RESEARCH ARTICLE

In-vitro Antimicrobial Activities of *Swertia chirata* C. B. Clarke and *Andrographis paniculata* (Burm.F.) Wall. Ex Nees Using Various Solvents

Shruti M. Parmar[1*], R. N. Nakar[1] and Jhanvee Chanpura[2]

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Abstract

Some plants have been used as a phytomedicine for thousands of years. *Swertia chirata* C. B. Clarke (Gentianaceae) and *Andrographis paniculata* (Burm.f.) Wall. Ex Nees (Acanthaceae) are respectively distributed in Nepal and Gujarat, and have potential medicinal properties. They are used in the development of new drugs and the use of combination chemotherapy targeted therapy along with improved adherence to treatment are all important to address strategies that might cope up against cancer treatment. In the study, the methanol, ethanol, and petroleum ether extracts of two plants, with their areal parts such as shoot and leaves, were tested for their antimicrobial activities against a variety of pathogenic bacterial strains and, the results showed that the extracts had varying degrees of antimicrobial activities. Methanol and ethanol extracts were found to be the most active, whereas petroleum ether extract didn't show any activity. However, further studies are needed and still required to identify the active compound(s) in the extracts.

Keywords: Antibacterial activity, Ethanol extract, Methanol extract, zone of inhibition, *Swertia chirata*, *Andrographis paniculata*.

Highlights

- The antimicrobial and bioactivity of any plant helps in inhibiting the growth of microbes and, by such activity plants fight and kill microorganisms, can be of immense use.
- Plants such as *Swertia chirata* and *Andrographis paniculata* have proved to be useful drugs in many studies.
- Methanol, ethanol and petroleum ether extracts of two plants showed that the extracts had varying degrees of antimicrobial activities.
- Antimicrobial potential of plant collected in winter season was more as compared to summer season plants.

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Introduction

Many studies on aromatic and phytomedicinal herbs are the source of potent bioactive phytoconstituents. They play a vital role in traditional treatment for several diseases. These plants have shown pharmacological with different types of worthwhile properties such as anti-inflammatory, antioxidant, antifungal, antibacterial, antidiabetic, analgesic, antipyretic, anticancer, antidiarrheal, antiviral, antimalarial activity (Ahirwal *et al.*, 2011, Joshi and Dhawan, 2005, Mishra *et al.*, 2009, and Reena *et al.*, 2001). Some of the plants have been shown to have hepatoprotective activity by improving the antioxidant status of the liver (Gupta *et al.*, 2005; Bhattacharjee and Sil, 2007). Liver disorders as a bitter tonic both plants have been shown to have immune modulatory activity, which can help to boost the Immune system. In a COVID-19 situation, most people used these plants. The antibacterial activity of *Andrographis paniculata* and *Swertia chirata* is thought to be due to a combination of factors. These factors include the presence of specific phytochemicals, such as Andrographolide and Swertiamarin, as well as the synergistic effects of the various compounds that are present in the extracts of these plants. Active compounds such as diterpenoids were recorded from *Andrographis* by Pholphana *et al.,* (2013) and they quoted that the most active diterpenoid AP_6 was found in the leaf of *Andrographis*. The market for herbal drugs is increasing like anything in India and it is estimated that, the herbal industry in India uses about 8000 medicinal plants so each medicinal plant is very unique and has quite useful properties (Sharma *et al.*, 2008). However, only a few of them are reachable to the needy world's market (Poulakou *et al.*, 2019).

Traditional herbal medicines have been used for centuries to treat a variety of diseases. In recent years, there has been a growing interest in the scientific study of herbal medicines. This research has shown that many herbal medicines have active ingredients that can have therapeutic effects. Many studies have shown that medicinal plants contain xanthones, Seco-iridoid glycosides, Flavonoids, Alkaloids, and Triterpenoids. Both these plants are controversial medicinal plants and has similar

[1]Sheth P.T. Arts and Science College, Godhra, Affiliated with Shri Govind Guru University, (State University of Gujarat), Gujarat, India.

