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EVALUATION OF SOLVENT-DEPENDENT ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF ROOT EXTRACT FROM *Arundinaria suberecta* Munro

B. R. Chhetri[1], S. Jha[1,✉], N. K. Bhattacharyya[2], K. G. Dolma[3], and A. M. Das[4]

[1]Department of Chemistry, Sikkim Manipal Institute of Technology, Sikkim Manipal University, Majitar, Rangpo-737136, (Sikkim), India.
[2]Assam Women's University, Jorhat-785004, (Assam), India
[3]Department of Microbiology, Sikkim Manipal Institute of Medical Sciences, Sikkim Manipal University, 5th Mile, Tadong, Gangtok-737102, (Sikkim), India.
[4]CSIR-North East Institute of Science and Technology, Jorhat-785001, (Assam), India
✉Corresponding author: sangeeta.j@smit.smu.edu.in

ABSTRACT

The emergence of drugs derived from traditional medicinal plants has increased in recent years. *Arundinaria suberecta* Munro (Poaceae) is one of the significant plants traditionally used by healers in Sikkim for treating kidney stones. In this investigation, the antioxidant and antimicrobial potential of *Arundinaria suberecta* Munro (*A. suberecta* Munro) root extracts were investigated using various solvents. A Fourier transform infrared spectrometer (FTIR) was used for the identification of functional groups. The absorption band showed the presence of hydroxyl groups (3325 to 3365 cm^{-1}), C-H stretching (2800 to 2920 cm^{-1}) and carbonyl groups (1700 cm^{-1}), indicating the existence of phenols, fatty acids, terpenoids, tannins and saponins. The antioxidant efficacy of the samples was investigated using both the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the metal chelation assay. The methanolic extract demonstrated the strongest DPPH radical scavenging activity (94%) and significant metal chelating activity (69%), showcasing the richness of bioactive components in the extract. The lowest IC_{50} values were observed in methanol (16.34 µg/mL) and ethyl acetate extracts (38.23 µg/mL), reflecting strong antioxidant properties. The assessment of antimicrobial properties was carried out against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* using the well diffusion method. The ethyl acetate extract revealed notable antibacterial activity against *E. coli* (18 ± 0.9 mm) and *S. aureus* (17 ± 02 mm), while no antifungal activity was detected against *Candida albicans*. These results highlight the root extracts of *A. suberecta* Munro as promising sources of natural antioxidant and antibacterial agents, supporting traditional uses and offering potential for pharmaceutical development.

Keywords: *Arundinaria Suberecta* Munro, DPPH Assay, Antimicrobial, Phytochemicals, Natural Products, IC_{50}

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INTRODUCTION

The rapid global rise of antimicrobial resistance (AMR) represents an urgent public health challenge, threatening the effective treatment of infectious diseases worldwide.[1] The World Health Organisation (WHO) has recognised AMR as a major global health threat and has implemented a global action plan to raise awareness, improve understanding of resistance mechanisms, and reduce the indiscriminate use of chemotherapeutic agents.[2] Although the discovery of antibiotics revolutionised medicine in the 20th century, their overuse has resulted in increasing resistance across numerous pathogenic species.[3] In response, researchers have increasingly turned to alternative sources—including plants, microbes, and natural oils—for novel antimicrobial compounds. Traditional medicinal practices continue to offer significant health benefits in developed countries, complementing mainstream healthcare, particularly for marginalised populations.[4] The growing threat of antibiotic-resistant bacteria has further highlighted the potential of herbal remedies as effective antimicrobial agents.[5,6] Despite long-standing ethnomedicinal use, many plant species remain underexplored for their antioxidant and antimicrobial properties.[7] *A. suberecta* Munro (syn. *Drepanostachyum khasianum* (Munro)) is a perennial, tufted bamboo species that belongs to

the Poaceae family. It is characterised by short, thick rhizomes and culms that grow to a height of 250–400 cm with a diameter of approximately 12 mm. This species thrives in subtropical regions of Assam, the Eastern Himalayas, Myanmar, Nepal, and Sikkim. Traditionally, its roots are used by local healers in Sikkim for the treatment of kidney stones, an application often associated with inflammation, suggesting potential antimicrobial activity.[8–11] Other parts of bamboo, such as leaves and sap, have historically been employed to treat fever and respiratory ailments, supporting their medicinal significance in Traditional Chinese Medicine.[12,13] Phytochemical investigations have revealed the presence of alkaloids, phenols, flavonoids, carbohydrates, steroids, proteins, saponins, and glycosides in various bamboo species.[14] However, no prior studies have examined the antioxidant or antimicrobial activity of *A. suberecta* Munro roots, highlighting a clear research gap. This work focuses on evaluating the bioactive potential of *A. suberecta* Munro root extracts, providing scientific validation for its traditional use by identifying plant-derived compounds with potential therapeutic applications.

