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Evaluation Of The Impact Of Various Extracts Of Swertia Chirata On Antimicrobial-Resistant Strains Of Pathogens

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Abstract:

In several Asian nations, as well as other regions of the world, the plant *Swertia chirata* is commonly utilized as a herbal remedy. The current study aimed to assess Swertia chirata's impact on a range of antimicrobial-resistant bacteria. To achieve this, the plant's leaves and stems were extracted using ethanol and methanol. Gram-negative bacteria (Escherichia coli) and Gram-positive bacteria (Staphylococcus aureus) were used as test organisms. The agar well diffusion method was utilized to determine the antibacterial activity and calculate the Minimum Inhibitory Concentration (MIC). Using the broth dilution procedure, MIC was determined. Bacterial sp. demonstrated a significant zone of inhibition against all extracts out of all the strains. Methanol and ethanol were employed as controls. The results indicated that the ethanol extract of the stem and leaves was superior to the methanol extract in terms of its ability to the methanol extract.

Keywords**: **Swertia chirata, methanol extract, Ethanol extract, Antimicrobial-resistant,

INTRODUCTION

Natural resources have traditionally been a significant source of therapeutic substances. Based on their application in conventional medicine, a remarkable number of contemporary medications have been separated from or derived from natural sources. Both the quantity of microbiological strains resistant to multiple drugs and the emergence of strains with decreased antibiotic susceptibility are constantly rising. Written documents dating back more than five millennia document the use of medicinal herbs as a means of healing sickness. Records from Northeast China and ancient Indian civilizations show that it is unquestionably a practice as old as humanity. Plant parts that are employed as sources of medicinal extracts include roots, stems, fruits, flowers, and twigs. the unrefined plant extracts In the context of therapeutic treatment, identifying recognized antibacterial properties is crucial. Swertia chirata is a seasonal herb that grows annually, biennially, or perennially in the Gentianaceae family. Its stem is elongated and can be anywhere between 60 and 150 cm in length. The stem rises in a quadrangular shape from a cylindrical base. When the plant is young, the stem is greenish-brown; as it ages, it becomes light brown or light violet in color. Typically, the roots are light brown, 5–10 cm long, slightly twisted, and taper off over time. Its opposing pair of leaves are about 10 cm long, pointy at the apex, and lack stalks. The seeds are tiny and range

in color from light brown to dark brown. The flowers are greenish-yellow with a hint of purple. It is typically found between 1200 and 3000 meters above sea level. Swertia chirata is an herbaceous plant. It is recognized to possess antibacterial properties against a range of microorganisms. The physiologically active components of Swertia chirata, which include gentiopicrin, oleanolic acid, and amarogentin-swerchirin triterpenoids, are thought to be responsible for its antibacterial properties (Abdul et al, 2011). Numerous illnesses, including bronchial asthma, liver problems, fever, anemia, diarrhea, and stomach problems, can be helped by it. Additionally, it demonstrates a number of biological actions, including hepatoprotective, antihelminthic, antibacterial, antioxidant, anticancer, and hypotensive properties.

A variety of factors, including human behavior, can either accelerate or exacerbate the development of resistance to various antimicrobial drugs. Microbes adapt to or die in response to any real or suspected infection when an antimicrobial agent is used, regardless of the dosage or duration of treatment. We refer to this procedure as "selective pressure." Microbes that are able to adapt and survive possess resistance genes, which can be passed on to other bacteria through reproduction. This study looks at how Swertia chirata extracts in ethanol and methanol affect different pathogen strains that are resistant to antibiotics. MIC was investigated in relation to these test microbes.

MATERIALS AND METHODS

Collection of plant material:

The Kauntalani Nursery, which is situated at N-300 45'123" latitude and E-0770 53'00" longitude, and is 2580 meters above sea level [Fortrex 201 GPS], is where the plant specimens of Swertia chirata were obtained. Under the aegis of the Uttarakhand Forest Development Corporation, the Aushdheeya Vanaspati Van Sanstha, Uttaranchal, and the Chakrata Forest Division, District Dehradun, Uttarakhand, jointly maintain the Kauntalani Nursery.

