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# Microbiological Investigations of *Girardinia palmata* (Forssk.) Gaudich. as a Potential Antimicrobial Agent Against MDR Pathogens

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## ARTICLE INFO

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## ABSTRACT

The use of natural products isolated from plants against infection, cancer diseases and other oxidative-stress-related diseases is one of the cornerstones of modern medicine. Therefore, we tried to establish a combination of medicinal plants for the development of antibacterial agents against these MDR strains. The aim of the research was to understand the Pharmacognostic features of dried stem, leaves and roots of *Girardinia palmata* (Forssk) Gaudich. Further antibacterial activity of Plant *Girardinia palmata* (Forssk) Gaudich. against MDR strains was determined via agar well diffusion assay. All experiments were conducted in triplicate. The results showed that the plant extract were shown to have zones of inhibition from 6 mm to 26 mm against multidrug-resistant pathogens such as *Acinetobacter baumannii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Staphylococcus epidermidis* and Salmonella typhi. The plant composites were active against most of the tested microorganisms. Hence, the present study suggested the possibility of employing compounds present in plant for the development of new antibacterial agents, as well as efflux pump inhibitors. The results showed that the Plant extract were have zones of inhibition from 6mm to 26mm against multidrug-resistant pathogens such as *Acinetobacter baumannii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Salmonella typhi and Staphylococcus epidermidis*. This study can be used as an effective tool for the authentication and identification of this plant. Further research should be done for the possibility of employing compounds present in plants for the development of new antibacterial agents, as well as efflux pump inhibitors.

## INTRODUCTION

In developing countries according to the World Health Organization about 65–80% of the population of the world depends on plants for their health maintenance due to absence and deficiency of recent health services. Plant-derived medicines are main aids of treating relatively harmless than artificial alternatives, present in reflective therapeutic advantages. Mostly all herbal preparations were derived from plants, both as a complex or simple form of plant parts or in the form of mixtures or crude extracts etc. (Kayani et al., 2014). The term Pharmacognosy was derived from two words in Greek i-e "Pharmako" and "gnosis" which means knowledge and medicine or drug. (Velmurugan and Radhakrishanan, 2015). Plants as a new source of biologically active chemical compound, pharmaceutical plants have been used as treatments for years. Therapeutic plants have the enormous properties of drugs, modern medicines, folk medicines, nutraceuticals, food supplements, pharmaceuticals, intermediate and chemicals allowed for synthetic drugs. (Das et al., 2010).

In Pakistan the study and collection of traditional uses of plants have been increasing in last few years. About 6,000 species of wild plants are present in Pakistan in which almost 400-600 plants are medically important. Remedial plants are usually treated by Tibbi dawakhanas (Abbasi et al., 2010. To verify the pureness of drugs, the microscopic and macroscopic structure have main part (Babu et al., 2018). In addition to their vital function, plants have antiviral, antiparasitic, anticoagulant, antiproliferative, antioxidant, anti-inflammatory,

antibacterial, hepato-protective, antifungal, anti-diabetic, antitumor and insecticidal properties. *Girardinia palmata* belongs to the nettle family Urticaceae. Worldwide it consists of 54 genera, and 1465 species comprises herbs, shrubs, small trees and vines spread mostly in temperate, tropical and subtropical belts. (Bhellum et al., 2016). The Girardinia Gaudich is a fibre producing, economically important genus. (Ambrish et al., 2016). *Girardinia palmata* is commonly recognized as Kairi or Bichu Booti. It has a height of approximately 12 -18 feet. It most frequently occurred in the mountainous and hilly regions at altitudes up to 3000m (J.Chen et al., 2003). therefore, the plant extract is rapidly developing antibacterial products that are biocompatible to overcome the obstacle of bacterial colonization and nano-engineering in the medicinal delivery of drugs. The objective of this study is to explore the antibacterial potential of synthesized plant extract against MDR pathogens.

## MATERIAL AND METHOD

### Collection Authentication and Preservation

The Plant was collected in the month of July 2019 from the hilly areas of Domail Siran Valley Mansehra. Prof. Dr. Manzoor Hussain Chairman of Botany Department Hazara University Mansehra identified this plant. Further experimental work was done after identification of plant. The plant was additional confirmed by the help of Flora of Pakistan and the herbarium specimen present in Hazara University Mansehra.

