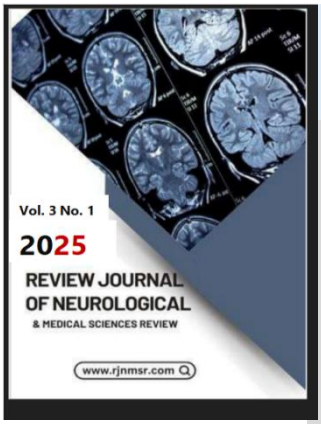

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Evaluation of Viburnum Grandiflorum Crude Extract as an Antibacterial Agent

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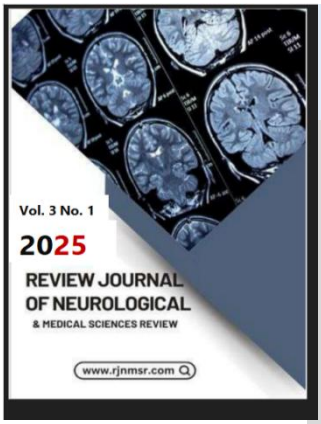

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Abstract

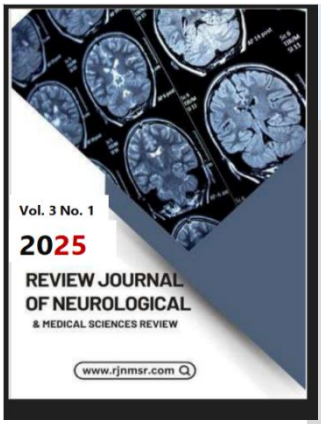
Bacteria impact the soil, animals, food consumers, and ecology as a whole and have developed a significant tolerance to overuse of antibiotics. In addition to resistance, they caused several toxicity issues. Around the world, chemical compounds are harming crops and agricultural commodities. In order to address these growing problems, it is becoming more and more necessary to find and create agricultural and/or botanical alternatives. Using an antibacterial agent derived from plant extract, which is a natural source of secondary metabolites and works well against bacteria that cause disease, is an alternate option. The most promising plant in terms of antibacterial properties is Viburnum grandiflorum. The aim of the proposed study is to investigate the antibacterial activity of the methanolic crude extract of Viburnum grandiflorum leaves and epicarp against the pathogenic effects of *P. aeruginosa* and *P. syringae,* which cause many illnesses in humans, animals, and plants. The maceration step produced the crude methanolic extract. The zone of inhibition against *P. syringae* and *P. aeruginosa* was evaluated by means of the agar well diffusion technique used to evaluate the antibacterial profile. The findings demonstrated that the plant's leaf and epicarp crude extract had the potential to be employed as an antibacterial agent due to its ability to inhibit bacterial growth and the presence of a zone of inhibition. In the future, various dilutions of various plant sections can be tested on various bacterial strains to further assess the plant's potential.

Key words: Viburnum grandiflorum, Crude extract, Antibacterial Agent

Introduction

The existence of chemical components inside medicinal plants determines their immense usefulness to humanity. Secondary metabolites, such as glycosides, volatile oils, tannins, vitamins, and minerals, are thought to be abundant in them and are utilized in the management and cure of several illnesses (Marrelli, 2021). Before modern medications, people used medicinal plants to cure a wide range of illnesses. Viburnum plants may be multiplied by layer branches in the fall and softwood cuttings in the summer (Spriggs *et al.,* 2018). The native name for the medicinal herb Viburnum grandiflorium is Guc. Only 21 species of the Adoxaceae plant family, which includes the genus Viburnum, have undergone chemical analysis. The family has approximately 200 species that are found in temperate and subtropical regions of Asia, North America, and Malaysia (Iqbal *et al.,* 2022). Pakistan is native to seven different species of viburnum, including Viburnum tinus, Viburnum opulus, Viburnum mullaha, Viburnum cotinifolium, and Viburnum grandiflorium. Viburnum cylindricum and Viburnum foetns are found in state of Jammu & Kashmir and northern Pakistan (Alam *et al.,* 2021).

