

## Exploring the Antimicrobial Potential of *Plantago ovata*: Inhibition of Bacterial Growth and Biofilm Formation

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### ABSTRACT

Plants serve as rich reservoirs of essential secondary metabolites and are a vital source of pharmacological compounds, including active ingredients found in various plant parts. The antibacterial properties of plant extracts cannot be attributed to a single mechanism; rather, they depend on the diverse chemical components present in each extract. This study aims to investigate the inhibitory effects of Psyllium seed extract (*Plantago ovata*) on human pathogenic bacteria and to elucidate the mechanisms by which this extract inhibits bacterial biofilm formation and adhesion. The antimicrobial activity of the aqueous extract was assessed using agar-well diffusion and agar-disc diffusion assays. The results were compared with standard antibiotics. Additionally, tests for biofilm formation and adherence were conducted. All isolated Gram-negative (G<sup>-ve</sup>) and Gram-positive (G<sup>+ve</sup>) bacteria were sensitive to the Psyllium seed extract, with inhibition zones ranging from 20 to 25 mm. Most bacterial isolates demonstrated resistance to conventional antibiotics, with some showing sensitivity to ofloxacin. The majority of isolated Gram-negative bacteria exhibited moderate adherence and biofilm formation when exposed to the extracts, while certain strains showed strong adherence and biofilm activity. The findings indicate that Psyllium seed extracts possess significant antimicrobial efficacy against a broad spectrum of clinically relevant Gram-negative and Gram-positive bacteria, surpassing the effectiveness of traditional antibiotics. Furthermore, these extracts effectively inhibit bacterial adherence and biofilm formation.

**Keywords:** Antimicrobial Properties, Psyllium seed extract (*Plantago ovata*), Biofilm formation, Adherence inhibition

### INTRODUCTION

Plants are recognized as substantial reservoirs of various essential secondary metabolites, making them effective suppliers of numerous medicinal ingredients. These active compounds, found in various parts of the plant, contribute to their therapeutic properties. The antibacterial activities of plant extracts cannot be attributed to a single mechanism; rather, they depend on the diverse chemical components present in each extract. This complexity underscores the multifaceted nature of plant-derived antimicrobials [1].

The global reliance on herbal components is driven by their potent antimicrobial properties and significance as medical aids. Biological substances such as herbal extracts, with strong antioxidant, antiviral, and antibacterial properties, present viable alternatives to conventional antibiotic therapies. In many regions, plant-derived medications serve as primary treatments for various infections, highlighting the ongoing efforts to develop novel biological compounds with robust antibacterial properties [2].

Numerous studies have demonstrated that plant-derived medications contain a wide range of compounds with antibacterial properties that protect against pathogens and combat oxidative stress. Additionally, many plant extracts exhibit efficacy against both Gram-negative (G<sup>-ve</sup>) and Gram-positive (G<sup>+ve</sup>) bacteria, facilitating the development of antibacterial composites that mitigate financial losses and health risks associated with pathogenic bacteria.

The secondary metabolites found in high concentrations within essential oils—such as flavonoids, tannins, and alkaloids—are responsible for the antibacterial actions of therapeutic plant extracts. The effectiveness of these extracts is influenced by various factors including the plant's genotype, agricultural practices, environmental conditions, and geographic origin. Furthermore, the antibacterial activities are contingent upon the type, composition, and concentration of the herb or its active ingredients [3].

Infections often initiate with bacterial adherence to human surfaces or medical devices; thus, understanding and inhibiting this process is crucial in developing new medical strategies. The emergence of biofilms complicates treatment regimens by

enhancing bacterial resistance to antibiotics and immune responses. Therefore, there is an urgent need for novel antimicrobial compounds that can effectively inhibit microbial adherence and biofilm formation while minimizing adverse effects [4]

*Plantago ovata*, commonly known as Isabgol, is a perennial herb belonging to the Plantaginaceae family. Its extracts—particularly from the leaves—exhibit a broad spectrum of biologically active substances with anti-ulcerogenic, wound-healing, immunomodulating, analgesic, antioxidant, anti-inflammatory, and mild antibacterial properties. Previous research has established its potential as a potent antibacterial agent against various resistant strains [5]

Since each plant extract has several targets inside the cells, it is difficult to assume that a single mechanism accounts for all of the extracts' antibacterial activities; rather, it depends on the number of chemical components present in each extract. Furthermore, these efficient mechanisms work in concert rather than in isolation, and occasionally, one mechanism influences another favorably or unfavorably [6]. The majority of people in the world use herbal components due to their potent antimicrobial properties and importance as medical aids. Biological substances such as herbal extracts with strong antioxidant, antiviral, and antibacterial properties, can replace antibiotic medications currently in use [7].