[2]Department of Microbiology, School of Sciences, ITM (SLS) Baroda University, Vadodara, Gujarat, India.

***Corresponding author:** Dr R. N. Nakar, Sheth P. T. Arts & Science College, Godhra Affiliated with, Shri Govind Guru University, Godhra, (A State University of Gujarat), Gujarat, India, Email: drrupeshresearch@gmail.com

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therapeutic action (Nagalekshmi *et al*., 2011). It is important that the phytoconstituents content of these plants can vary depending on the growing conditions, harvesting methods and processing techniques. In recent year, infections have increased to a great extent and antibiotic resistance effects become an ever-increasing therapeutic problem (Mahesh and Satish, 2008). Many study have investigated the medicinal and pharmacological properties of *S. chirata* and *A. paniculata* (Brahmachari *et al*., 2004; Mishra *et al*., 1992 & 2009; Shihabudeen *et al*., 2010; Niranjan *et al*., 2010). The research on these plants is ongoing and there is still much that we don't know about their potential health benefits. However, the research that has been done so far suggests that these plants have a wide range of potential medicinal properties. The main active compounds, Andrographolide (Bhan *et al.,* 2006) and Swertiamarin, are found in *A. paniculata* and *S. chirata,* and have potent antibacterial activities against a variety of gram-positive and gram-negative bacteria.

Methanolic and ethanolic extracts were widely used for bioactivity studies. In one such study, Methanolic extracts were useful in antioxidant activities (Ahirwal *et al*., 2014). The author further stated that petroleum ether can also be good for studying such bioactivities. Alam *et al.,* (2009) carried out work of the antimicrobial activity of *Swertia* sp. using ethanol extracts and found a large zone of inhibition (ZOI) against *Staphylococcus aureus*. Work on *Andrographis* was in bits, but in one work, a yield of ten accessions of *Andrographis* were assessed at different growth stages for andrographiloides (Bhan *et al.,* 2006). *Andrographis* and *Swertia* were checked for hepatoprotective activities against ethanol and other drugs and produced good results (Bhardwaj, *et al.,* 2011). In one review work, Brahmachari *et al.,* (2004) presented that there are vast applications and uses of *Swertia* plant, especially for biological activities. There are many active chemicals present in *Andrographis* sp. and such show anti-inflammatory tumor suppressor activity along with antihepatotoxic activity (Chao and Lin, 2010). Methanol extracts are widely used for different activities in different plants, and in one such plant *Caesalpinia bonducella*, an antioxidant defense system, was evaluated (Gupta *et al.,* 2005). Antihepatotoxic effects were studied by Kapil *et al.,* (1993), who found *Swertia* as useful drug. There is need to work more and more on plants such as *Swertia* and *Andrographis* and for that, need of conservation and techniques like micropropagation were suggested in different articles (Joshi and Dhawan, 2005; Kumar and Johannes, 2016). Keeping in mind, *Swertia* and *Andrographis,* which are known as Kadiyatu and Khotu kadiyatu in Gujarat, were selected for antimicrobial screening.

Materials and Methods

Plant collection in different two seasons

Both mature plants, i.e., *S. chirata* were collected at the Nutan Ayurvedic Research Centre, Bhumel, Gujarat and *A. paniculata* were collected at the Shree Darshanam, Navli, Anand. *S. chirata was* collected from the same site and *A. paniculata* was collected at the Ajod Vruksh Mandir, Vadodara at a vegetative stage in two different seasons winter and summer seasons, respectively.

After collection, mature, vegetative, fresh, healthy and disease-free plants were washed thoroughly in tap water, followed by distilled water. The plants should be dried in a shady spot with good air circulation so, later on completely dried leaves, stems and roots were separated. It was dried and homogenized into a fine powder by using a grinder and stored in an airtight dry container because it preserves the plant material for a long period for further use.