The use of pharmaceuticals and the hunt for plant-based dietary supplements have both surged in recent years. Thousands of phytochemicals have been discovered in labs worldwide that have inhibitory effects on different types of bacteria in vitro. Additionally, anti-rheumatic, anti-inflammatory, anti-hyperlipidemic, antifungal, anthelmintic, antibacterial, antimalarial, antioxidant, and antimicrobial properties



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were demonstrated by the plant extract (Mehmood *et al.,* 2022). *Pseudomonas syringae* is a gram-negative bacterium that is well-known for its variety and interactions with certain plants. *P. syringae* multiplies in the intercellular space to reach large population numbers in susceptible plants after entering host tissues (often leaves) through wounds and natural apertures like stomata. Water-soaked patches appear on infected leaves, which eventually wither away. Depending on the bacterial strain, widespread chlorosis may surround necrotic lesions. Galls and ulcers can also be caused by *P. syringae* strains (Xin *et al.,* 2018). However, in resistant plants, *P. syringae* can cause an allergic reaction (HR), which is a rapid death associated with the defense of plant cells in contact with the pathogen (Katagiri *et al.,* 2002).

Globally, *Pseudomonas aeruginosa* is a prevalent gram-negative bacillus. Throughout the past 20 years, P. aeruginosa has become a significant pathogen. In the majority of hospitals, it is the cause of 10% to 20% of infections. Pseudomonas infections are commonly caused by burns, cystic fibrosis, acute leukemia, organ donation, and intravenous drug use (Krell and Matilla, 2024). Numerous aspects in the hospital environment can be linked to the epidemic, which is caused by a frequent hospital contaminant. Patients who are hospitalized for an extended period of time are often colonized by this bacteria, which raises the risk of illness. Penicillins (like ticarcillin, piperacillin), cephalosporins (like ceftazidime, cefepime), carbapenems (like imipenem, meropenem), and single lactams (like aztreonam) are among the β-lactams that are often used to treat *P. aeruginosa* infections. Drug resistance is rising (El Zowalaty *et al.,* 2015).

P. aeruginosa, which is only responsive to colistin, is becoming more and more resistant to drugs, and its infection incidence is rising. Regretfully, certain novel medications that have anti-pseudomonas activity such as the MBL inhibitor ME1071, the anti-pseudomonas cephalosporin CXA-101, and the siderophore-monobacterium combination BAL30072—are susceptible to established resistance mechanisms (Bassetti *et al.,* 2018). Despite the fact that the lack of traditional antibacterial alternatives has spurred research into novel anti-pseudomonas tactics and compounds, such as phage treatment, KB001, cationic antimicrobial peptides, efflux pump inhibitors, virulence modifiers, humanized anti-P. P. aeruginosa Fab antibody fragments (Reynolds and Kollef, 2021).

Materials and Method

Plant Collection

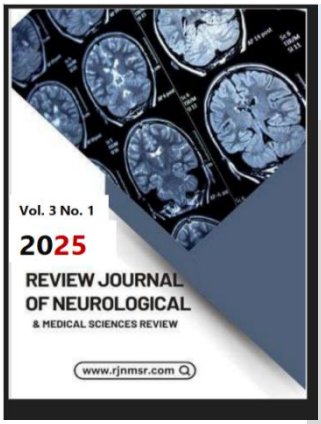
Plant *Viburnum grandiflorum* was being collected from Hazara, Thandiani District, (KPK) Pakistan.

Bacteria

Pathogenic strains of *P. aeruginosa* and *P. syringe* were obtained from Microbiology Lab of Abbottabad university of Science and technology.

Culture Media and Chemicals

Two types of growth media were used in this study to check the antibacterial activity and find the inhibition zones: Nutrient Agar and TSB.



