khanal et al., 2015), the methanolic extract of *Amomum subulatum* showed the total flavonoid content of 15.2 ± 0.6 mg of QE/g of the extract (Belew, 2023) and 10.07 ± 0.21 mg of QE/g of the extract (Kanthlal et al., 2021), and the methanolic extract of *Astilbe rivularis* showed the total flavonoid content of 40 ± 0.9 mg of QE/mg of the extract (Mitra et al., 2017) and 857.26±10.38 mg of QE/g of the extract (L. Subedi et al., 2014); which supports our findings.

Among the studied species, *Swertia chirayita* shows the highest flavonoid concentration, further confirming its strong antioxidant and pharmacological potential. The findings provide scientific validation for its traditional medicinal use and highlight the species as a valuable candidate for natural drug development.

3.5 Zone of Inhabitation of Antibacterial Assay

The antibacterial assay of three Indigenous plant extracts of different concentrations was tested against four different bacteria (two gram-negative i.e. *Escherichia coli* and *Salmonella typhi*; two gram-positive bacteria i.e. *Staphylococcus aureus* and *Bacillus subtilis*), by agar well diffusion method.

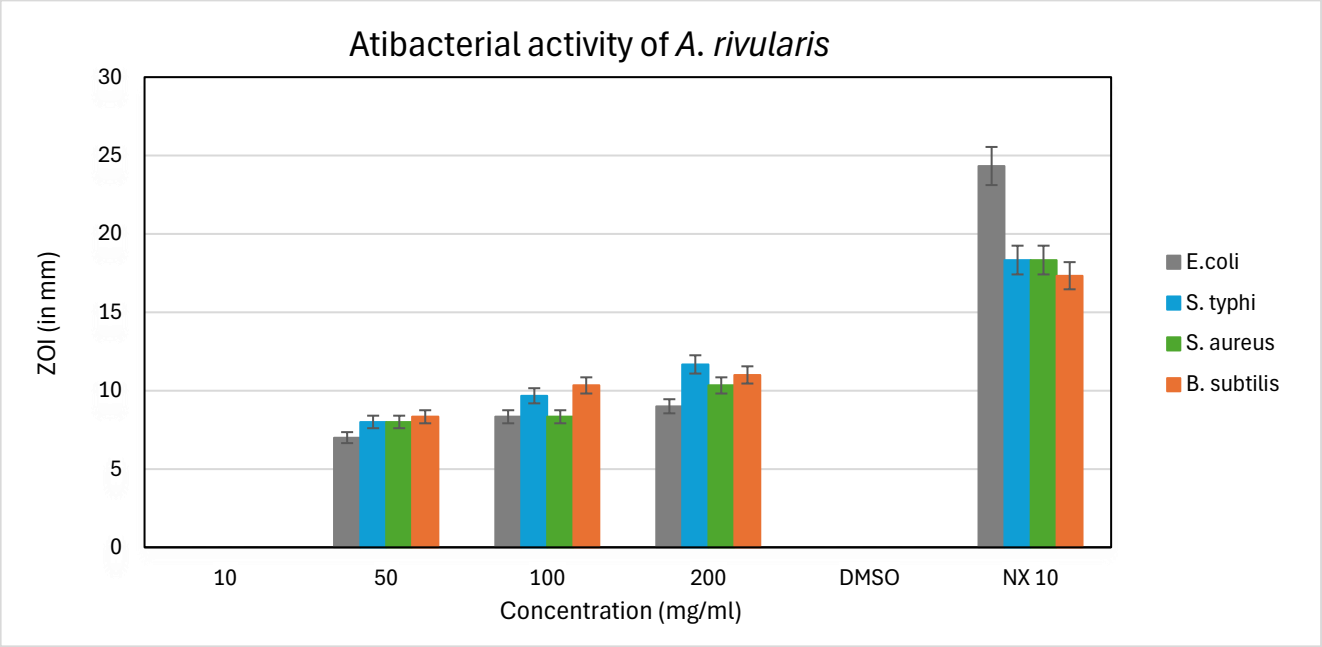

Figure 2
Bar diagram showing antibacterial activity of methanolic extracts of *Amomum subulatum* (L.) compared to Norfloxacin (10mcg).