Extraction Method

The methodologies of Harborne (1988) were used to process the ethanolic, methanolic and Petroleum ether extracts from two plants *i.e. S. chirata* and *A. paniculata.* Leaves and, stems and roots of *S. chirata* were subjected to cold maceration methods (Harborne,1973) to extract their active ingredients. The air-dried and ground plant material (Leaves, Stems and roots) was extracted with methanol, ethanol and petroleum ether by using cold maceration method. About 50 g of both powdered plant materials (Leaves, Stems and roots) was mixed with 250 mL of pure ethanol, methanol and petroleum ether in a conical flask at 37°C for 72 hours. The conical flask web kept in a shaker for shaking the mixture was filtered by using Whatman no. I filter paper. Concentrated filtrate when filtrate was concerted, then processed using rotary evaporator at 40°C. The dried extracts were scraped and stored in sterile container and kept in a refrigerator till further use.

Testing Microorganism and Maintenance of bacterial strains

Both gram-positive (*Bacillus subtilis*, *B. megaterium*, *Staphylococcus aureus*) and gram-negative (*Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi A*, *S. typhi B, Escherichia coli*) microorganisms were collected from IAR- The University for Innovation, Gandhinagar. The microbial strains were maintained by periodic sub-culturing in nutrient agar slants and active cultures were prepared in nutrient broth and, incubated at 37°C for 24hrs, and stored at 4°C.

Antimicrobial activity by agar well diffusion method

The effect of different concentrations of ethanol, methanol and petroleum ether extract of leaves, stems and roots of the plants on several bacterial strains can be studied using on agar well diffusion assay. The plant extract was added to the well in an agar plate that had been seeded with the bacterial strains. The plates were then incubated for some time and the diameter of the zone of inhibition around each well was measured. The larger the zone of inhibition the more effective the plant extract was at inhibiting the growth of the bacteria. In the process, 20 mL of sterile N. Agar with 0.2 mL broth culture of the test organisms was added to sterile Petri plates and allowed to solidify. Then, using a sterile Cork borer, a well of the agar was made in a plate at the desired location. 100 mL of different concentrations of the plant extracts (ethanol, methanol, and petroleum ether of leaf, stems and root ranging from 1000 to 5000 µg/mL were added into the well. The positive control well was filled with respective solvents (ethanol, methanol, and petroleum ether) and the negative control well was filled with distilled water. All the plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the zone of inhibition and, was noted after the next day. The result size of the zone of inhibition was an indication of the effectiveness. The

Experiment was performed in triplicates and the same procedure was followed for both seasons, i.e., season 1 and 2. The results of both season's activity were analyzed using MS Excel 2021.

Results and Discussion

The results of the antibacterial activity of ethanol, methanol, and petroleum ether extract of leaves, stems and roots of the two plant species under study against the 8 various pathogenic bacterial strains are interpreted in Table 1 and Figs 1 to 9 with graphs. Seasonally, plants were collected, and then plant extracts were subjected to antibacterial activity. The study showed that the different solvents extracted different compounds from the plant material. The ethanolic extract extracted the most polar compounds and, the methanolic extract extracted a mixture of polar and non-polar compounds whereas, petroleum ether extract extracted the most non-polar compounds.

In Table 1 and Fig. 1a and 1b show ethanol extract of the root of *A. paniculata,* which shows significant activity against *Salmonella typhi A* and B with a zone of inhibition is 11 to 14 mm and 9 to 11 mm for *P. aeruginosa E. coli, B. subtilis, B. meghaterium* and *S. aureus*. The lower zone of inhibition was seen only at higher concentrations.