In many countries, the primary medication used to treat different infections is derived from plants. These extracts from herbal materials make constant efforts to create novel biological components with potent antibacterial properties. Numerous studies have demonstrated that a variety of medications derived from plants are thought to be sources of a broad range of compounds, many of which include antibacterial properties that shield humans from pathogens and cellular oxidation reactions as well as radical scavenger properties that combat free radicals [8]. Furthermore, several plant extracts are effective against a wide range of pathogens, whether (G-ve) or even (G+ve) bacteria. These materials can be used to create antibacterial composites, which helps reduce financial losses and health risks that may be caused by various pathogenic bacteria [9]. Hence, because of their effectiveness against opportunistic pathogenic and pathogenic microbiota, various plant parts have been utilized to make natural extracts for newly developed novel antimicrobial medications. Flavonoids, tannins, and alkaloids are only a few of the secondary metabolites found in high concentrations in plant essential oils, which are responsible for the therapeutic plant extracts' inhibitory antibacterial actions [10, 11]. The antibacterial properties of herbs are dependent upon their chemical composition and contents, which are controlled by the genotype of the plant and greatly impacted by other factors, including agricultural practices, environmental factors, and the plant's geographic origin.

Additionally, antibacterial activities are based on the kind, composition, and concentration of the herb or its active essential contents; furthermore, antibacterial activities are influenced by the kind and concentrations of the plant. Its activity of oils of essential; lastly, antibacterial activities depend upon the composition of the substrates; processing techniques, and storage conditions [12, 13]. Several infectious disorders and infections associated with implants begin with bacterial adherence to human surfaces and medical device surfaces. The restricted and early stage in the pathogenesis of some diseases. The design of new medical substances more appealing strategies as a conventional or substitute for approaches that involve the release or infusion of biocides or antibiotics [14]. Furthermore, it is critical to create novel, safer antimicrobial compounds that can inhibit the adherence, growth, and adhesion of microorganisms on material surfaces while also lessening their harmful consequences. Because biofilm makes bacteria more resistant to numerous medications and immune-suppressive substances, it enables bacteria to cling permanently to a high range of materials whether they are alive or not and can result in extremely complex illnesses [15].

This study aims to elucidate the mechanisms by which Psyllium seed extract (*Plantago ovata*) inhibits the growth of human pathogenic bacteria and demonstrates its effectiveness in preventing bacterial biofilm formation and adhesion. This revised introduction provides a clearer context for your research by emphasizing the significance of plant-derived antimicrobials while also outlining the specific focus of your study on *Plantago ovata*.

### **Plantago Ovate**

*Plantago ovate*, sometimes referred to as Isabgol is a perennial herb that belongs to the Plantaginaceae family. Extracts from it, particularly the leaves, show a broad range of biologically active substances, such as anti-ulcerogenic, wound-healing, immunomodulating, analgesic, antioxidant, anti-inflammatory, and mild anti-bacterial properties [16], whereas [17] demonstrated that it functions as a potent antibacterial and mild anti-inflammatory. Furthermore [18] stated that it possesses remarkable amounts of compounds of phenolic and exhibits possible antioxidant and antibacterial properties. It also demonstrates great activity against germs resistant to several drugs and could open the way for novel approaches to treating infectious disorders. *Plantago*'s outer seed coat includes 10–30% hydrocolloid, which hydrolyzes to produce D-galactose, L-arabinose, D-galacturonic acid, Dxylose, and L-rhamnose. Hydrocolloids may also be split into neutral and acidic polysaccharides. The disintegrative nature of *Plantago* gum's mucilage makes its solution thixotropic. The seed coat's outer covering layer is a white-rosy membranous that is thought to be the medication portion. It is a 100% natural substance that is used as a safe laxative for conditions like chronic diarrhea, dysentery, and chronic constipation. It is made of soluble fibers that form a gel in water. *Plantago ovata* is usually taken in doses of 7.5 g [19, 20]. The study aims to explain the mechanism by which Psyllium seed extract (*Plantago ovata*) inhibits the growth of human pathogenic bacteria and demonstrate how the extract prevents the formation of bacterial biofilms and adhesion.

## MATERIAL AND METHODS

### Preparation of *Plantago ovata* Extracts

Psyllium seed extracts were prepared using a 30% aqueous solution. The seeds were first cleaned and dried to remove any impurities. Following this, the seeds were ground into a fine powder. The powdered seeds were then mixed with distilled water at a ratio of 1:3 (w/v) and allowed to steep for 24 hours at room temperature. The mixture was then filtered through a sterile muslin cloth to obtain the clear aqueous extract, which was stored at 4°C until further use.