Processing of sample:

To get rid of any unwanted materials or dust particles that adhered to the leaves and stems of the plant sample, they were separated and cleaned using sterile distilled water. The leaves and stems were then dried in the shade, milled into a fine powder. For later usage, the powdered samples were kept in sterile, dry, and clean containers.

Preparation of alcoholic extract:

Native plant extracts were prepared using alcohol and the methods described by Davis. The alcoholic extract was obtained through the "Soxhlet Extraction" method, which entails continuous hot percolation. The dried and ground plant material was added to an extractor along with a cylinder of filter paper for extraction. The higher location of the plant material was carefully positioned below the upper section of the syphon. The menstruum, or solvent, ethyl alcohol, which was inside the flask that was boiling. Heat caused the menstruum to evaporate. The extractor was filled and emptied fourteen or fifteen times before the active components were completely extracted from the plant material. This process took four to six hours. Following the process, the concentrated active components from the plant material were transferred to sterile test tubes and stored there until they were required once more. For one hour, the tubes were kept at 500°C to burn out any leftover ethanol. After the dried and powdered stem and leaves were extracted using methanol and ethanol in a soxhlet apparatus, they were kept at 60°C for 48 hours. Whatman No. 1 filter was used to filter the extracts once they had cooled to room temperature. One filter paper and the filtrate it generated were evaporated until they were completely dry in a flash evaporator. The dried extracts were then scraped off and stored in a clean, sterile container for future use.

Ethanol extraction

Following minor adjustments to take into consideration the peculiarities of the lab, the ethanol extraction process was completed in compliance with previously released guidelines. The pulverized powder was weighed in 100-gram increments, immersed in ten times its volume of ethanol, and allowed to cool on a magnetic stirrer for an entire day. The extracts were first passed through two layers of muslin fabric before being filtered through Whatman No. 1 filter paper. The filtrate was air-dried at a low temperature of 500°C and stored at -200°C until needed again.

Methanol extraction

With only minimal adjustments made to account for the unique conditions of the laboratory, methanolic extraction was carried out in accordance with previously published protocols. Every 100 grams of pulverized powder were weighed, submerged in ten times as much methanol, and left to cool on a magnetic stirrer for a whole day. The extracts were filtered using Whatman No. 1 filter paper after initially passing through two layers of muslin fabric. After the entire amount of methanol had evaporated, the filtrate was air-dried at a low temperature of 50°C. The powder was then weighed to determine the yield and redissolved in methanol to the proper concentration. The extract was diluted to a preset concentration and kept cold (-200°C) until needed.

Test microorganisms

Using different antibiotics or antimicrobial medications, both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) organisms were grown on sterile media, creating strains that were resistant to medicines.

Antibacterial activity by agar well diffusion method

The Agar Well Diffusion method was used to test the antibacterial activity of various concentrations of ethanol and methanol extracts from the leaves and stems of the plant under study on a number of bacterial strains. To determine the minimum concentration of extracts that can inhibit the growth of microorganisms, the MIC (minimum inhibitory concentration) of each extract was calculated. Sterile petri plates were filled with 20 milliliters of sterile Mueller Hinton Agar and left to solidify. Next, use a sterile cotton swab to swab the test organisms' overnight broth culture. Using a sterile cork borer, wells were created, and 20 µl of ethanol and methanol extracts at various concentrations (ranging from 100 to 5000 µ g/ml) were applied to each well. After that, the plates were incubated for 24 hours at 37°C. The zone of inhibition was measured to determine the antibacterial activity. To determine the effectiveness of the plant under study's leaves and stem, the outcomes of the ethanol and methanol extracts of the leaves and stems were examined.