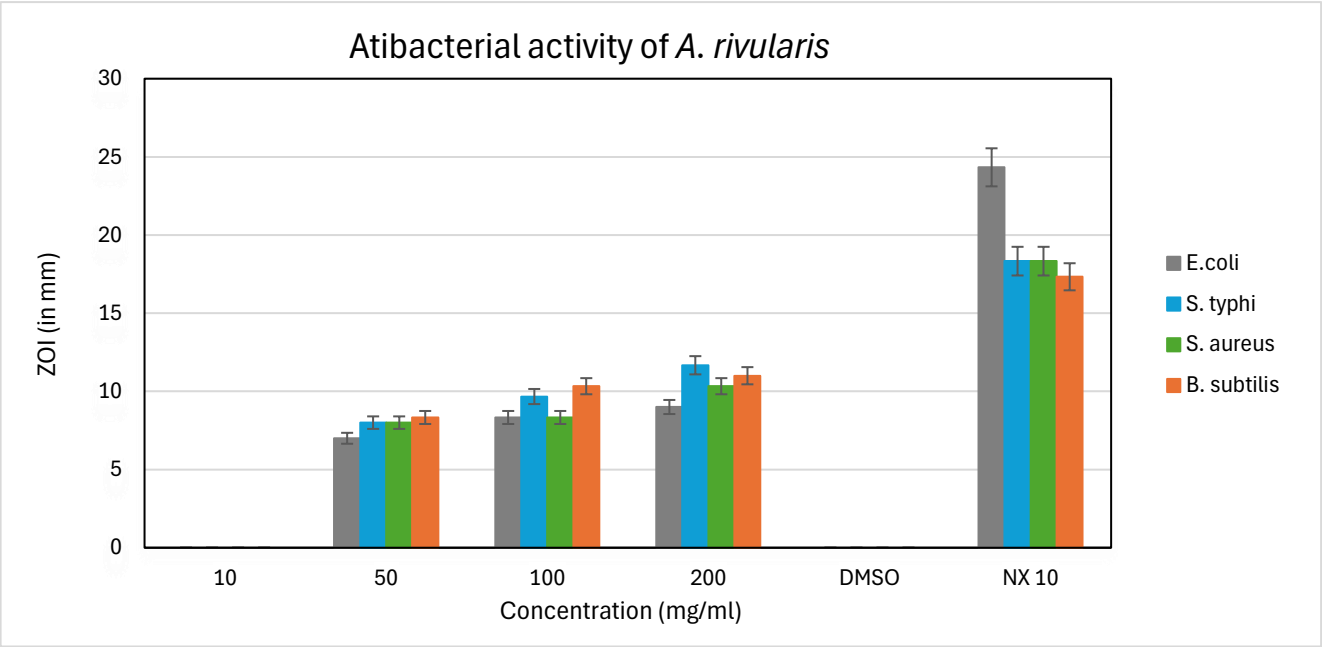

Figure 3
Bar diagram showing antibacterial activity of methanolic extracts of *Astilbe rivularis* (R.) compared to Norfloxacin (10mcg).