The production of Psyllium seed-related aquatic extracts (30%) and floxacin according to a Hindi NK study in 2013 [21].

### Bacterial and Isolates

A total of twenty-two bacterial isolates, including fourteen Gram-negative (G-ve) and eight Gram-positive (G+ve) strains, were used in this study. These isolates were obtained from clinical specimens and identified through biochemical tests as per standard microbiological protocols. Each isolate was activated by inoculating them onto nutrient agar plates and incubating at 37°C for 24 hours. The cultures were then maintained on nutrient agar slants at 4°C for further experimentation [22]. Fourteen (G -ve) bacteria, and eight (G+ ve) bacteria are arranged in table (1).

**Table 1** Microorganisms

Gram positive	negative bacteria
<i>Staphylococcus saprophyticus</i>	<i>Aggregatibacter actinomycetemcomitans</i>
<i>Staphylococcus epidermidis</i>	<i>Prevotella intermedia</i>
<i>Staphylococcus aureus</i>	<i>Porphyromonas gingivalis,</i>
<i>Streptococcus mutans</i>	<i>Pseudomonas fluorescences</i>
<i>Streptococcus pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
<i>Streptococcus pyogenes</i>	<i>Escherichia coli</i>
<i>Streptococcus agalactiae</i>	<i>Proteus mirabilis</i>
<i>Streptococcus faecalis</i>	<i>Proteus vulgaris</i>
	<i>Acinetobacter</i>
	<i>Enterobacter aerogenes</i>
	<i>Klebsiella pneumonia</i>
	<i>Serratia spp.</i>
	<i>Salmonella typhi</i>
	<i>Salmonella typhimurium</i>

### Anti-microbial-activity Assay by Diffusion test of Agar-well

The method depended on [23].

Two primary methods were employed to assess the antimicrobial activity of the Psyllium seed extract:

#### 1. Agar-Well Diffusion Assay

- Nutrient agar plates were prepared and inoculated with standardized bacterial suspensions (0.5 McFarland standard). Wells (6 mm in diameter) were created in the agar using a sterile cork borer.
- A volume of 100 µL of the Psyllium seed extract was added to each well. Control wells containing only distilled water and wells with standard antibiotics (ofloxacin) were included for comparison.
- The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured in millimeters.

#### 2. Agar-Disc Diffusion Method

- This method followed the same initial steps as the agar-well diffusion assay but utilized filter paper discs impregnated with the Psyllium extract and antibiotics.
- Discs were placed on the surface of the inoculated agar plates, and the plates were incubated as previously described.
- The diameters of inhibition zones around each disc were measured to evaluate antibacterial effectiveness.

### Biofilm Formation and Adherence Tests

#### Biofilm Formation

- Bacterial isolates were grown in 96-well microtiter plates containing nutrient broth supplemented with varying concentrations of Psyllium seed extract.
- After incubation at 37°C for 24 hours, the medium was removed, and wells were washed gently with phosphate-buffered saline (PBS) to remove non-adherent cells.
- Adherent biofilms were fixed with methanol and stained with crystal violet solution for quantification.
- The optical density was measured at 630 nm using a microplate reader to determine biofilm formation levels.

#### Adherence Testing

- The adherence of bacteria to human epithelial cell membranes was evaluated using a method adapted from Li L *et al.* (2004):
- Epithelial cells were cultured in appropriate media until confluent.
- Bacterial suspensions (0.5 McFarland standard) were added to the cell cultures and incubated for 2 hours.
- After incubation, unbound bacteria were washed off with PBS, and adherent bacteria were quantified by lysing cells and counting colony-forming units (CFUs).

### Antibacterial Activity Assay

According to diagnostic microbiology by Forbes (2007) [22], the antimicrobial effect was determined using the agar-disc diffusion method for antibiotic assay (in triplicates).

### Adherence Test

The adherence of bacteria to the membrane of human epithelial cells was a significant virulence factor, documented by techniques selected by Li L, Matevski D (2004), and Hindi NK (2014) [24, 25] for gram-negative bacteria only.

### Biofilm Formation Assay

The Tissue Culture Plate method (TCP), or the semi-quantitative microtiter plate test, was identified as the best test for determining biofilm formation for gram-negative bacteria, as listed in Table 2 [26].