MIC (Minimum Inhibitory Concentration):

Test tubes were filled with 5 mL of sterile nutrient broth and varying doses of plant extract (100-5000µg/mL) to determine the Minimum Inhibitory Concentration (MIC). Following this, 100 µl of an overnight broth culture containing multiple test organisms were added. Afterward, each microbial culture was incubated in test tubes for 18 to 24 hours at 37ºC. The Microorganism-killing concentration (MIC) is the lowest concentration of the extract that inhibits the growth of microorganisms.

RESULTS AND DISCUSSION

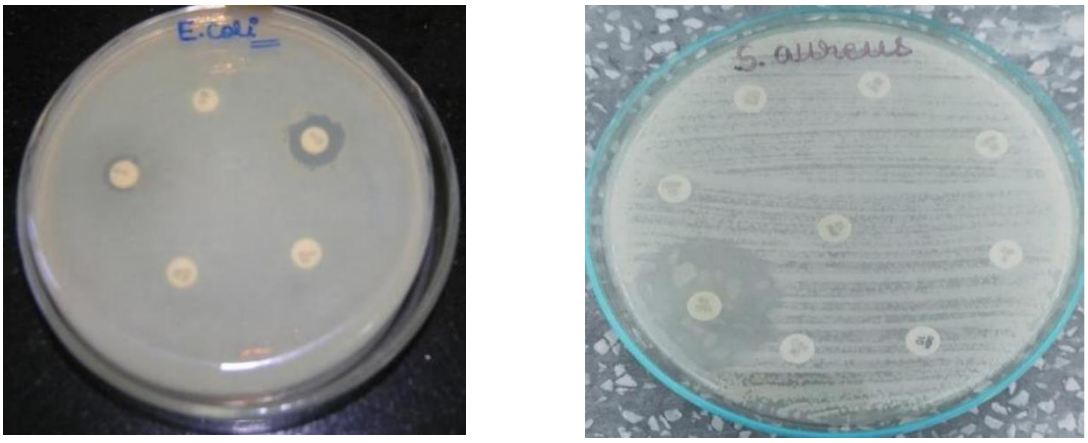
Tables I–IV show the outcomes of the antimicrobial sensitivity tests conducted on the leaves and stem of the plant Swertia chirata, using both ethanol and methanol extracts, against strains of antimicrobial-resistant bacteria.

The antibiotic sensitivity patterns of the test bacteria.

The test organisms, including Staphylococcus aureus and Escherichia coli isolates, were examined for antibiotic sensitivity and resistance. The results of the antibiotic sensitivity patterns of the bacterial isolates under investigation are shown in Table 1.

Table 1: Antibiotic sensitivity and resistance of different test organisms in the SensiDisc diffusion test.

S.NO.	Test Organism	Resistant	Sensitive
1	Staphylococcus aureus	Amoxycillin,	Bacitracin,
		Ampicillin,	Chloramphenicol,
		Ciprofloxacin,	Ciprofloxacin,
		Doxycycline,	Gentamicin,
		Methicillin,	Kannamycin,
		Penicillin,	Neomycin,
		Sulfadizine	Vancomycin
2	Escherichia coli	Amoxycillin	Amoxycillin
		Bacitratin,	Chloramphenicol,
		Ciprofloxacin,	Ciprofloxacin,
		Penicillin,	Gentamicin,
		Sulfadizine,	Kannamycin,
		Vanocomycin,	Methicillin,
		Ciprofloxacin	Neomycin



(A) **(B)**

Figure 1: Antibiotic resistance of bacterial strains. (A) *E. coli.* (B) S. aureus.

Antibacterial activity of methanol and ethanol extracts.

Significant efficacy was demonstrated by the ethanol leaf extract against these bacterial strains that were resistant. Zones of inhibition were only observed at higher doses, and S. aureus and E. coli were resistant at lower concentrations. All of these bacteria were effectively inhibited by the stem's ethanol extract, with S. aureus showing the largest zone of inhibition (20 mm at 5000µg/ml). The methanol leaf extract exhibited a moderate level of efficacy against S. aureus and E. coli. A moderate level of activity was demonstrated by the stem methanol extract against S. aureus (9-10 mm at 2500-5000 µg/ml).

