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# Analysis of Antibacterial Effects of Methanolic Extract of *Drynaria propinqua* on Different Bacterial Strains

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## ABSTRACT

*Traditionally, Drynaria propinqua has been utilized for treating various conditions and for culinary purposes in the local community. The plant is recognized for its significant levels of phenol and flavonoids as well as its strong antioxidant properties. The primary aim of the research was to evaluate the antibacterial effects of plant root extracts on both Gram positive and Gram-negative bacteria. The plant sample was gathered in May 2023 from Kavresthali, Kathmandu and additional processing was carried out at the laboratory of Valley College of Technical Sciences, also located in Kathmandu. The samples were subjected to Soxhlet extraction to obtain the methanolic extract, which was then mixed with dimethyl-sulfoxide. The in vitro antibacterial activity against S. aureus, E. coli, P. aeruginosa, and K. oxytoca of various extract concentrations was assessed using the agar well diffusion method. The sample demonstrated noteworthy antibacterial activity against the organisms used in the test. This research demonstrated antibacterial effects on both Gram positive and Gram-negative bacteria.*

**Keywords:** *Drynaria propinqua, Methanolic, Bacteria, Soxhlet, Antibacterial, Laboratory.*

## ABBREVIATIONS

*E. coli-Escherichia coli*
*S. aureus-Staphylococcus aureus*
*P. aureginosa: Pseudomonas aureginosa*
*K. oxytoca: Klebsiella oxytoca*
*MHA-Muller Hinton Agar*
*CLSI: Clinical and Laboratory Standard Institute*
*AST: Antibacterial Susceptibility Test*
*DMSO-Dimethyl Sulfoxide*

# INTRODUCTION

*Drynaria propinqua* can be seen growing as an epiphyte on tree trunks covered with moss. It can be seen in the mountainous areas of Nepal, Bhutan, Tibet, South and Central China, Vietnam, Thailand, Malaysia, Myanmar, and Northern India. In Nepal, it is frequently seen in the forests of hilly and Himalayan areas at elevations between 800 and 3500 meters. [1]. *Drynaria propinqua* is a plant species belonging to family Polypodiaceae, epiphytes, growing in sunny places, compound leaves with 6-18 minor leaves per strand, dark green and hard texture, smooth edges and orderly two linear sori under leaf. It has fibrous, adventitious and

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dark brown roots. Rhizomes are long creeping, up to 1 cm diameter, very densely scaly throughout [2].

Different studies have shown the pharmacological activity of the plant. The roots and rhizomes of the plant are used locally to treat urinary infections, backache, headache, bone dislocation and sprains. The paste of the rhizome is used in fractured bone. The rhizome is found non-toxic and the extract is used as antidote for food and meat poisoning and also as antipyretics during food poisoning [2]. The extracts of the plant contain multiple organic components including flavonoids, phenols, alkaloids all of which are known to have antibacterial affects [3]. As the extract is rich in phenolic compounds, it shows high antimicrobial properties. The main antibacterial mechanism of phenolics is thought to be focused on the presence of cytoplastic membrane and the outer lipid membrane of the Gram-negative bacteria which provides additional protection to these bacteria from antibacterial compounds [4].

Phytochemical screening of *Drynaria* species shows the presence of alkaloids, flavonoids, phenols, resin, and tannins. Bacterial pathogen has developed different types of resistance to antibacterial agents, thereby causing significant increase in the resistant to different pharmaceutical agents. Antibacterial resistant threatens the effective treatment of an ever-increasing range of infections caused by different bacteria. *Drynaia* species have been used locally for treating different diseases. The purpose of this research was to evaluate data on the capacity of *Drynaria propinqua* extracts and their efficacy on the different microorganisms based on the agar gel diffusion method. Drynaria propinqua is an epiphytic or terrestrial fern of the family Polypodiaceae (Fig. 1). Rhizome is long, wide, creeping, terete, 1–2 cm in diameter; scales appressed, brown, peltate, 3–6 mm, 1–1.5 mm, margin dentate; fronds dimorphic, glabrous; basal fronds orbicular or ovate, 10–20 cm, 7–18 cm, pinnatifid up to 2/3 or more, margin irregularly dentate.