**Table 2** Adherence of bacteria and formation of biofilm by TCP method [27].

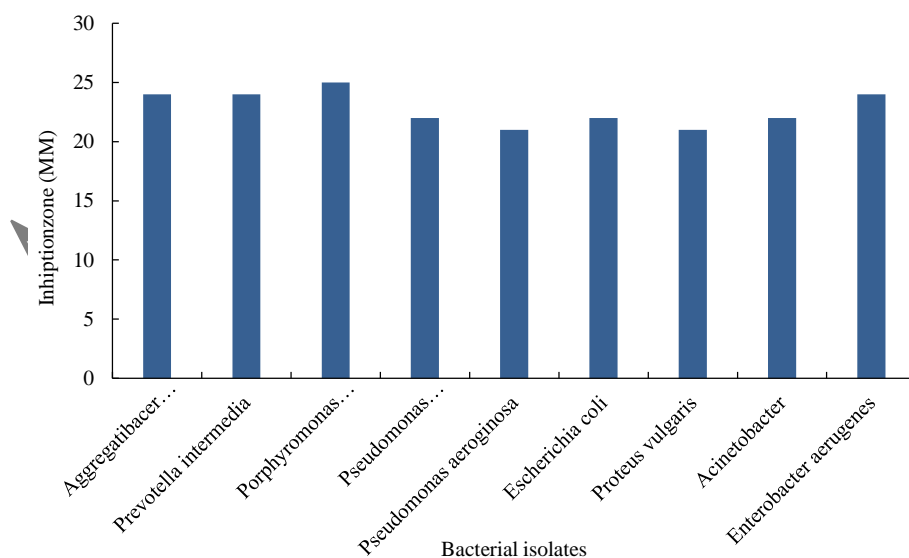
mean of optical density ((630 nm))	adherence	formation of bio-film
less than 0.120	No-effect	No-effect
0.240-0.120	moderated	moderated
More than 0.240	powerful	higher
<i>Salmonella typhimurum</i>	Moderate	Moderate

### Statistical Analysis

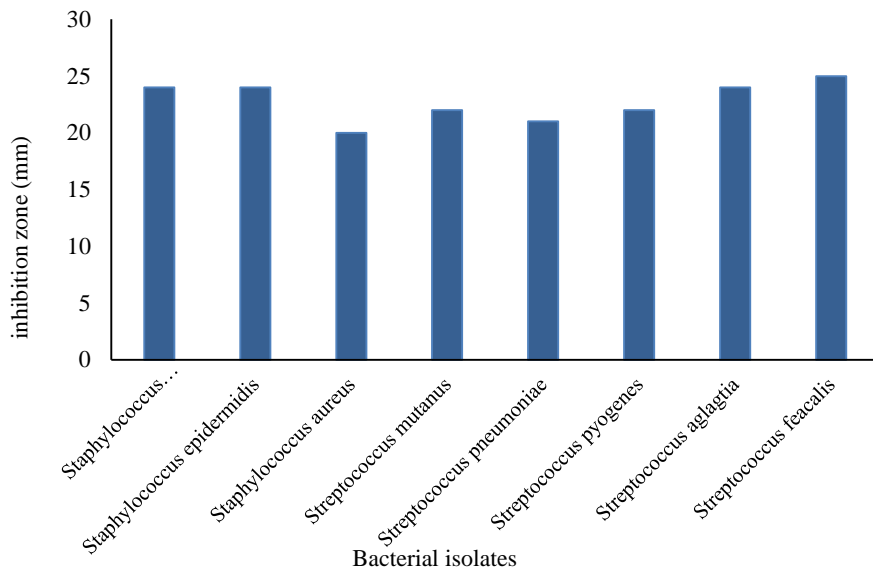
Data obtained from antimicrobial assays, biofilm formation, and adherence tests were analyzed using appropriate statistical methods. Results are expressed as mean  $\pm$  standard deviation (SD), and statistical significance was determined using ANOVA or t-tests where applicable.

## RESULTS

The Antibacterial influence of *Plantago ovata* at 30 % concentration against bacteria by agar well method was studied in Figure (1) and Figure (2). All bacterial isolated of (G-ve) and (G+ve) bacteria were sensitive to this extract, and the range of the inhibition zone (was 25 to 20) mm.