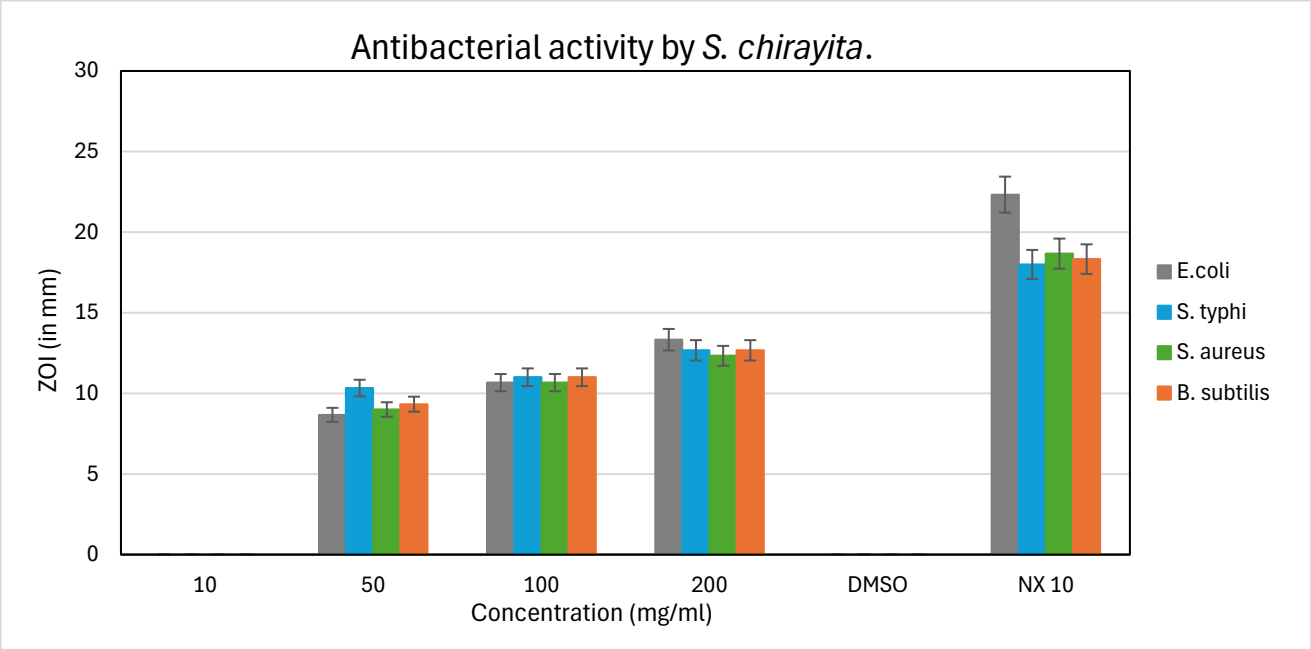

Figure 4
Bar diagram showing antibacterial activity of methanolic extracts of *Swertia chirayita* (L.) compared to Norfloxacin (10mcg).

The results show that all the extracts showed action against four different bacteria (two gram-negative i.e. *Escherichia coli* and *Salmonella typhi*; two gram-positive bacteria i.e. *Staphylococcus aureus* and *Bacillus subtilis*). Among concentrations (10mg/ml, 50mg/ml, 100mg/ml, and 200 mg/ml) examined, a concentration of 10mg/ml of all the extracts doesn't show any effect in all four bacteria. The concentration of 50, 100, and 200mg/ml of plant extracts showed antibacterial effects, as the Zone of Inhibition (ZOI) increased as the concentration was higher. Broad-spectrum antibiotic Norfloxacin (10mcg) showed a larger zone of inhibition compared to all tested concentrations of plant extract where DMSO control does not show any inhibition.
Across all tested bacteria, *Swertia chirayita* consistently demonstrated the highest antibacterial activity, with ZOI values increasing proportionally with concentration. Against *E. coli*, it exhibited ZOIs of 8.67 mm, 10.67 mm, and 13.33 mm at 50, 100, and 200 µg/ml respectively (Figure 4). In comparison, *Astilbe rivularis* showed the lowest activity with ZOIs of 7 mm, 8.33 mm, and 9 mm (Figure 3), while *Amomum subulatum* showed moderate inhibition of 8.33 mm, 10.33 mm, and 11.33 mm (Figure 2). A similar trend was observed against *Salmonella typhi*, where *Swertia chirayita* again exhibited superior activity with ZOIs of 10.33 mm, 11 mm, and 12.67 mm across increasing concentrations. *Astilbe rivularis* displayed lower activity with ZOIs of 8 mm, 9.67 mm, and 11.67 mm, while *Amomum subulatum* showed intermediate activity with ZOIs of 8.67 mm, 10.67 mm, and 11.67 mm. Notably, at 200 µg/ml, both *Astilbe rivularis* and *Amomum subulatum* exhibited equal inhibition zones of 11.67 mm (Figures. 3 and 2). In the case of *Staphylococcus aureus*, *Swertia chirayita* again showed the highest antibacterial activity with ZOIs of 9 mm, 10.67 mm, and 12.33 mm (Figure 4). *Astilbe rivularis* showed comparatively lower inhibition (8 mm, 8.33 mm, and 10.33 mm), while *Amomum subulatum* recorded slightly lower or similar activity with ZOIs of 7.33 mm, 8.67 mm, and 11 mm. It is worth noting that at the lowest concentration (50 µg/ml), *Amomum subulatum* exhibited a lower ZOI than *Astilbe rivularis*. Finally, against *Bacillus subtilis*, *Swertia chirayita* continued to show the most potent antibacterial effects with ZOIs of 9.33 mm, 11 mm, and 12.67 mm (Figure 4). *Astilbe rivularis* followed with values of 8.33 mm, 10.33 mm, and 11 mm (Figure 3), while *Amomum subulatum* showed the least inhibition at higher concentrations with ZOIs of 8.33 mm, 9 mm, and 10.33 mm (Figure 2).
Overall, the results indicate a clear dose-dependent antibacterial activity in all three plant extracts, with *Swertia chirayita* exhibiting consistently superior inhibition across all bacterial strains tested. *Astilbe rivularis* generally showed the least antibacterial activity, while *Amomum subulatum* demonstrated moderate effects that occasionally matched or slightly exceeded those of *Astilbe rivularis* at higher concentrations.
Earlier (Laxmi et al., 2011) investigated that the methanolic extract of *S. chirayita* exhibits antibacterial activity against *E. coli, S. aureus, B. subtilis, S. typhi, V. cholerae, S. pyogenes, P. mirabilis, P. alkalifaciens, B. polymyxa* and *P. aeruginosa* and (I. Subedi & Karki, 2018) analyzed that the methanolic extract of *S. chirayita* exhibits strong antibacterial activity against pathogenic microbial species *Klebsiella pneumonia, Salmonella typhi, Citrobacter freundii, Escherichia coli, Staphylococcus aureus*, and *Shigella dysenteriae*. (Tijjani et al., 2012) investigated the effectiveness of the extract of *A. subulatum* against bacteria such as *Streptococcus pneumoniae, Bacillus subtilis, Candida albican senegalensis, Klebsiella pneumoniae,*