Table 1: Antibacterial activity of ethanol extract root of *A. paniculata* (Season 1 & 2)

Bacterial strain	*Sample con. (µg/mL)*	*S1*	*S2*	*Mean*	*SD*	*SDE*
E. coli	5000	9.00	8.33	8.67	0.47	0.34
	3000	7.50	7.67	7.59	0.12	0.09
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B. subtilis	5000	8.50	8.67	8.59	0.12	0.09
	3000	7.50	7.33	7.42	0.12	0.09
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B. meghaterium	5000	10.00	9.33	9.67	0.47	0.34
	3000	9.00	9.00	9.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. aureus	5000	9.50	9.00	9.25	0.35	0.25
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. vulgaris	5000	9.00	9.00	9.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. aeruginosa	5000	11.00	9.67	10.34	0.94	0.67
	3000	10.00	8.67	9.34	0.94	0.67
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. typhi A	5000	12.00	9.33	10.67	1.89	1.34
	3000	10.00	9.00	9.50	0.71	0.50
	2000	8.50	8.33	8.42	0.12	0.09
	1000	0.00	0.00	0.00	0.00	0.00
S. typhi B	5000	14.00	8.67	11.34	3.77	2.67
	3000	9.50	8.33	8.92	0.83	0.59
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00

Note: Table (1-9) Here, S1 and S2 are mean values of season-1 and season-2 whereas, mean represents average of season1 and 2. SD and SDE are Average standard deviation and Average standard error recorded during both seasons. In all tables from 1 to 9, such symbols will be same.

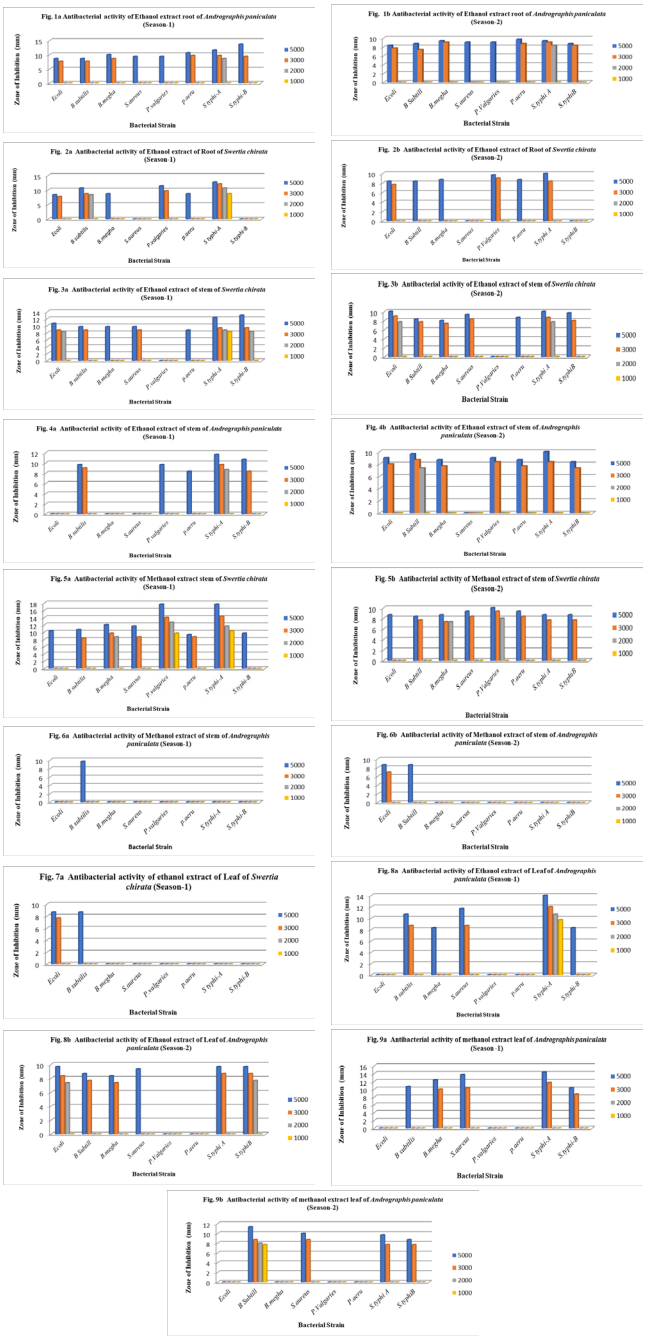

Figure 1 to 9: Zone of inhibition- antimicrobial activities against eight different strains

