

## In-Vitro Antimicrobial Activity And Phytochemical Study Of An Indigenous Plant Species Of Meghalaya

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### ABSTRACT

**Objective:** Antimicrobial activity refers to the ability of substance, such as a drug, chemical, or plant extract to inhibit the growth or destroy microorganisms, including bacteria, fungi, viruses and parasites. The plant *Anemone rivularis* Buch.-Ham. ex. DC., indigenous to the northeastern state of Meghalaya, is traditionally believed to possess pharmacological properties for the treatment of microbial infections, inflammations, and cancer related conditions. This study aims to evaluate the in-vitro antimicrobial potential and identify the active phytoconstituents present in the leaves of *A. rivularis*.

**Methods:** The leaves were subjected for methanolic extraction. The crude extracts were tested against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli* for antimicrobial activity using agar well diffusion and UV spectrophotometer methods. Minimum inhibitory concentrations (MICs) for active crude extracts were done using agar well diffusion technique.

**Results:** It was observed that the crude extracts showed highest activity against the selected bacterial strains with minimum inhibitory concentrations (MIC) of 60 µL. The activity indices and zones of inhibition were compared with commercially used standard antibiotic azithromycin and Penicillin G and provide evidence of the methanolic extract's antibacterial efficacy. The UV spectrophotometry method also showed significant inhibition zone at a wavelength of 600nm against the bacterial strains. TLC with phytochemical study showed the presence of active phytoconstituents like flavonoids, tannins, phenolic compounds, triterpenoids & steroids.

**Conclusion:** These results suggest that *A. rivularis* contains different phytoconstituents having medicinal properties and justify the traditional use for the treatment of antimicrobial diseases. However, research on in-vivo methods along with toxicity study is recommended to validate the safety for society.

**Keywords:** *A. rivularis*, thin layer chromatography, minimum inhibitory concentration, antimicrobial activity

### 1. INTRODUCTION

According to the World Health Organization, traditional medicine is the total of all knowledge, skills, and practices based on various beliefs, theories and experiences native to different tribes or cultures, whether understandable or not. It is applied in maintaining health as well as in prevention, diagnosis, improvement, or treatment of mental and physical disease. Different traditional medicine systems are backed up by a plethora of literature and documents of theoretical knowledge and practice skills and some are transmitted generation to generation orally. Plants have been used by humans to treat infectious diseases that are common since ancient times, and some of these old remedies are even now used to treat a number of diseases (1). Microbes are found in all domains of life and include bacteria, fungi, protozoa, algae, and viruses. An antimicrobial is an agent that kills microorganisms (microbicide) or stops their growth (bacteriostatic agent). The evaluation for antimicrobial agent of plant origin begins with thorough biological evaluation of plant extracts to ensure efficacy and safety. Antimicrobial

phytochemicals fall into several categories like phenolics/polyphenols, terpenoids and essential oils, alkaloids, and lectins and polypeptides.

Infectious diseases caused by microbes remain a significant global health challenge. Historically, such diseases have posed serious threats to both human and animal health, contributing substantially to morbidity and mortality worldwide. The use of natural products for therapeutic purposes dates back to ancient times; for centuries, minerals, plants, and animal-derived substances were the primary sources of medicine. Traditional healing practices, particularly plant-based remedies, have played a crucial role in healthcare, with approximately 80% of the world's population still relying on traditional plant-based medicine today. In recent years, the rise of antibiotic resistance has become a critical global concern. This issue, combined with the problem of microbial persistence, underscores the urgent need for the development of novel antimicrobial agents. These new drugs must not only combat drug-resistant pathogens but also effectively eliminate persistent microorganisms and reduce the duration of treatment (2).

Traditional medicine refers to healing practices, knowledge, and beliefs using natural resources, primarily plant-based remedies developed over generations within various cultures. On the other hand, Western medicine is based on scientific research, evidence based practices, and standardized pharmaceutical drugs or surgical interventions. Therefore, traditional medicines viewed as an efficient and acceptable system from cultural perspective. This research aim to investigate the antimicrobial efficacy and evaluate the active phytoconstituents that are responsible for different medicinal properties present in *A. rivularis*. *A. rivularis* has been traditionally employed in herbal formulations in powdered or decocted state. It is used extensively in traditional medicine to treat gastrointestinal disorders, improve blood circulation, and heal wounds. The plant is extensively used in traditional medicine, primarily for diseases such as arthritis, because of its anti-inflammatory properties in reducing swelling and alleviating pain in the joints. Its extensive range of therapeutic applications in various healing systems is evidenced by the fact that traditional healers also employ it to cure fever and respiratory diseases (3). Even though it has been used for centuries, scientific studies are in their early stages.