### Drying and preparation of powder

After collection the plant specimen was then rinsed with tap water to remove the dust. The plant parts were then removed and left in shade for drying for almost 3 weeks. After drying, make it into fine powder with the help of electric grinder and kept in polythene bags.

### Extracts Preparation

Plants were first washed properly using distilled water and kept in a shady area to dry for several weeks because plants take a long time to dry. Then, the dried plants were ground to powder form using an electric grinder and, for further experiments, stored in plastic zipper bags. Around 50 g of root, stem and leaves was diluted in 250–400 mL of polarity-based solvents (methanol, Ethyl Acetate and n-hexane). The solutions were incubated afterward in a shaker incubator for 72 h at 40 °C and 120 rpm. Nylon and Whatman filter paper was utilized to filter the extract mix. The obtained filtrate at 60 °C was rotary desolventized. A fractional crude extract was obtained after drying the semi-solid composite for 3 days in an oven at 60 °C. After removing the residue, final composites were refrigerated at 4 °C until antibacterial testing.

### Bacterial Strain

The bacterial strains acquired from Microbiology department of Hazara University Mansehra *(Acinetobacter baumannii, Proteus mirabilis, Klebsiella pneumoniae, Salmonella typhi (gram-negative) Staphylococcus epidermidis, Staphylococcus aureus* (gram-positive) were maintained overnight in Luria Bertani liquid media, and 1 × $10^8$ cells $mL^{-1}$ were maintained.

### Antibacterial Test

The well diffusion technique was utilized to conduct antibacterial tests against MDR bacteria. The prepared media were mixed and autoclaved at 121 °C for 15 min. After cooling, media were added into Petri plates to solidify for 10 min. Then, 4 wells were created in every plate, using a 6 mm cork borer, one for the negative control and 3 for the plant composites employed in the ratio of 1:60 dissolved in a solution containing 50% DMSO. Bacteria were inoculated on each Petri plate using a sterilized spreader or cotton swab. DMSO solutions were used as controls. Antibiotics, ciprofloxacin and levofloxacin for gram-negative strain and lincomycin for gram-positive strain were used as a reference.

## RESULT AND DISCUSSION

Due to excessive antibiotic usage and inappropriate prescription, MDR infections represent a substantial threat to the therapeutic system worldwide [(Ali Syed, 2023)]. In this study, Plant extract was tested for their antibacterial activities. Our screening results confirmed that Plant extract exhibited moderate to remarkable antibacterial activity against several bacteria, including resistant and multidrug-resistant strains. No antibacterial analysis of *Girardinia palmata* has been conducted to date. Comparisons of our results have been made with the antibacterial activity of plant extracts belonging to different taxonomic groups.

### Antibacterial Potential of Methanolic Solvent

*Girardinia palmata* methanolic extract has strong anti-MDR pathogen activity. The ZOI of root, stem and leaves of *Girardinia palmata* against *Proteus mirabillis* was 17 mm,22 mm and 17 mm and the largest ZOI of root, stem and leaves of *Girardinia palmata* methanolic extract was 18 mm, 14mm and 25mm against ***Staphylococcus epidermidis*** (see Table 01) (**Figure 1**). Methanolic extracts were shown to have the most common antibacterial actions against Gram-negative bacteria as compared to Gram-positive pathogens. Gram-negative bacteria contain an outer membrane that is hydrophilic because of the occurrence of lipopolysaccharide molecules that enable smaller lipophilic compounds to move via the membrane, which is necessary for any antibacterial solute to penetrate the microorganisms' membrane. Methanol has low water solubility and can easily penetrate into the outermost membrane of Gram-negative pathogens, disrupting their metabolism and cellular function, leading to the death of the microbes (Khan, et al., 2023).