Table 2: Antibacterial activity of ethanol extract of Root of *S. chirata* (Season-1&2)

Bacterial Strain	*Sample con. (µg/mL)*	*S1*	*S2*	*Mean*	*SD*	*SDE*
Ecoli	5000	8.50	8.33	8.42	0.12	0.09
	3000	7.50	0.00	3.75	5.30	3.75
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B Subtilis	5000	11.00	8.33	9.67	1.89	1.34
	3000	9.00	0.00	4.50	6.36	4.50
	2000	8.50	0.00	4.25	6.01	4.25
	1000	0.00	0.00	0.00	0.00	0.00
B.meghaterium	5000	9.00	8.67	8.84	0.23	0.17
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S.aureus	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P.vulgaris	5000	11.50	9.67	10.59	1.29	0.92
	3000	10.00	9.00	9.50	0.71	0.50
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P.aeruginosa	5000	9.00	8.67	8.84	0.23	0.17
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S.typhi A	5000	13.00	10.00	11.50	2.12	1.50
	3000	12.00	8.33	10.17	2.60	1.84
	2000	11.00	0.00	5.50	7.78	5.50
	1000	9.00	0.00	4.50	6.36	4.50
S.typhi B	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00

Table 3: Antibacterial activity of Ethanol extract of stem of *S. chirata* (Season-1&2)

Bacterial strain	*Sample con. (µg/mL)*	*S1*	*S2*	*Mean*	*SD*	*SDE*
E. coli	5000	11.00	10.00	10.50	0.71	0.50
	3000	9.00	9.00	9.00	0.00	0.00
	2000	8.50	7.67	8.09	0.59	0.42
	1000	0.00	0.00	0.00	0.00	0.00
B. subtilis	5000	10.00	8.33	9.17	1.18	0.84
	3000	9.00	7.67	8.34	0.94	0.67
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B. meghaterium	5000	10.00	8.00	9.00	1.41	1.00
	3000	0.00	7.33	3.67	5.18	3.67
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. aureus	5000	9.50	9.33	9.42	0.12	0.09
	3000	8.50	8.33	8.42	0.12	0.09
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. vulgaris	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. aeruginosa	5000	8.50	8.67	8.59	0.12	0.09
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. typhi A	5000	12.50	10.00	11.25	1.77	1.25
	3000	9.50	8.67	9.09	0.59	0.42
	2000	9.00	7.67	8.34	0.94	0.67
	1000	8.50	0.00	4.25	6.01	4.25
S. typhi B	5000	13.50	9.67	11.59	2.71	1.91
	3000	9.50	8.00	8.75	1.06	0.75
	2000	8.50	0.00	4.25	6.01	4.25
	1000	0.00	0.00	0.00	0.00	0.00

The ethanol extract of the root of *S. chirata* plant is shown in Table 2 and Fig. 2a and b, was effective with maximum zone of inhibition for *S. typhyi A* (10–12 mm at 5000 µg/mL). *E. coli*, *B. subtilis*, *P. vulgaris, B. Meghaterium*, *S. aureus* also showed significant zone of inhibition. *P. aeruginosa* and *S. typhi B* was resistant at higher concentrations.

Ethanol extract of stem of *S. chirata* is shown (Table 3 and Fig. 3a and b) where moderate activity was noted against *E. coli*, *B. subtilis, B. meghaterium, P. aeruginosa, S. typhi A, S. typhi B and P. vulgaris,* were resistant to the extract at all concentration.