## 2. MATERIAL AND METHODS

### 1. COLLECTION OF PLANT

The plant *A. rivularis* Buch. Ham. ex. DC was collected from a village in Mihmyntdu, Jowai, West Jaintia Hills, Meghalaya. The plant specimen was authenticated by Botanical survey of India (BSI), Shillong and voucher specimen (BSI/ERC/Tech/2022-23/209) deposited.

### 2. EXTRACT PREPARATION

After collection the leaves were air dried under shade at room temperature, ground into powder and extracted by following two methods- maceration and percolation using methanol according to standard extraction methods (4). The powdered plant material was mixed thoroughly with the solvent and kept for extraction separately. For maceration method the mixture was kept for 72 hours with moderate shaking. For percolation method Soxhlet apparatus was used and standard procedure was followed. Both the extracts were filtered and dried crude extracts were obtained after evaporating the solvent using rotary evaporator followed by lyophilizer for uniform drying.

### 3. ANTIMICROBIAL ACTIVITY

#### 3.1 Sources of microorganism

Pure cultures of bacteria; *Staphylococcus aureus* ATCC BAA976, *Escherichia coli* ATCC 25922, *Streptococcus pyogenes* ATCC 19615 and *Pseudomonas aeruginosa* ATCC 27853 (from School of Applied Sciences, University of Science & Technology, Meghalaya) were maintained on nutrient broth at 4°C. (5)

#### 3.2 Agar well diffusion method

##### 3.2.1 Preparation of nutrient broth

1.5 grams of nutrient broth was weighed and taken in a conical flask. 100 ml of distilled water was added and mixed properly. With the help of cotton plug the mouth of the conical flask containing the media was sealed properly to make it airtight. The conical flask was then put inside the autoclave for sterilization for around 15-20 minutes at 37 °C at 15 psi.

##### 3.2.2 Incubation of microorganisms

Four cultural tubes were autoclaved for sterilization. After making sure the cultural tubes were completely dry the nutrient broth was added equally. Add the microorganisms namely *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* with help of micropipette. While adding the microorganisms the pipette tip was changed after a single use. Then the cultural tubes were left in the incubator overnight at 37-39°C which is an optimal temperature for the growth of bacteria.

##### 3.2.3 Preparation of media

All the apparatus including conical flask, beaker, spreader and petri dishes were washed properly. With the help of aluminum

foil, the apparatuses were covered and autoclaved for sterilization. 3.9 grams of Muller-Hinton Agar were weighed and transferred into conical flask. Add 100ml of distilled water in the conical flask containing the Muller-Hinton Agar and mixed properly avoiding any lumps. Cover the conical flask with cotton plug to avoid spillage. All the washed apparatus including the media prepared were autoclave for 25 minutes at 121°C at 15 psi.

### 3.2.4 Inoculation of microorganisms

After autoclaving process was completed, all the apparatus including the media were put in laminar air flow to evaporate all the moisture present in them. All the apparatus (petri dishes, cork borer, scissor, lamp, micropipette, micropipette tips, marker, and cotton) were left under UV in laminar air flow for 10 minutes to avoid any sort of contamination. Pour the media equally into the petri dishes and allow them to solidify. Using micropipette, pipette out the microbial suspension and spread evenly on the surface of solidified media with the help of a spreader. After spreading, the spreader was put under lamp or swab with alcohol to avoid any sort of cross contamination. It is repeated for all the four bacteria. After the spreading was over, the petri dishes were covered and marked properly.

### 3.2.5 Well formation

Using a sterile cork borer or pipette tip, four wells of 6-8mm in diameter were punched out. Remove the agar plugs carefully to avoid distribution in surrounding areas.

### 3.2.6 Addition of test sample

Each well was filled with a sample of volume 30ml, 60ml, 90ml and 120 ml. Add the antibacterial agent namely Azithromycin for *Escherichia coli* and *Pseudomonas aeruginosa* and penicillin-G for *Staphylococcus aureus* and *Staphylococcus pyogenes*, in the middle of the plate.