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Preparation of Plant Materials

To get rid of debris and contaminants, freshly harvested viburnum leaves were cleaned with tap water. After washing, let it dry for approximately three weeks in the shade, then use a mortar and pestle to grind it into a fine powder and sift it. The finely ground plant material was then kept in an airtight bottle (Krakowska-Sieprawska *et al.,* 2022).

Preparation of Plant Extracts

The dipping technique, which involved soaking 50 grams of dried plant leaves in 250 milliliters of methanol in a flask for five days and vigorously stirring at room temperature every twelve hours, was used to carry out the extraction. Five days later, the methanolic extract was filtered, and Whatman filter paper was used to separate the plant material from the solvent. This procedure is carried out three times until methanol has extracted every component. After being gathered, the crude extracts were examined for antibacterial properties (Krakowska-Sieprawska *et al.,* 2022).

To prepare agar medium, dissolve 3.5g nutrient agar in 125ml distilled water and autoclave. Measure pH to 7.5, then transfer agar medium to a petri dish (25ml/plate) and proceed in a laminar flow hood to prevent any type of contamination. After the plates have solidified, place them in an incubator at 28°C for one day (Devika *et al.,* 2021).

Bacterial Strains Culturing

A small bacterial colony in which *Pseudomonas syringae and Pseudomonas aeruginosa* was grown was picked up from the already prepared media plates with the help of a sterilized loop and was mixed in 5ml LB broth which is used for bacterial growth. This activity was performed in the Laminar flow hood and then it was kept in a shaking incubator at 37°C overnight. After incubation, 200µl of LB broth containing bacteria were spread over the entire surface of the plate through the spreader, which was sterilized with 70% ethanol, and the procedure was performed under a laminar flow hood (Tong *et al.,* 2023).

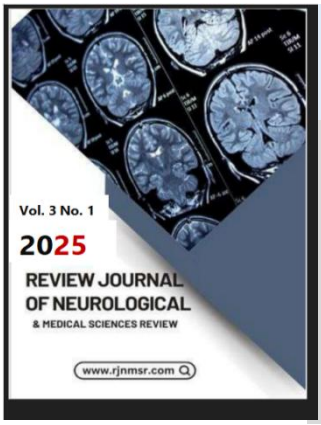
Preparation of Crude Extract Dilutions

A 2%stock solution was made in which 6mg plant extract was used in 294 microliters of DMSO. It is a solvent that is used for the dissolution of different polar as well as non-polar compounds. It has a high boiling point, so it evaporates slowly at room temperature. The stock solution of leaf extract was further diluted before their use to three different fixed amounts of diluted solutions i.e. 100 ppm,300 ppm, and 400 ppm.

Media Preparation for Antibacterial Activity

Preparation of Tryptone Soya Media

TSA is a versatile growth medium used for the isolation, cultivation and maintenance of microorganisms supporting the growth of both fastidious and non-fastidious bacteria. A quantity of 3g of TSB fine powder and 2 g of bacteriological agar were weighed and dissolved in 100 *ml* of autoclaved water. The flask head was properly covered with aluminum foil. The flask was then placed in the autoclave, which was set to 121℃ .



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Composition of TSB

Reagent	Weight/ Volume
Distilled water	100 ml
Bacteriological agar	2 grams
Tryptone Soya Broth	3 grams

Antibacterial Activity Test

Antibacterial activity profiles of the extracts were conducted by agar well diffusion assay under laminar flow hood in aseptic conditions. The inoculation of test organisms *P. syringae and P. auriginosa* on nutrient agar plate was done by cork borer wells of about 5mm created in plate surfaces. The plant extract was introduced at a specific concentration with the help of a micropipette. The agar plates were firstly incubated at 37 C for the period of 24 hours. The diameter of the zone of inhibition was checked and compared with control. All the experiments were conducted in triplicates and data was represented as Standard Error of mean (SEM) (Imon *et al.*, 2023).