The ethanol extract of stem of *Andrographis paniculate* against *E. coli*, *B. subtilis, B. meghaterium*, S. *aureus, P. vulgaris, P. aeruginosa S. typhi A and B* was recorded and zone of inhibition ranged from 7 to 11 (at 2000-5000µg/ml). It is indicated in Table 4 and Fig. 4a and 4b.

Methanol extract of stem of *S. chirata* showed (in Table 5 and Fig. 5a and 5b) higher antibacterial activity against *P. vulgaris* and *S. typhi A,* it showed moderate activity against *E. coli*, *B. meghaterium, S. aureus, P. aeruginosa, S. typhi B* with a zone of inhibition from 8 to 13 mm (2000–5000 µg/mL). Methanol extract of stem of *Andrographis* sp.

The Methanol extract of the stem of *A. paniculata* was effective against only two bacterial strains with a lower zone of inhibition for *E. coli* and *B. subtilis*. All other strains were found

Table 4: Antibacterial activity of ethanol extract of stem of *A. paniculata* (Season-1&2)

Bacterial strain	*Sample con. (µg/mL)*	*S1*	*S2*	*Mean*	*SD*	*SDE*
E. coli	5000	0.00	9.00	4.50	6.36	4.50
	3000	0.00	8.00	4.00	5.66	4.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B. subtilis	5000	10.00	9.67	9.84	0.23	0.17
	3000	9.00	8.67	8.84	0.23	0.17
	2000	0.00	7.33	3.67	5.18	3.67
	1000	0.00	0.00	0.00	0.00	0.00
B. meghaterium	5000	0.00	8.67	4.34	6.13	4.34
	3000	0.00	7.67	3.84	5.42	3.84
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. aureus	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. vulgaris	5000	10.00	9.00	9.50	0.71	0.50
	3000	0.00	8.33	4.17	5.89	4.17
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P.aeruginosa	5000	8.50	8.67	8.59	0.12	0.09
	3000	0.00	7.67	3.84	5.42	3.84
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S.typhi A	5000	11.50	10.00	10.75	1.06	0.75
	3000	9.50	8.33	8.92	0.83	0.59
	2000	8.50	0.00	4.25	6.01	4.25
	1000	0.00	0.00	0.00	0.00	0.00
S.typhi B	5000	10.50	8.33	9.42	1.53	1.09
	3000	8.50	7.33	7.92	0.83	0.59
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00

Table 5: Antibacterial activity of methanol extract stem of *S. chirata* (Season-1&2)

Bacterial Strain	*Sample con. (µg/mL)*	*S1*	*S2*	*Mean*	*SD*	*SDE*
E. coli	5000	10.50	8.67	9.59	1.29	0.92
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B. Subtilis	5000	10.50	8.33	9.42	1.53	1.09
	3000	8.50	7.67	8.09	0.59	0.42
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B.meghaterium	5000	12.50	8.68	10.59	2.70	1.91
	3000	10.00	7.33	8.67	1.89	1.34
	2000	8.50	7.33	7.92	0.83	0.59
	1000	0.00	0.00	0.00	0.00	0.00
S.aureus	5000	11.50	9.33	10.42	1.53	1.09
	3000	9.00	8.33	8.67	0.47	0.34
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. vulgaris	5000	18.00	10.00	14.00	5.66	4.00
	3000	14.50	9.33	11.92	3.66	2.59
	2000	13.00	8.00	10.50	3.54	2.50
	1000	10.00	0.00	5.00	7.07	5.00
P. aeruginosa	5000	9.50	9.33	9.42	0.12	0.09
	3000	8.50	8.33	8.42	0.12	0.09
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. typhi A	5000	18.00	8.67	13.34	6.60	4.67
	3000	14.50	7.67	11.09	4.83	3.41
	2000	11.50	0.00	5.75	8.13	5.75
	1000	10.50	0.00	5.25	7.42	5.25
S. typhi B	5000	10.00	8.67	9.34	0.94	0.67
	3000	0.00	7.67	3.84	5.42	3.84
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00

to be resistant to this extract. Also mentioned in Table 6 and Fig. 6a and b.