### 3.2.7 Incubation

Incubate the plates in an incubator overnight at 37 °C for 24 hours. After 24 hours the plate was taken out and the zone of inhibition or diameter was measured for different concentrations. Activity indices for different concentrations were measured and the MIC (Minimum inhibitory Concentration) was calculated. Activity index is measured using equation as follows-

Activity index = Zone of inhibition by sample/Zone of inhibition by standard (5)

## 3.3 UV spectrophotometer method

Prepare 30ml of nutrient broth and autoclave it for 20-25 minutes. After autoclaving the nutrient broth, divide it equally into two parts in two separate cultural tubes. Then 100 µl of the bacteria *S. aureus* and *E. coli* were added in the cultural tube consisting of nutrient broth. These two cultural tubes consisting of bacteria and nutrient broth were left for 24 hours for incubation. Again prepare 50 ml fresh nutrient broth in a conical flask and autoclave it for 20-25 minutes. Eight separate culture tubes were taken (4 cultural tubes for each bacterium) and 4.5 ml of nutrient broth was added in each of the culture tubes. Rest of the nutrient broth is used as a blank solution. 500µl of *S. aureus* was added in 4 cultural tubes and 500µl of *E. coli* was added in 4 cultural tubes. After completion of the adding process, incubate the cultural tubes along with the remaining broth for 24 hours. After the incubation is done, the absorbance was checked in UV spectrophotometer using the remaining broth as a blank solution. The result should be decreased to its minimum inhibition (5).

## 3.4 Thin Layer Chromatography (TLC)

### 3.4.1 Phytochemical analysis

The active compounds were primarily identified by some preliminary phytochemical tests- for alkaloids (Dragendorff's test, Mayer's test and Wagner's test), for steroids and triterpenoids (Salkowski's test and Sulphur test), for flavonoids (alkaline reagent test, concentrated sulfuric acid test and lead acetate test), for tannins and phenolic compounds (Ferric Chloride, Lead Acetate, Gelatin, Bromine and Iodine tests), for saponins (froth test) and for proteins and amino acids (Biuret test, Millon's test, Ninhydrin test and Xanthoproteic test)(4).

### 3.4.2 TLC analysis

The bioactive constituents were finally confirmed and identified using TLC method on commercially available Aluminium silica gel TLC plates. For mobile phase preparation a mixture of chloroform: methanol: water in 70:20:10 ratio was prepared. The separated spots were observed under UV chamber in different wavelengths.

## 3. RESULTS

### ANTIMICROBIAL ACTIVITY

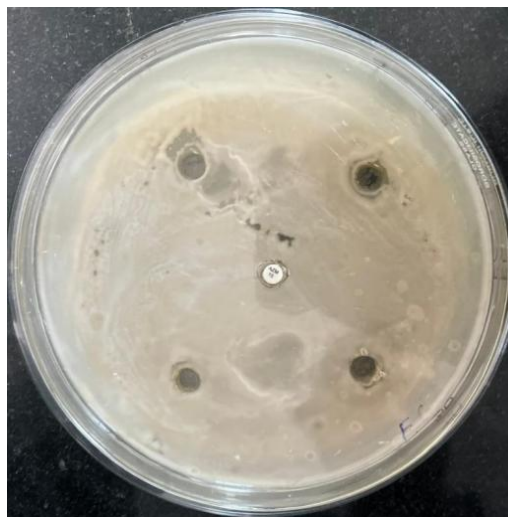
The antimicrobial activity was performed using agar well diffusion method and UV spectrophotometer method with the bacterial strains that are *E. coli*, *S. aureus* and *S. pyogenes*. The activity index and MIC (Minimum inhibitory concentration) were found out.

### 3.2 Agar well diffusion method

Agar well diffusion method was performed using two bacterial strains *E. coli* (gram negative) and *S. pyogenes* (gram positive) against standard antibiotics azithromycin and penicillin-G respectively. The inhibition zone of sample and standard against both *E. coli* and *S. pyogenes* were showed in figures as follows-



**Fig:1 Zone of inhibition of sample and standard against *E. coli***



**Fig:2 Zone of inhibition of sample and standard against *S. pyogenes***

The inhibition zone of the sample against *E. coli* was found to be 0.5cm at concentration 60  $\mu$ l in comparison with standard drug Azithromycin (15 mg) at 1.2cm. The MIC was found to be 60  $\mu$ l as shown in the table 1.

**Table 1. Activity Indices of different concentration of sample against *E. coli***

Sample	Concentration	Diameter	Activity Index
Methanolic extract of <i>A. rivularis</i>	30 $\mu$ L	0cm	0
	60 $\mu$ L	0.5cm	0.41
	90 $\mu$ L	0.8cm	0.66
	120 $\mu$ L	1.1cm	0.91

The inhibition zone of the sample against *S. pyogenes* was found to be 0.8 cm at concentration 60 µl in comparison with standard drug Penicillin-G (10mg) at 0.8cm. The MIC was found to be 60 µl as shown in the table 2.