Salmonella typhi, Pseudomonas aeruginosa, and *Staphylococcus aureus*. (Garg et al., 2016) tested for antibacterial properties against enteropathogenic and food-spoiler bacteria, including *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* and *Bacillus pumilus* which supports the use of *Amomum subulatum* to treat illnesses caused by pathogens, as its phytochemicals were discovered to have anti-bacterial activities. (Adhikary et al., 2011) Examined the biological properties of *Astilbe rivularis* collected in the Kavrepalanchowk district, Nepal, where the bactericidal activity of the methanolic extract was assessed. It showed the most potent antibacterial activity *against E. coli, Klebsiella spp.,* and *Serratia spp*. and Antimicrobial susceptibility tests performed by (Gyawali et al., 2014) showed that *Astilbe rivularis* had a prominent effect against *Klebsiella pneumoniae, E. coli, Salmonella typhi, Staphylococcus spp.,* and *Pseudomonas spp.* These prior findings align with our results.

4. CONCLUSION

The present study evaluated the phytochemical profile and antibacterial activity of three indigenous medicinal plants from Eastern Nepal *Amomum subulatum* (F.), *Astilbe rivularis* (R.), and *Swertia chirayita* (L.) against common pathogenic bacterial strains. The investigation aimed to determine the presence of secondary metabolites and assess the potential antibacterial effects of their methanolic extracts. The results demonstrated that all three plants possessed varying degrees of antibacterial activity, with *Swertia chirayita* showing the highest zones of inhibition across all tested strains. Phytochemical screening confirmed the presence of bioactive compounds such as phenols, flavonoids, tannins, alkaloids, and saponins, which are known for their pharmacological properties. Quantitative analysis revealed that *Swertia chirayita* had the highest total phenolic and flavonoid content, which likely contributed to its enhanced antibacterial performance compared to *Amomum subulatum* and *Astilbe rivularis*. These findings support the traditional ethnomedicinal use of these plants and underscore their potential as natural sources of antibacterial agents. The study highlights the importance of preserving indigenous knowledge while promoting the sustainable use of Himalayan plant biodiversity. Further research is needed to isolate and characterize the specific bioactive constituents and to explore their potential in the development of plant-based alternatives to conventional antibiotics.

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CONFLICTS OF INTEREST

The authors declare that they do not have any conflict of interest.

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