Ethanol extract of the leaf of *S. chirata* showed (Table 7 and Fig. 7a) moderate activity against *E. coli* and *B. subtilis* strains with a zone of inhibition ranging from 7 to 8 mm. All other strains were completely resistant to the extract all concentration with this extract.

Ethanol extract of leaf of *A. paniculata* was effective against *E. coli, B. subtilis, B. meghaterium, S. aureus, S. typhi A, S.typhi B.* It is shown in Table 8 along with Fig. 8a and 8b.

Methanol extract of leaf *A. paniculata* is shown in Table 9 and Fig. 9a and b, was effective against *S. typhi A* with a maximum zone of inhibition at 14mm at higher concentration. Two strains *E. coli* and *P. vulgaris* were resistant at all concentrations. All other strains were found to have a significant zone of inhibition.

Petroleum ether extract of stem of *A. paniculata* did not show any activity against all these strains. All strains were found to be completely resistant to this extract. Methanol extracts of the roots of both plants gave irregular results, so they are not presented here. However, from the other data, it is quite obvious that both these plants have good potential for antimicrobial activities.

The highest zone of inhibition value was recorded in *S. chirata* plant, with stem material in methanol extract in the

Table 6: Antibacterial activity of Methanol extract of stem of *A. paniculata* (Season-1&2)

Bacterial Strain	*Sample con. (µg/mL)*	*S1*	*S2*	*Mean*	*SD*	*SDE*
E. coli	5000	0.00	8.67	4.34	6.13	4.34
	3000	0.00	7.33	3.67	5.18	3.67
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B. subtilis	5000	10.00	8.67	9.34	0.94	0.67
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B. meghaterium	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. aureus	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. vulgaris	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. aeruginosa	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. typhi A	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. typhi B	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00

Table 7: Antibacterial activity of ethanol extract of leaf of *S. chirata* (Season-1&2)

Bacterial Strain	*Sample con. (µg/mL)*	*S1*	*S2*	*Mean*	*SD*	*SDE*
E. coli	5000	8.67	0.00	4.34	6.13	4.34
	3000	7.67	0.00	3.84	5.42	3.84
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B. subtilis	5000	8.67	0.00	4.34	6.13	4.34
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B. meghaterium	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. aureus	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P.vulgaris	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. aeruginosa	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. typhi A	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. typhi B	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00

winter season with a value of 17.67mm with strains *P. vulgaris and S. typhi A* at 5000 µg/mL concentration on the other hand, *A. paniculata* showed the highest zone of inhibition value 14.33mm with strain *S. typhi A* in winter season in methanol extract of a leaf at 5000 µg/mL concentration.

Roy *et al.,* (2015) worked on *S. chirayita* and *S. cordata*, and found a good amount of antibacterial and antidiabetic potential. Additionally, they reported *S. chiratiya* species as a better species than *S. cordata*. Even in the current study, among *A. paniculata* and *S. chirayita*, *Swertia* proved to be better and more sensitive among different results. Nayak *et al.,* (2015) reported that among methanol and ethanol extracts, ethanol extract gave better results and was more effective against test microorganisms, whereas in the current study, methanol and ethanol both gave good effectiveness and sensitivity against test microorganisms. In the useful study, of *A. paniculata*, Singha *et al*., (2003) reported various activities such as antibacterial and antifungal activities. Similarly, *Andrographis* represented antimicrobial sensitivity in different extracts. In another interesting study, Mishra *et al.,* (2013) advocated that the methanol extract of *Andrographis* leaves exhibited strong antibacterial activities against gram-positive bacterial strains. Current results are also in the line work where *Andrographis* proved sensitive in antimicrobial studies.