**Table 2. Activity Indices of different concentration of sample against *S. pyogenes***

Sample	Concentration	Diameter	Activity Index
Methanolic extract of <i>A. rivularis</i>	30µL	0cm	0
	60µL	0.8cm	1
	90µL	0.9cm	1.12
	120µL	1cm	1.25

### 3.4 Thin Layer Chromatography

#### 3.4.1 Phytochemical Study

To confirm the presence of active phytoconstituents some preliminary phytochemical tests were performed. The result indicates the presence of various phytoconstituents like flavonoids, saponins, tannins and phenolic compounds, steroids and triterpenoids.

#### 3.4.2 TLC analysis

TLC was performed by using commercially available Aluminium silica gel TLC plates as stationary phase and for mobile phase a mixture of chloroform: methanol: water (70:20:10) was prepared. The spots were separated based on their polarity towards the stationary phase, observe under UV chamber and  $R_f$  values were calculated. Based on the  $R_f$  values phytoconstituents like triterpenoids, saponins, tannins, flavonoids, steroids and phenolic compounds were found to be present in the leaves of *A. rivularis*.

## 4. DISCUSSION

The organic extract of leaves of *A. rivularis* provide valuable insights into the bioactive compounds through phytochemical analysis and thin layer chromatography technique. These results suggest the presence of various active phytoconstituents as triterpenoids, saponins, tannins, flavonoids, steroids, phenolic compounds and their potential pharmacological activities associated with *A. rivularis* with wide therapeutic applications as antimicrobial agent.

The organic extract further provide evidence on the antimicrobial efficacy of the plant species. The methods used were agar well diffusion method against two bacterial strains, *E. coli* (Gram-negative) and *S. pyogenes* (Gram-positive) and UV spectrophotometer method against two bacterial strains, *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). The results suggest that these organic extracts could be used for management of bacterial diseases caused by *S. pyogenes* and *S. aureus* such as infection in skin, throat and blood. The organic extract (for agar well diffusion method) showed high activity against *S. pyogenes* when compared with *S. aureus*, with the minimum inhibitory concentrations (MIC) of 60 µL against standard antibiotic Penicillin-G. The comparison of activity indices and zones of inhibition signifies the antibacterial efficacy of the organic extract. The MIC of 60 µL for both bacterial strains indicate the lowest concentration at which the extract inhibits bacterial growth. For UV spectroscopic method the highest activity showed by *S. aureus* when compared with *E. coli*. The activity indices of absorbance of the organic extract against *S. aureus* was found to be +0.842 at a concentration of 30 µL when compared against *E. coli*.

These results validate the use of *A. rivularis* as traditional medicine. The finding of this study reported potent antimicrobial effect of organic extracts of the leaves of *A. rivularis* against selected bacterial strains *E. coli* (Gram-negative), *S. pyogenes* (Gram-positive) and *S. aureus* (Gram-positive) by comparison of different activity indices and zones of inhibition. This justified the traditional use of the plant for the treatment of skin, throat and blood infections. The literature survey done on the plant species elicited no previous research work on the antimicrobial activity of the crude extracts of leaves of *A. rivularis* from the Northeastern state Meghalaya. As such, this could be the first report on such activity and could be a start point for future drug research on a wide diversity of microbial pathogens.

The crude extract against *E. coli* were less active when compared to *S. pyogenes* and *S. aureus* respectively. These findings were correlated with the previous study by Zhao,2012, who reported moderate activity against the gram-positive bacteria and potent activity against gram-negative bacteria on the same plant species from China. However, the organic extracts were not active against the bacterial strain *P. aeruginosa*.

## 5. CONCLUSION

This study demonstrated efficacy of the crude extract of the leaves of *A. rivularis*, which were collected using available information on literature and has justified the ethnomedicinal property that were used by traditional practitioner in China for the treatment of inflammation and cancerous diseases. The information on traditional uses are important and can be used a prime source for research in drug discovery. The findings from this study justifies that *A. rivularis* contains a diverse range of bioactive compounds as evidenced by preliminary phytochemical study and TLC analysis. The in-vitro antimicrobial tests revealed that the organic extracts exhibit significant higher activity against both *S. aureus* and *S. pyogenes*. The Minimum Inhibitory Concentration (MIC) was found to be 60 µl (for *S. pyogenes*). The zone of inhibition for *S. pyogenes* was 0.8 cm when compared with standard antibiotic Penicillin-G. Notably, the extract showed comparable or superior efficacy to standard antibiotics, particularly against *S. pyogenes*. These findings support the traditional medicinal usage of the plant and underline its potential for developing natural antimicrobial agents. Further scientific evidences are needed for future research and benefit for the society.

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## 7. CONFLICT OF INTEREST

The author declares no conflict of interest.

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