Results

Plant Extraction

The extraction of fresh plant leaves of *Viburnum grandiflorum* was done to evaluate their antibacterial activities. For this purpose, fresh leaves of *Viburnum grandiflorum* were gathered, washed, and dried. The dried leaves were crushed into fine powder form, which was then extracted using 70:30 ethanol-water solution. After a two-week incubation period, the extract was filtered, and the ethanol was evaporated at 60°C to yield a thick leaf extract. The extract was preserved for biological activity tests.

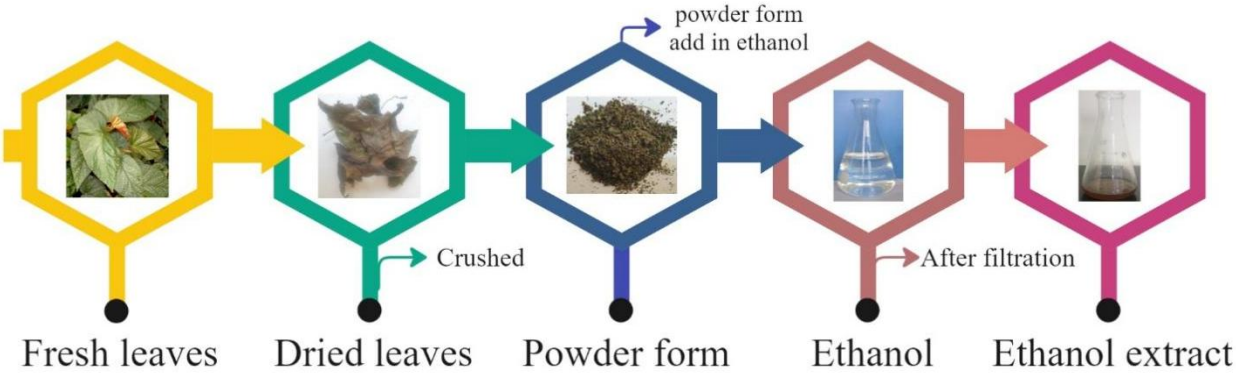
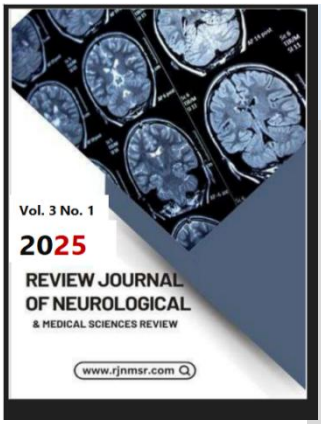


Figure 4.1 Fresh *Viburnum grandiflorum* leaves and their ethanolic extract

Extract and its Antibacterial Activity

The extract of leaves and epicarp was prepared in methanol. Leaf and exocarp extracts contain secondary compounds with antibacterial activity. The extract was dissolved in DMSO. The bacterial culture was grown on nutrient agar medium in a petri dish, and antibacterial activity was against *P. aureginosa and P. syringae* using agar hole diffusion method. A zone of inhibition showing the activity of the extract



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was observed.

Figure 1: *Viburnum* Leaves Extract

Antibacterial Activity of *V. Grandiflorum* Leaf and Epicarp Extract Against *Pseudomonas Syringae*

The assay was first performed on *P. syringae* but there was no growth of bacteria on plates due to some reasons, so we moved toward *Pseudomonas aeruginosa*.

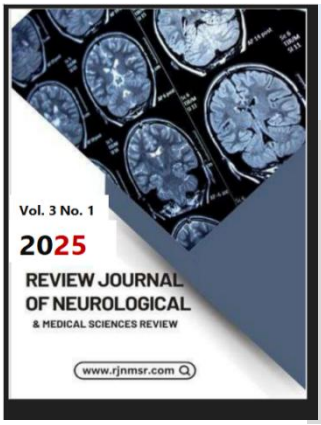
Antibacterial Activity of *V. Grandiflorum* Leaf Extract against *P. Auriginosa*

In addition to antibiotics, the crude methanol extract of *Viburnum* leaf has antibacterial activity due to the existence of a bacteriostatic zone against *P. auriginosa*. The antibacterial zone of the antibiotic ciprofloxacin is 19mm.