## MATERIALS AND METHODS

### Study Site and Duration

The study was conducted at Valley College of Technical Sciences from June to July 2023. In this timeframe, plant roots were collected from Kavresthali, Kathmandu, and later confirmed by the National Herbarium Centre in Godawari, Lalitpur, Nepal.

## Processing of Sample

### Washing, Drying and Grinding of Sample

The sample collected was first washed thoroughly with normal water. After washing, the sample was spread under the shade at room temperature to dry. The dried sample was then ground to obtain fine powder.

## Extraction of the potential plant material

### Soxhlet extraction (methanol extraction)

50 g of powdered root was extracted with methanol (800 ml) in Soxhlet apparatus. The boiling point of the solvent was set up to 70°C for 24 hours. The solvent was recycled, thereby extracting the compounds present in the sample. They were continuously extracted until solvent becomes colorless. Extract was concentrated by rotary evaporator at 85°C and diluted in DMSO [5].

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**Collection of Test Organism**

The bacterial culture was collected from Deparment of Microbiology laboratory, GoldenGate International College. The organisms in the study included were *S. aureus, E. coli*, *K. oxytoca* and *P. aeruginosa*.

**Preparation of Bacterial Cultures**

*E. coli, S. aureus, K. oxytoca* and *P. aeruginosa* were used as test organisms. Active cultures for each bacterial species were prepared by transferring a loopful of cells from the stock culture to the test tubes of nutrient broth and incubated at 37°c for 24 hours. The tubes were then compared with 0.5 McFarland Standard [6].

## Antibacterial Susceptibility Test

**Screening the antibacterial activity of methanol extracts of *D. propinqua***

Using the agar well diffusion method, *D. propinqua* root extract (methanol) was screened. According to the CLSI 2013's recommendation, the antibacterial activity of plant crude methanol extract was initially evaluated against the four tested bacteria using the agar well diffusion method. The bacterial suspension made on nutrient broth was swabbed on sterile MHA media with cotton swabs. The boreholes of 6 mm diameter were punched at equivalent distances on the surface by using a sterile cork-borer. The wells were fed with 30 µl of the plant extracts and DMSO as a control. These were then incubated at 37°C for 24 hours. A clear inhibition zone around the well suggested antibacterial activity.

**Confirmation by combined disk diffusion method**

Inoculum was prepared in normal saline by matching with 0.5 MacFarland turbidity standard. Lawn culture was made on MHA plate. Different types of antibiotics were placed on MHA plate keeping at a distance of 25 mm from center to center. The plate was incubated at 37°C for 24 hours. The size of inhibition was measured and was interpreted.

## RESULT

**Phytochemical Screening Result**

| S.N. | Test | Inference |
|---|---|---|
| 1 | Mayer's test (For alkaloids) | Negative (Absence of cream precipitate) |
| 2 | Ferric chloride test (For Phenols) | Positive (Presence of Intence purple to redish color) |
| 3 | Legal's test (For Glycoside) | Negative (Absence of violet color complex) |
| 4 | Alkaline reagent test (For Flavonoids) | Positive (Presence of intense yellow color) |
| 5 | Gelatin test (For Tannins) | Positive (Medium flows when the tube is tilted) |

## ANTIBACTERIAL ACTIVITY

**Antimicrobial activity against Gram positive bacteria**

Methanol extraction of *Drynaria propinqua* showed the antimicrobial activity against the gram positive Bacteria – *S. aureus*. Methanol extraction was tested as undiluted, double dilution and four times dilution i.e. 1, 1:1 and 1:3. Dilution was carried out in DMSO. 30µl of each diluted extract was taken for antimicrobial activity testing. Plant extract was diffused in

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6 mm well (by cork borer) in MHA. Among the dilution, undiluted methanol extraction of *D. propinqua* showed highest antimicrobial activity and antimicrobial activity was observed up to double dilution. Size of zone of inhibition and details are presented in Table 1.