**Table 1**

Antibacterial Potential of Methanolic extract of *Girardinia palmata Against MDR pathogens*

| Tested bacteria | Leaf zone of inhibition (mm) | Stem zone of inhibition (mm) | Root zone of inhibition (mm) | Antibiotic ZOI (mm) |
|---|---|---|---|---|
| **Gram negative strains** | | | | |
| *Acinetobacter baumannii* | 11 mm | 17 mm | 18 mm | 33 mm |
| *Proteus mirabillis* | 17 mm | 22 mm | 17 mm | 30.3 mm |
| *Klebsiella pneumoniae* | 12 mm | 14 mm | 11 mm | 35.6 mm |
| *Salmonella typhi* | 9 mm | 14 mm | 11 mm | 31.6 mm |

| | | | | |
|---|---|---|---|---|
| **Gram positive strains** | | | | |
| *Staphylococcus epidermidis* | 25 mm | 14 mm | 18 mm | 24 mm |
| *Staphylococcus aureus* | 10 mm | 12 mm | 10 mm | 00 mm |

**Figure 1**
*Graph represent the antibacterial activity of methanolic extract Against MDR pathogens*

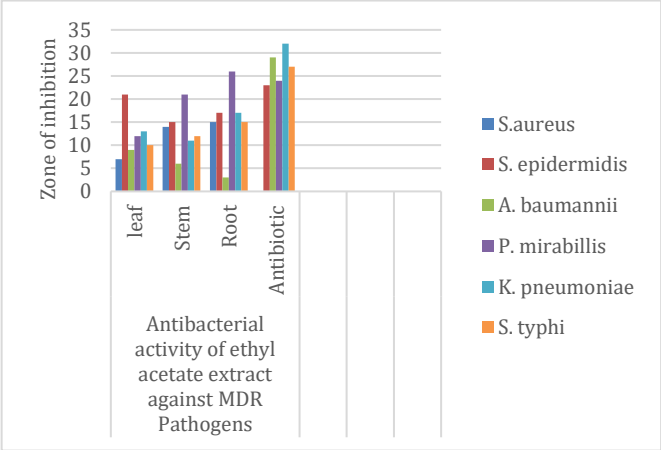



**Figure 2**
*Antibacterial activity of methanolic extract against P. mirabilis and A. baumannii*


**Antibacterial Potential of Ethyl Acetate Extract**

The antibacterial effect of ***Girardinia palmata*** ethyl acetate showed significant efficacy against ***Proteus mirabillis*** and ***Staphylococcus epidermidis*** among tested microorganisms. The maximum ZOI was displayed against ***Proteus mirabillis*** (26 mm) and minimum against ***Staphylococcus aureus*** (7 mm). (Table 2). Nevertheless, minimal action was shown against *A. baumannii*. In previous research, ethyl acetate extracts of oyster exhibited minimal and *reishi* showed no inhibitory zone against most of examined organisms (Ali, 2024). Based on our results and earlier investigations, ethyl-acetate mushroom extract was ineffective against most MDR bacteria. This might be because the antimicrobial compounds include polar flavonoids and terpenoids that can be extracted using a less polar solvent (Shah, 2023).

**Table 2**
*Antibacterial Potential of Ethyl Acetate Extract of Girardinia palmata Against MDR pathogens*

| Tested bacteria | Leaf ZOI (mm) | Stem ZOI (mm) | Root ZOI (mm) | Antibiotic ZOI (mm) |
|---|---|---|---|---|
| **Gram negative strains** | | | | |
| *Acinetobacter baumannii* | 09 mm | 06 mm | 03 mm | 29 mm |
| *Proteus mirabillis* | 12 mm | 21 mm | 26 mm | 24.2 mm |
| *Klebsiella pneumoniae* | 13 mm | 11 mm | 17 mm | 32.7 mm |
| *Salmonella typhi* | 10 mm | 12 mm | 15 mm | 27.6 mm |
| **Gram positive strains** | | | | |
| *Staphylococcus epidermidis* | 21 mm | 15 mm | 17 mm | 23 mm |
| *Staphylococcus aureus* | 07 mm | 14 mm | 15 mm | 00 |