Figure 2: Zone of Inhibition of Leaf

Antibacterial Activity of *V. Grandiflorum* Epicarp Extract Against *P. Aeruginosa*

In addition to the addition of antibiotics, the crude methanol extract of *Viburnum*



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grandiflorium has antibacterial activity due to the presence of zone of inhibition against *P. auriginosa*. The inhibition zone of the antibiotic ciprofloxacin was 20mm.

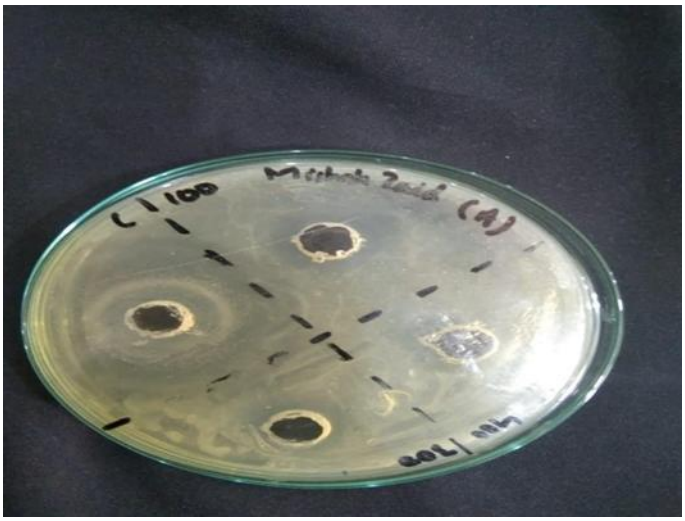


Figure 3: Zone of Inhibition of Epicarp

Table 1: Zone of Inhibition of Leaf Extract in Addition to Control

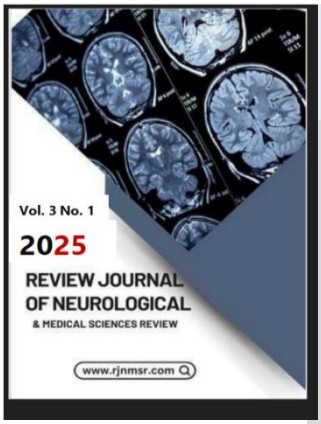
Control		100 l (mm)	300 l (mm)	400 l (mm)
1.9mm	R1	11	13	16
19mm	R2	10.5	14	15.5
19mm	R3	11	14.5	16.5
Mean		10.8	13.8	16

Table 2: Zone of Inhibition of Epicarp Extract in addition to Control

Control	Replication	100 l Mm	300 l Mm	400 l mm
20mm	R1	16	17	18
20mm	R2	15.5	17.5	17.5
20mm	R3	15	17.3	18
Mean		15.5	17.2	17.8

Discussion

Antibiotic resistance is the condition in which bacteria develop a kind of resistance against antibiotics, such bacteria are known as antibiotic resistance bacteria. Different types of microbes are present in nature that may cause harm to human beings. The problem of antibiotics resistance is a big threats in the sector of healthcare in developed as well as in developing countries (Mostafa *et al.*, 2021).



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Many bacteria have developed multidrug resistance some of which are *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumonia* which is methicillin-resistant and mostly acquired by patients during their stay in hospitals (Marasini *et al.,* 2021).