**Table 1 Size of zone of inhibition and details of Gram-positive bacteria**

| S.N. | Organism | Dilution | Zone of Inhibition | Inference |
|---|---|---|---|---|
| 1 | *S. aureus* | Undiluted | 18 mm | Antimicrobial activity positive |
| | | 1:1 Dilution | 6 mm | Antimicrobial activity positive |
| | | 1:3 Dilution | 0 mm | No antimicrobial activity |
| 2 | DMSO (Control) | ---------- | 0 mm | No antimicrobial activity |

**Antimicrobial Activity Against Gram Negative Bacteria**

Methanol extraction of *D. propinqua* showed the antimicrobial activity against the gram negative bacteria – *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella oxytoca*. Methanol extraction was tested as undiluted, double dilution and four times dilution i.e. 1, 1:1 and 1:3. Dilution was carried out in DMSO. 30µl of each diluted extract was taken for antimicrobial activity testing. Among the dilution, undiluted methanol extraction of *D. propinqua* showed highest antimicrobial activity and antimicrobial activity was observed up to double dilution. Size of zone of inhibition and details are presented in below Table 2.

**Table 2 Size of zone of inhibition and details of Gram negative bacteria**

| S.N. | Organism | Dilution | Zone of Inhibition | Inference |
|---|---|---|---|---|
| 1 | *E. coli* | Undiluted | 10 mm | Antimicrobial activity positive |
| | | 1:1 Dilution | 8 mm | Antimicrobial activity positive |
| | | 1:3 Dilution | 0 mm | No antimicrobial activity |
| 2 | *K. oxytoca* | Undiluted | 8 mm | Antimicrobial activity positive |
| | | 1:1 Dilution | 6 mm | Antimicrobial activity positive |
| | | 1:3 Dilution | 0 mm | No antimicrobial activity |
| 3 | *P. aeruginosa* | Undiluted | 8 mm | Antimicrobial activity positive |
| | | 1:1 Dilution | 5 mm | Antimicrobial activity positive |
| | | 1:3 Dilution | 0 mm | No antimicrobial activity |

**Antibacterial Susceptibility Pattern of Test Gram Negative Bacteria**

*S. aureus* used in determination of antimicrobial activity of plant extract was subjected for determination of antimicrobial susceptibility pattern against set of 5 antibiotics using standard microbiological procedure. The test *S. aureus* was found to be resistant to Ceftazidime (30 µg) and Penicilin G (10 µg) and sensitive to Erythromycin (15 µg), Gentamicin (10 µg) and Ciprofloxacin (5 µg).

**Table 3 Antibacterial Susceptibility Pattern of Test Bacteria: S. aureus**

| | Cetazimed | Penicillin G | Erythromycin | Gentamicin | Ciprofloxacin |
|---|---|---|---|---|---|
| *S.aureus* | R (14 mm) | R (28 mm) | S (23 mm) | S (18 mm) | S (21 mm) |

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| | Cetazimed | Meropenem | Imipenam/ Piperacilin+ Tazobactam | Gentamicin | Ciprofloxacin |
|---|---|---|---|---|---|
| *E. coli* | S (21 mm) | S (23mm) | S (21 mm) | S (15 mm) | S (26 mm) |
| *K. oxytoca* | R (13 mm) | S (24 mm) | S (34 mm) | R (12 mm) | R (18 mm) |
| *P. aeruginosa* | R (18 mm) | S (19 mm) | S (21 mm) | S (15 mm) | S (25 mm) |

**Antibacterial Susceptibility Pattern of Test Gram Negative Bacteria**

Three different types of gram-negative bacteria were used for determination of antimicrobial activity of the extract. The test bacteria include *E. coli*, *K. oxytoca* and *P. aeruginosa.* These test bacteria were subjected for determination of antibacterial susceptibility pattern against Ceftazidime (30 µg), Penicilin G (10 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg) and Imipenam (10 µg) for *K. oxytoca*; Ceftazidime (30 µg), Penicilin G (10 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg) and Meropenam (10 µg) for *E. coli* and Penicilin G (10 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg) and Piperacillin+ Tazobactam (10 µg) for *P. aeruginosa.*

**Antibacterial Susceptibility Pattern of Test Gram Negative Bacteria**


*Figure 1 Antibacterial Susceptibility testing of S. aureus*


*Figure 2 Antibacterial Susceptibility testing of E. coli*

## DISCUSSIONS

Medicinal plants are nature's hidden and unexplored treasures that has been used throughout history for curing various ailments. The recent report of WHO shows that more than 80% of

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global population still rely on preparations made from plants [7]. The use of herbal and traditional medicines derived from natural sources is most common phenomenon in South Asia, and their uses have been widely reported among South Asian countries [8].