EXPERIMENTAL

Apparatus and Reagents

The materials used in this study included the root of *Arundinaria suberecta* Munro, DPPH, ascorbic acid, gentamycin, fluconazole, methanol, ethyl acetate, chloroform, petroleum ether, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* (ATCC 25922), *Candida albicans* (obtained as a clinical isolate from the microbiology laboratory), Muller Hinton Agar (MHA), Lactose Broth (LB), Potato dextrose Agar (PDA), 6 mm diameter paper discs, filter paper, and distilled water. The experiments were performed using standard laboratory equipment, including a digital analytical balance, a Soxhlet, a rotary evaporator (R II, V29/32, BUCHI, Switzerland), a refrigerator, an incubator, an Attenuated Total Reflection Fourier Transform Infrared Spectrophotometer (AT-FTIR Shimazu) and a complete set of glassware. Graphical and statistical analyses were conducted with Origin software.

Preparation of *A. suberecta* Munro Extract

The root of *A. suberecta* Munro was procured locally from Uttarey, West Sikkim, India, during September and October, under the supervision of the Forest and Environment Department, Government of Sikkim. The collected plant specimens were authenticated and deposited at the Regional Ayurveda Research Institute, Tadong, Gangtok, Sikkim, with accession number RARI 5498. The collected roots were shade-dried at ambient temperature and finely pulverised using a mechanical grinder. A total of 100 g of powdered root material was processed through sequential Soxhlet extraction using petroleum ether, chloroform, ethyl acetate, and methanol solvents. The resulting extracts were filtered, concentrated to dryness using a vacuum evaporator and used for further testing.

Fourier Transform Infrared Spectrometer (FTIR)

The identification of various functional groups in the extracts was determined from ATR-FTIR. The spectra were recorded on samples prepared as KBr disks in the 500 cm^{-1} to 4000 cm^{-1} range.

Antimicrobial Activity

The antimicrobial properties of the root extracts were investigated by the agar well diffusion method.[15] MHA plates were swabbed with *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *C. albicans* (obtained as a clinical isolate from the microbiology laboratory) in a PDA plate.[16] Each extract was prepared at a concentration of 50 $mgmL^{-1}$ in 10% DMSO, and wells measuring 6 mm were made with a cork borer. After adding 100 µL of the extracts to each well, the plates were incubated at 37°C for 48 hr for fungal strains and 24 hr for bacterial strains. After incubation, the zone of inhibition observed in the plates was measured in millimetres. The 10% DMSO served as the negative control, fluconazole (10 mcg) for fungal and gentamycin (10 mcg) for bacterial as positive controls.

Antioxidant Activity

2,2-diphenyl-1-picrylhydrazyl Assay

The DPPH scavenging assay was used to investigate the antioxidant potential of the root extracts.[17] Stock solutions of the root extracts and ascorbic acid (1 mg/mL) were prepared. For the assay, 3 mL of each sample solution (20-100 µg/mL) was combined with 1 mL of freshly prepared DPPH reagent and kept in

the dark for 30 minutes at room temperature. The optical density of the reaction mixtures was then recorded at 517 nm using a spectrophotometer. All experiments were conducted in triplicate. The antioxidant activity of the samples was quantified relative to ascorbic acid. The absorbance of the control solution, containing DPPH without any antioxidant, was also recorded. The DPPH radical inhibition percentage was evaluated using the following equation.

$$\text{DPPH RSA}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

Where $A_{control}$ is the control absorbance, and A_{sample} is the sample absorbance.

The inhibition data for each sample and ascorbic acid were plotted against concentration, and IC_{50} values were calculated from the resulting curves.

$$Y = mx + c \quad (2)$$

$$IC_{50} = \frac{(50 - c)}{m} \quad (3)$$

The sample having the minimum IC_{50} value indicates better antioxidant activity.