Both the ethanol and methanol extracts proved efficient against these bacterial strains in the current investigation. When it came to these bacteria, the ethanol extract outperformed the methanol extract. From the research mentioned above, it can be inferred that the plant extract's potential efficacy may stem from the presence of broad-spectrum antibiotic components or secondary metabolites. The potential antibacterial activity seems to be significantly influenced by the solvent's polarity (Ahirwal et al, 2011). The ethanol extract of the leaf was tested in this study against various bacterial species, and at increasing doses between 100 and 5000 µg/ml, it showed substantial efficacy against them.

Table 2 presents a correlation and explanation of the results of the antibacterial assay and the Minimum Inhibitory Concentration (MIC). In the current study, the ethanol and methanol extracts were effective against a range of bacterial species. The ethanol extract was more successful in killing the bacteria than the methanol extract.

Table 2 : The ethanol extract of Swertia chirata leaves has antibacterial properties against a range of microorganisms.

	Dilution(µg/ml)	**Zone of inhibition (mm)**	
		Staphylococcus aureas	***Escherichia coli***
	100	-	-
	250	-	-
	500	-	-
	1000	-	-
	2000	-	-
	2500	10	10
	3000	11	11
	3500	12	13
	4000	13	15
SCE (LEAF)	**5000**	14	15

Table 3: The ethanol extract of Swertia chirata's stem has antibacterial action against a variety of bacterial species.

Plant extract	Dilution(µg/ml)	Zone of inhibition (mm)	
		Staphylococcus aureus	***Escherichia coli***
	100	-	-
	250	-	-
	500	-	-
	1000	12	-
	2000	12	-
	2500	15	12
	3000	15	13
	3500	16	14
	4000	17	14
SCE (STEM)	**5000**	20	15

Table 4: The methanol extract of Swertia chirata leaves has antibacterial activity against a range of bacterial species.

Plant Extract	Dilution(µg/ml)	Zone of inhibition (mm)	
		Staphylococcus aureus	***Escherichia coli***
	100	-	9
	250	-	9
	500	-	10
	1000	-	10
SCM (LEAF)	2000	-	10
	2500	-	11
	3000	-	11
	3500	-	11
	4000	-	11
	5000	-	11

Table 5: The methanol extract of Swertia chirata's stem has antibacterial activity against a range of bacterial species.

Plant Extract	Dilution(µg/ml)	Zone of inhibition (mm)	
		Staphylococcus aureus	***Escherichia coli***
	100	-	-
	250	-	-
	500	-	-
	1000	-	-
	2000	-	-
SCM (STEM)	2500	9	-
	3000	9	-
	3500	9	-
	4000	10	-
	5000	10	-

CONCLUSION

We found that there was moderate action against *Escherichia coli* and *Staphylococcus aureus* in our investigation. Using an ethanol extract, modest efficacy against *Staphylococcus aureus and Escherichia coli* is shown. Our results were comparable to those of Nik et al. (2013), who found that *Escherichia coli* and *Staphylococcus aureus* were sensitive to higher doses, and of Jesmin et al. (2007) and Lwin et al. (2013), who found that *Escherichia coli* and *Staphylococcus aureus* were sensitive. Regarding the methanol extract, *Staphylococcus aureus* showed resistance.

This was corroborated by Ahirwal et al. (2011), who also discovered that *Staphylococcus aureus* were resistant. Furthermore, considering the increasing demand for new medications, plant extracts may provide a compelling alternative for treating a variety of illnesses and chronic diseases. These investigations can assist in the identification of new therapeutic agents with minimal or no side effects, as a result of MDR bacteria and the adverse effects of synthetic medications.

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