The use of plants for treating various diseases is as old as human species. Also, in many developing countries, traditional medicine plays an important role in meeting the primary health care needs of the population, and specific types of traditional medicine have been used for a long time [6,9]. Among different medicinal plants, *Drynaria* species have also been used as medicine esp. for the bone healing, cough, fever and urinary complications in different parts of the world [6]. They are a rich bio-resource for drugs of traditional medicinal systems, modern medicines, food supplements, folk medicines, pharmaceuticals and chemicals for synthetic drugs [10].

The global burden of infectious diseases caused by bacterial agents is a serious threat to public health. The emergence of antimicrobial resistance and toxicity issues subside the use of antibacterial agents. Safety and efficacy related limitations to antibiotics augment biological research on the antimicrobial role of plants due to comparable toxicity and efficacy [11].

From different studies, it was found that *Drynaria* extract is rich in phenol and flavonoids and are highly antioxidant which suggest their therapeutic use in antibacterial properties [12].

The main antimicrobial mechanism of phenolics is thought to be focused on the presence of cytoplasmic membrane and the outer lipid membrane of the Gram negative bacteria which provides additional protection to them [13,14].

The study on root extract of *D. propinqua* showed a range of different effects towards the bacterial organisms. The undiluted and double dilution extract of the plant showed antimicrobial activity against both gram positive and gram negative bacteria with clear zone of inhibition. However, the four- fold dilution showed no effect over the organism.

The finding agrees with previously published results for D. quercifolia that exhibited broad and concentration dependent antimicrobial activity [15].

All the bacteria isolates were subjected for antimicrobial susceptibility test against set of 5 antibiotics. Among the antibiotic used, Ceftazidime was found resistant over *S. aureus, K. oxytoca* and *P.aeuroginosa* but it was found to be sensitive over *E. coli.* Penicilin G was found resistant over *S. aureus, K. oxytoca, P.aeuroginosa* and *E.coli.* Meropenem was found sensitive over *E. coli* and *Klebsiella* whereas *Pseudomonas* was sensitive to Piperacillin Tazobactam combination. Gentamycin was found to be sensitive over *S. aureus, E. coli* but resistant over *Klebsiella.* Erythromycin was found to be sensitive over *S. aureus*. Ciprofloxacin was found to be sensitive over *S. aureus* and *E. coli* but resistant over *Klebsiella.*

It was concluded from the above experimental observation that the plant showed antibacterial activity over different strains at different concentration.

The properties of *D. propinqua* to inhibit bacterial growth can be related to their chemical components including phenols, flavonoids, glycosides, essential oils, tannin, saponin and

steroids. Antioxidant activity is one of the most significant biological properties of *D.propinqua*. A high correlation between phenolics and flavonoids content and antioxidant power of *Drynaria* species has been confirmed by several researchers [14, 15].
Findings of the present study are preliminary and for the investigation require to determine the exact nature of compound which may be present in the different parts of the plant.

Hence, it is clear that the *D. propinqua* possess number of health benefits and can prove to be potential medicine for various treatments. It showed significant inhibition against both gram-positive and gram-negative bacteria. So, proper exploration and experimentation of *D. propinqua* can be done to produce better medicinal treatments.

## CONCLUSION

Resistance to antibiotics poses a worldwide public health issue. Antibiotic resistance is on the rise in both nosocomial and community-acquired isolates. Researchers are attempting to find different methods or substances to combat antibiotic resistance. Research papers have consistently shown the numerous health benefits of the *Drynaria* plant. It has been extensively studied and has a long history of traditional use, making it a promising candidate in the fight against microorganisms. According to the findings from the current research, the methanol extracts of *Drynaria* demonstrated strong antimicrobial properties and could be developed into a therapeutic treatment. The benefits found in *Drynaria* can be used to help society by promoting health and preventing various illnesses. The current research demonstrates a thorough assessment of *Drynaria* plants in a laboratory setting to facilitate their commercialization. The effectiveness of plants in fighting bacteria is diverse and influenced by the levels of phytochemicals, bioactive qualities, and both synergistic and antagonistic effects concurrently. The results of the current research are initial and more studies are needed to ascertain the specific characteristics of the bioactive compounds that might exist in the root.

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## Conflict of Interest

The authors declare no conflict of interest

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