Metal Chelating Assay

Metal chelating activity is based on the binding of ferrozine to form a red complex with ferrous ions. The test was performed as described earlier with slight modifications.[18] Initially, 1 $mgmL^{-1}$ of the corresponding extract was prepared as a stock solution, and aliquots were diluted to concentrations of 2, 4, 6, 8, and 10 μgmL^{-1}. Subsequently, 1 mL of methanol was added to the samples, followed by 200 µL of 5 mM ferrozine and 50 µL of 2 mM ferrous sulfate heptahydrate. After allowing the reaction mixture to stand at ambient temperature for 10 minutes, the absorbance was recorded at 562 nm with a spectrophotometer. A standard solution of EDTA was prepared and analysed under the same conditions. A decrease in absorbance of the reaction mixture indicated higher Fe^{2+} chelation. For the control, all components were included except the test sample. The % inhibition in the metal chelating assay was determined using the equation below.[19] All experiment was done in triplicate.

$$\%\ \text{metal chelating} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (4)$$

Where $A_{control}$ = Absorbance of control and A_{sample} = Absorbance of sample. The graph of % chelating activity against concentration was plotted for each extract and standard.

The IC_{50} value was calculated by plotting the graph between % metal chelating vs. concentration. The sample with the lowest IC_{50} value indicates high chelating activity.

RESULTS AND DISCUSSION

FTIR Spectroscopy

The FTIR spectra shown in Fig.-1, depict a broad peak around 3325 to 3365 cm^{-1} of O-H stretching modes, indicating the existence of some polysaccharides and phenols. The peaks at 2800 to 2920 cm^{-1} result from stretching vibrations of aliphatic C-H bonds, characteristics of alkanes. Carbonyl groups were observed around 1700 cm^{-1}, indicating the presence of ketones, aldehydes, esters and carboxylic acids. The peaks around 1456 cm^{-1} are associated with aromatic rings or phenolic compounds, usually found in tannins and saponins. The peaks at 1110 to 1200 cm^{-1} are associated with C-H deformation and C-H bending vibration of fatty acids. The observed spectral peaks suggest the occurrence of alkaloids, phenols, flavonoids, tannins, and fatty acids, in agreement with earlier studies using spectroscopic techniques.[20] The existence of these phytochemicals significantly contributes to the biological activities of *A. suberecta* Munro.

Antimicrobial Activity

The antimicrobial properties of the different root extracts were investigated using the well diffusion method according to their inhibition zone against *S. aureus*, *E. coli* and *C. albicans* (Fig.-2 and Fig.-3). The results were compared with the standards, viz., Gentamycin (10 mcg) and Fluconazole (10 mcg). The results showed ethyl acetate extract at 50 $mgmL^{-1}$ exhibited strong antibacterial activity, with 18 ± 0.9

mm inhibition zone against *E. coli* and 17 ± 0.2 mm against *S. aureus,* respectively. The petroleum ether and methanol extracts were not sensitive to the tested organism. The chloroform extract was only sensitive to *S. aureus* with a 17 ± 0.2 mm zone of inhibition (Table-1).

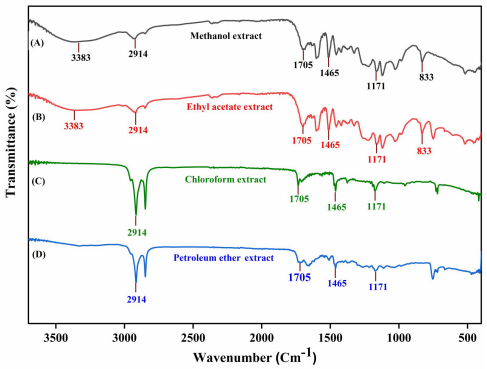

Fig.-1: FTIR Spectrum: Methanol (A); Ethyl Acetate (B); Chloroform (C), and Petroleum Ether (D) extract of Root *A. suberecta* Munro

These results strongly suggest ethyl acetate extract has bioactive components effective against the tested microorganism. Similar results were reported from the *C. serrulate*, where ethyl acetate extract exhibited strong antibacterial potency against *E. coli, S. aureus, K. pneumoniae,* etc., compared to other extracts.[21] Another report stated that ethyl acetate extract from *S. inducus* and *A. racemosa* exhibited significant inhibition zone against *E. coli, S. aureus* and other pathogens, suggesting the solvent's role in extracting the bioactive compounds for bacterial activity.[22,23] All the tested extracts were sensitive against *Candida albicans*, which may be due to inadequate quantities of bioactive phytochemicals in the extracts that target fungal components like ergosterol in the cell membrane or *β*-glucans in the cell wall. Also, *C. albicans* is a highly adaptable fungal pathogen due to several defence mechanisms, including biofilm formation and immune evasion.[24] Similar findings have been reported in studies where *C. albicans* was resistant to extracts of *Vicia faba*, *Morinda tomentosa*, and *Syzygium cumini* seeds.[24] By systematically investigating the phytochemical composition of plant extracts and optimising their preparation, future studies could explore ways to enhance their antifungal potential.