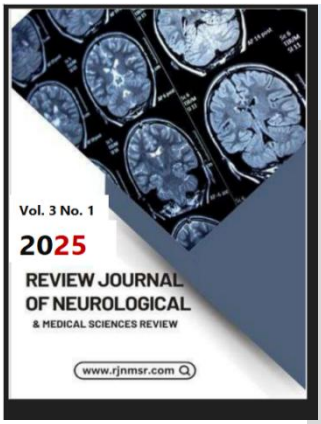
Apart from the resistance of bacteria, there are some other problems like synthesized chemicals which are used against bacteria are damaging crops and agricultural product globally, increasing the level of toxicity of drugs on host tissues. Antibacterial drugs can be used but due to pathways bacteria has developed these drugs are not effective against the. Nature has developed many solutions or remedies to cure these diseases caused by bacteria. One of these natural remedies is the antimicrobial activity of plants. Plants produce different secondary bioactive compounds that are well known for their therapeutic properties (Marasini *et al.,* 2021).

During the current study *Viburnum grandiflorum* was being collected from Hazara, Thandiani District, (KPK) Pakistan. Fresh Viburnum grandiflorum plant leaves were extracted in order to assess their antibacterial properties. Fresh Viburnum grandiflorum leaves were collected, cleaned, and dried for this purpose. After being crushed into a fine powder, the dried leaves were extracted using a 70:30 ethanol-water solution. A thick leaf extract was obtained by filtering the extract after two weeks of incubation and evaporating the ethanol at 60°C. For testing of biological activity, the extract was maintained. Similar study was carried out by Rehman *et al.,* 2015 which shows *Viburnum grandiflorum* was collected from Murree KPK Pakistan to check their antibacterial activity.

Viburnum grandiflorium has these bioactive secondary metabolites and is screened for its biological activity (Rahman *et al.*, 2019). The studies have shown that stem extract shows efficiency in the reduction of microbial growth as compared to root extracts (Iqbal *et al.,* 2022). We evaluated the crude extract of leaf and epicarp of *Viburnum grandiflorium* plant against *Pseudomonas syringae* and *Pseudomonas auriginosa* which cause different diseases in human and plants. For the antibacterial activity we have used agar well diffusion process. Results indicated that both leaves and epicarp have antibacterial activity as it showed zone of inhibition against *P. auriginosa*. There was no growth of *P. syringae*. Similar study was conducted by Alam *et al.,* 2018 which shows *Viburnum grandiflorium* plant activity was checked against *Pseudomonas syringae* and *Rizocotonia solani*. *Viburnum grandiflorium* plant extract show antibacterial activity as it showed zone of inhibition against *Pseudomonas syringae* and *Rizocotonia solani*.

Conclusion

Pseudomonas is one of the best-studied plant pathogens and serves as a model for understanding host–microorganism interactions, bacterial virulence mechanisms and host adaptation of pathogens as well as microbial evolution. Individual strains of the plant pathogenic bacterium vary in their ability to produce toxins, nucleate ice, and resist antimicrobial compounds. Plant extracts have been used for the long treat to treat such plant diseases. Viburnum grandiflorum is of the highest promise with antibacterial activities. Results indicated that both leaves and epicarp have



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antibacterial activity as it shown zone of inhibition against *P. auriginosa*. It means the plant have secondary metabolites which have antibacterial activity. There was no growth of *P. syringae*, first, a possible reason could be that the culture of bacteria was old, second, the conditions for the growth of bacteria were not suitable. however, the freshly prepared culture of *Pseudomonas syringae* and proper temperature and nutrient media, and other conditions can give best growth of bacteria.

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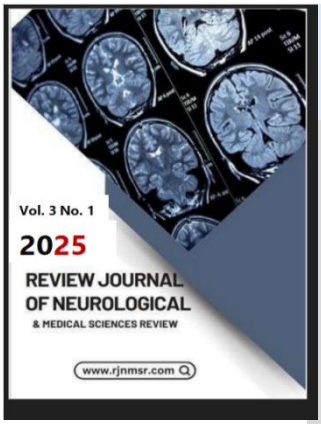

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