**Figure 3**
Graph represent the antibacterial activity of Ethyl Acetate extract Against MDR pathogens

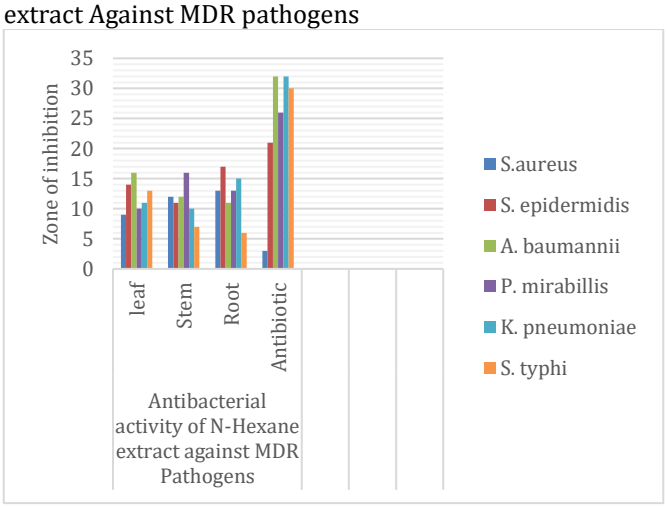
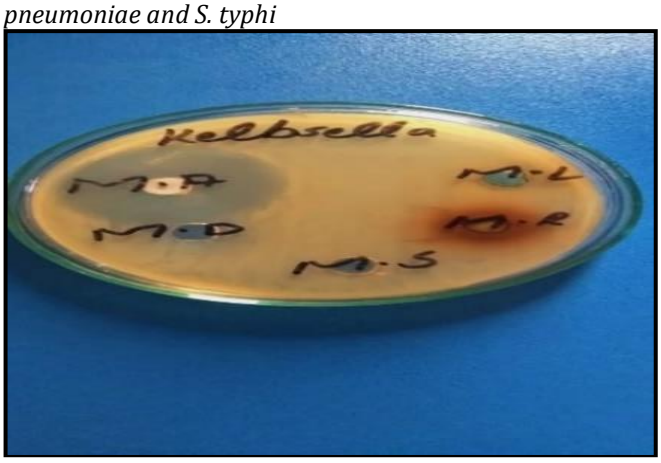



**Figure 4**
Antibacterial activity of Ethyl Acetate Extract against *K. pneumoniae and S. typhi*




### Antibacterial Potential of n-Hexane Extract

The n-hexane-derived extract exhibits strong antibacterial potential against examined MDR bacteria. The highest ZOI was examined against ***Staphylococcus epidermidis*** (17 mm), while the smallest was 06 mm against ***Salmonella typhi*** (see **Table 3**). n-hexane extracts were less effective against all tested pathogens than ethyl-acetate extracts. At 50 µL, the n-hexane extract of *V. sativa* showed the maximum antibacterial activity at 100 mg/mL concentration against *E. coli* (40 mm) [ (khan, 2023)]. Same as Methanolic extracts, n-hexane-derived extract were shown to have the most common antibacterial actions against Gram- negative bacteria as compared to Gram-positive pathogens.

**Table 3**

*Antibacterial Potential of N-hexane extract of Girardinia palmata Against MDR pathogens*

| Tested bacteria | Leaf ZOI (mm) | Stem ZOI (mm) | Root ZOI (mm) | Antibiotic ZOI (mm) |
|---|---|---|---|---|
| **Gram negative strains** | | | | |
| *Acinetobacter baumannii* | 16 mm | 12 mm | 11 mm | 32 mm |
| *Proteus mirabillis* | 10 mm | 16 mm | 13 mm | 26.2 mm |
| *Klebsiella pneumoniae* | 11 mm | 10 mm | 15 mm | 32.6 mm |
| *Salmonella typhi* | 13 mm | 07 mm | 06 mm | 30.6 mm |
| **Gram positive strains** | | | | |
| *Staphylococcus epidermidis* | 14 mm | 11 mm | 17 mm | 21 mm |
| *Staphylococcus aureus* | 09 mm | 12 mm | 13 mm | 03 |

**Figure 5**

*Graph represent the antibacterial activity of N-Hexane extract Against MDR pathogens*