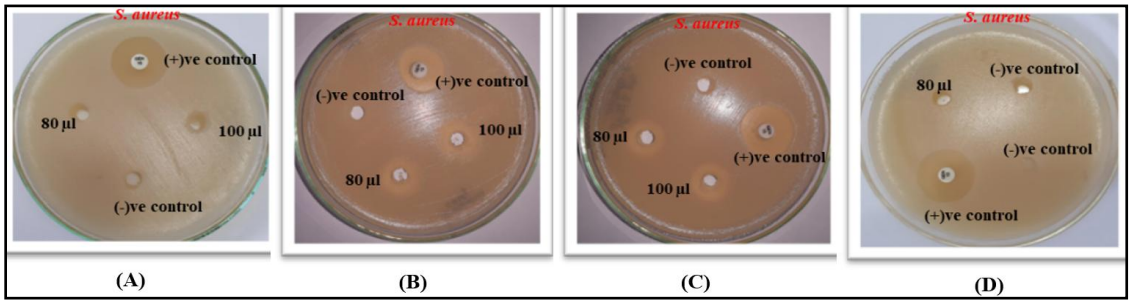

Fig.-2: Antibacterial Activity by Well Diffusion Method: Petroleum Ether (A); Chloroform (B); Ethyl Acetate (C), and Methanol (D) extract against *S. aureus*

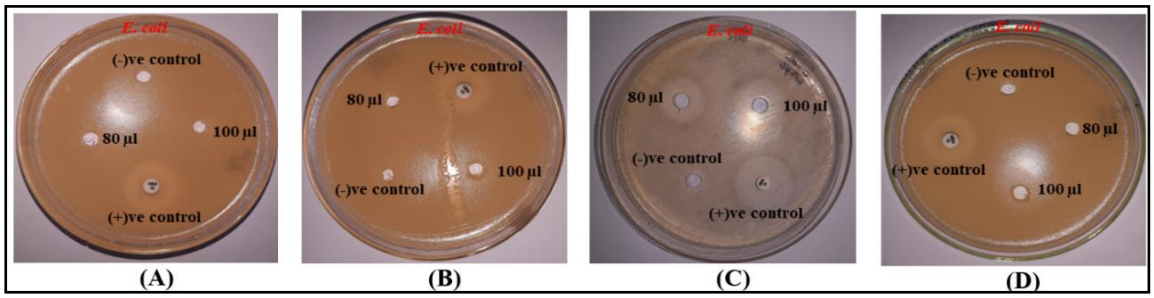

Fig.-3: Antibacterial Activity by Well Diffusion Method: Petroleum Ether (A); Chloroform (B); Ethyl Acetate (C); and Methanol (D) extract against *E. coli*

Table-1: Antimicrobial Activity of Root Extract of *A. suberecta* Munro

Extract / Standard	ZOI against microbes (mean ± SD) (mm)		
	E. coli	*S. aureus*	*C. albicans*
Pet. ether	NA	NA	NA
Chloroform	NA	15 ± 0.1	NA
Ethyl acetate	18 ± 0.9	17 ± 0.2	NA
Methanol	NA	NA	NA
Gentamicin (μgmL^{-1})	27 ± 0.3	23 ± 0.1	ND
Fluconazole (μgmL^{-1})	ND	ND	20 ± 0.1