Latif *et al.,* (2011) worked on *Swertia* and MRSA and found

Table 8: Antibacterial activity of ethanol extract of leaf of *A. paniculata* (Season-1&2)

Bacterial Strain	*Sample con. (µg/mL)*	*S1*	*S2*	*Mean*	*SD*	*SDE*
E. coli	5000	0.00	9.67	4.84	6.84	4.84
	3000	0.00	8.33	4.17	5.89	4.17
	2000	0.00	7.33	3.67	5.18	3.67
	1000	0.00	0.00	0.00	0.00	0.00
B. subtilis	5000	11.00	8.67	9.84	1.65	1.16
	3000	9.00	7.67	8.34	0.94	0.67
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B. meghaterium	5000	8.50	8.33	8.42	0.12	0.09
	3000	0.00	7.33	3.67	5.18	3.67
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. aureus	5000	12.00	9.33	10.67	1.89	1.34
	3000	9.00	0.00	4.50	6.36	4.50
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P.vulgaris	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. aeruginosa	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. typhi A	5000	14.50	9.67	12.09	3.42	2.41
	3000	12.50	8.67	10.59	2.71	1.92
	2000	11.00	0.00	5.50	7.78	5.50
	1000	10.00	0.00	5.00	7.07	5.00
S. typhi B	5000	8.50	9.67	9.09	0.83	0.59
	3000	0.00	8.67	4.34	6.13	4.34
	2000	0.00	7.67	3.84	5.42	3.84
	1000	0.00	0.00	0.00	0.00	0.00

Table 9: Antibacterial activity of methanol extract leaf of *A. paniculata* (Season-1&2)

Bacterial Strain	*Sample con. (µg/mL)*	*S1*	*S2*	*Mean*	*SD*	*SDE*
E. coli	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
B. subtilis	5000	11.00	11.33	11.17	0.23	0.17
	3000	0.00	8.67	4.34	6.13	4.34
	2000	0.00	8.00	4.00	5.66	4.00
	1000	0.00	7.67	3.84	5.42	3.84
B. meghaterium	5000	12.50	0.00	6.25	8.84	6.25
	3000	10.00	0.00	5.00	7.07	5.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. aureus	5000	13.50	10.00	11.75	2.47	1.75
	3000	10.00	8.67	9.34	0.94	0.67
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. vulgaris	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
P. aeruginosa	5000	0.00	0.00	0.00	0.00	0.00
	3000	0.00	0.00	0.00	0.00	0.00
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. typhi A	5000	14.50	9.67	12.09	3.42	2.41
	3000	12.00	7.67	9.84	3.06	2.17
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00
S. typhi B	5000	10.50	8.67	9.59	1.29	0.92
	3000	9.00	7.67	8.34	0.94	0.67
	2000	0.00	0.00	0.00	0.00	0.00
	1000	0.00	0.00	0.00	0.00	0.00

that the methicillin-resistant *Staphylococcus aureus* strain was sensitive to *S. chirata* plant and it showed a significant zone of inhibition compared to the standard drug. Similarly, in the current study, ZOI was recorded and showed sensitivity against different strains. In the review carried out by Kumar *et al.,* (2010), they expressed that *Swertia* is a very useful drug because of its medicinal uses. From our study, it is quite clear that both *Swertia* and *Andrographis* are biologically sensitive drugs that can be of immense use for treating various diseases.

Conclusion

In the study, ethanol and methanol extracts from two plants exhibited higher toxicity against specific bacterial strains. Both plant extracts demonstrated effectiveness against *Bacillus subtilis*. The observed antibacterial activity is likely due to active secondary metabolites and notably, solvent polarity plays a crucial role in showcasing potential antibacterial properties, possibly due to the presence of broad-spectrum antibiotic compounds. Interestingly, during the summer season, extracts from *S. chirata* and *A. paniculata* showed reduced antimicrobial activities at higher concentrations, while winter-season plant extracts yielded better results.

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Conflicts of Interest

The authors declare no conflict of interest.

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