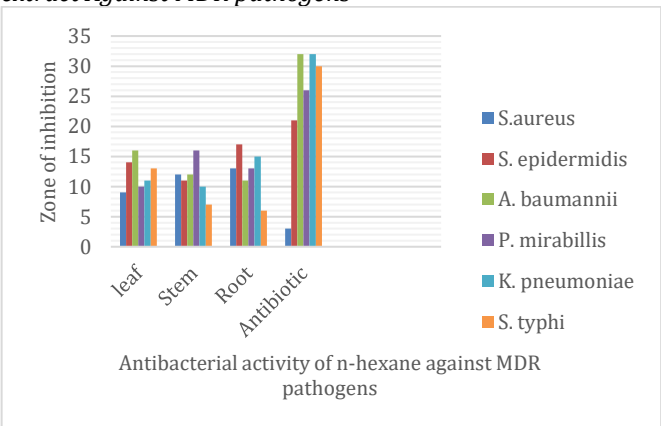
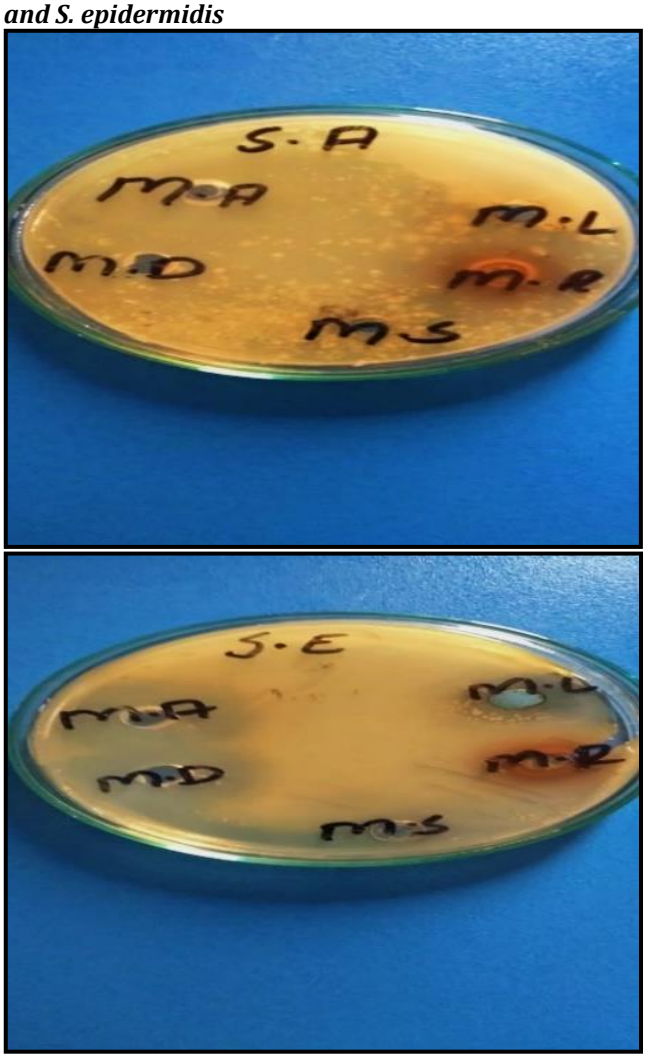



**Figure 6**

*Antibacterial activity of N-Hexane extract against **S. aureus and S. epidermidis***




## CONCLUSIONS

Due to their minimal side effects and safe impact on human health, natural products with medicinal properties are highly valued. However, there is always a risk of contamination, posing a significant threat to the pharmaceutical industry. To mitigate this risk, conducting Pharmacognostic screenings of plants can be beneficial. The six bacterial strains employed in this study cause skin, wound, GI, urinary and respiratory infections. These MDR pathogenic bacteria were suppressed by a bioactive component in plants composites. The antibacterial activity of plants composites opens up new treatment possibilities for infectious illnesses, allowing plant to be employed as therapeutic agents, even when it is no longer effective itself. The findings clearly revealed that green-synthesized plant extract may occasionally compete with commercial antibiotics for the treatment of pathogenic diseases. Furthermore, the phytochemical tests were conducted have confirmed the presence of numerous biologically active compounds in this plant, which could potentially lead to the development of innovative medicines in the future.

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