ZOI = Zone of inhibition in mm, NA= Activity not found, ND = Not Determined

Antioxidant Activity

The antioxidant property of *A. suberecta* Munro root extracts was investigated using DPPH radical scavenging and the metal chelating assay. The principle of the DPPH assay involves antioxidants transferring electrons or hydrogen to the free radical, leading to a visible colour change from violet to pale yellow or colorless. In the current study, all the extracts demonstrated dose-dependent DPPH scavenging activity, with the methanol extract displaying the maximum radical inhibition (94%), ethyl acetate (80%), chloroform (60%) and petroleum ether (43%), respectively. In comparison, ascorbic acid showed 97% radical scavenging (Fig.-4a). As compared to other extracts, the methanol extract demonstrated the lowest IC_{50} values (16.34 μgmL^{-1}), indicating strong radical scavenging potential. In comparison, IC_{50} values of ethyl acetate, chloroform and petroleum ether extracts were 45.62 μgmL^{-1}, 39.53 μgmL^{-1} and 112.5 μgmL^{-1}, respectively, with ascorbic acid having an IC_{50} of 28.40 μgmL^{-1}. The strong antioxidant potential of the ethyl acetate and methanol extract can be associated with the existence of phenols, flavonoids, and saponins. These phytochemicals can donate electrons and are known for their redox properties, which effectively neutralise free radicals. This observation is consistent with the previous study, where phenolic-rich extract showed significant radical scavenging activity.[25] For instance, methanol extracts (200 mgmL^{-1}) of *H. pinifolia*, *E. acoroides*, and *C. rotundata* demonstrated up to 70% scavenging activity.[26] In addition to radical scavenging, the metal chelating activity of the different extracts was determined, reflecting their competence to bind metal ions such as Fe^{+2}. Of all the tested extracts, the methanolic extract exhibited the highest metal chelating activity (69%), followed by ethyl acetate (30%), while chloroform and petroleum ether extracts showed minimal chelation (9%) (Fig.-4b). The IC_{50} value of the metal chelating activity for methanol, ethyl acetate, chloroform and petroleum ether root extract was 74 μg/mL, 38.23 μgmL^{-1}, 77.29 μgmL^{-1} and 699.15 μgmL^{-1}, respectively, with EDTA having a much lower IC_{50} value of 13.81 μgmL^{-1}. In both assays, the IC_{50} values correspond to the concentration of extract needed to scavenge 50% of DPPH radical activity or metal ion complex formation. Although ethyl acetate extract showed a lower DPPH scavenging activity than methanol, it displayed a comparatively better metal chelating IC_{50} value. This indicates that various classes of phytochemicals might be responsible for contributing differently to the two antioxidant mechanisms. The results support that *A. suberecta* root extracts, particularly the methanol extract, possess better antioxidant potential through both radical scavenging and metal ion chelation, which may be associated with their phenolic and flavonoid content.

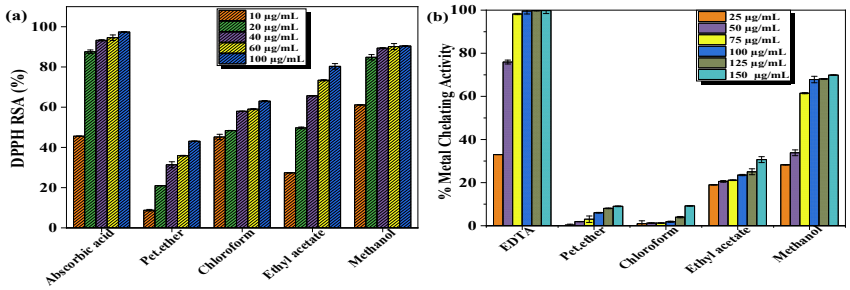

Fig.-4: Dose-dependent (**a**) DPPH Radical Scavenging Activity and (**b**) Metal Chelating Activity of Methanol, Ethyl Acetate, Chloroform, and Petroleum Ether Root Extract of *A. suberecta* Munro.

CONCLUSION

The present study highlights the significant presence of pharmaceutical phytochemicals in the root of *A. suberecta* Munro. Fourier Transform Infrared (FTIR) spectra confirm the existence of essential functional groups that are typically found in bioactive phytocompounds. Notably, the ethyl acetate extract exhibited strong antibacterial properties against various bacterial pathogens, demonstrating its potential as an antimicrobial agent. Additionally, DPPH and metal chelating assay results showed that both methanol and ethyl acetate extracts show higher antioxidant activity compared to others, suggesting that these solvents are particularly effective in extracting compounds with potent antioxidant properties. However, further phytochemical investigations are required to determine the specific bioactive compounds contributing to the plant's antimicrobial and antioxidant effects, which could lead to its broader application in modern medicine.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

B. R. Chhetri https://orcid.org/0000-0003-3816-0424
S. Jha https://orcid.org/0000-0003-1663-9354
N. K. Bhattacharyya https://orcid.org/0000-0002-4590-5298
K. G.Dolma https://orcid.org/0000-0001-7007-0774
A. M. Das https://orcid.org/0000-0001-6186-6981

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