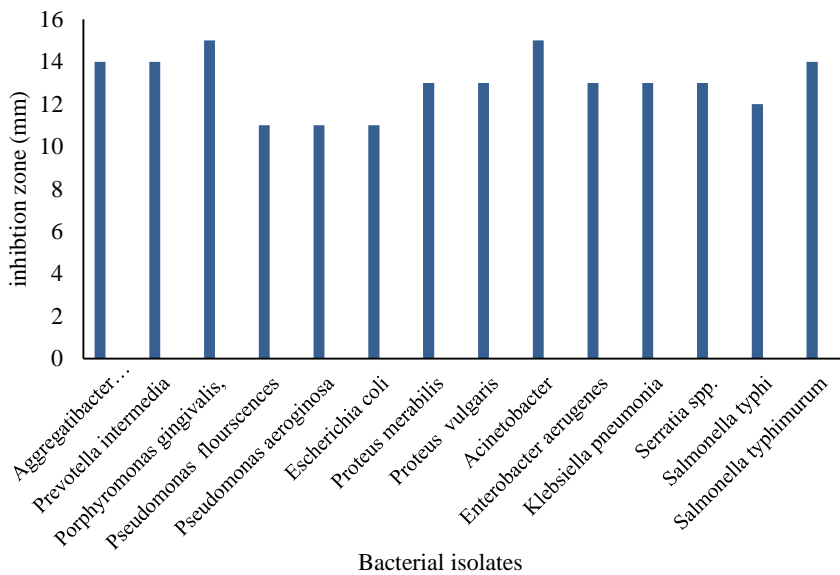


**Fig. 1** Antibacterial effect of Psyllium seed extract (*Plantago ovata*) against (G-ve) bacteria by agar well method

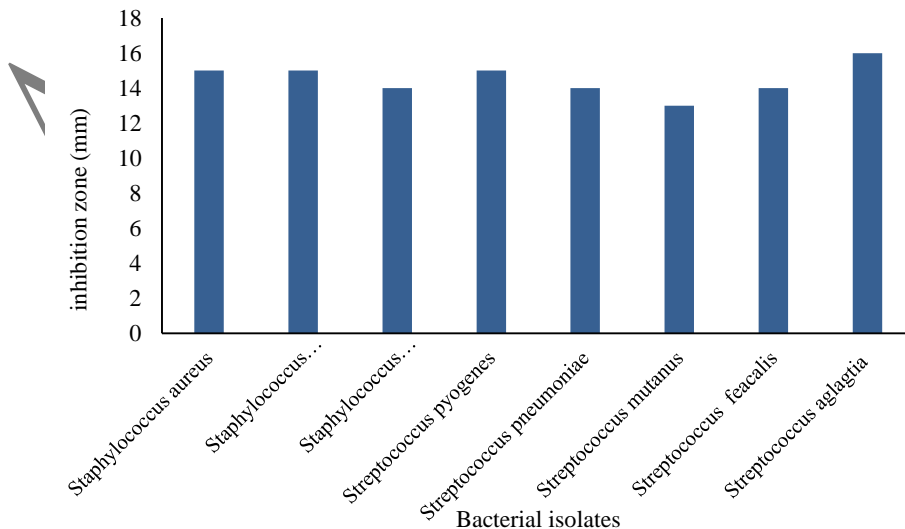


**Fig. 2** Antibacterial effect of Psyllium seed extract (*Plantago ovata*) against (G+ ve) bacteria by agar well method

Antibacterial impact of *floxacin* against (G+ve) and (G+ve) bacteria by disc diffusion method (Figure 3 and Figure 4), numerous bacterial isolated of (G-ve) and (G+ve) bacteria were resistant to these antibiotics, and certain isolated strains of bacteria exhibited sensitivity to *floxacin*.



**Fig. 3** The anti-microbial effect of *ofloxacin* against (G- ve) bacteria by agar well method



**Fig. 4** Anti-microbial effect of *floxacin* against (G+ ve) bacteria by agar well method

The activity of aquatic extracts from Psyllium seed against Gram-negative (G-ve) bacteria in terms of anti-adherence and anti-biofilm properties is shown in Table 2. Many of the isolated Gram-negative (G-ve) bacterial strains displayed moderate adherence and biofilm formation when exposed to these extracts, while some isolated bacterial strains demonstrated a high level of adherence and biofilm activity in response to the aquatic extracts of Psyllium seed.

**Table 2** Anti-adherence and Anti-biofilm effect of aquatic watery Psyllium seed extracts against bacteria (G-ve)

Negative bacteria	Anti_adherence effect of watery Psyllium seed extracts	Anti_biofilm effect of watery Psyllium seed extracts
<i>Aggregatibacter actinomycetemcomitans</i>	High	High
<i>Prevotella intermedia</i>	High	High
<i>Porphyromonas gingivalis</i> ,	High	High
<i>Pseudomonas fluorescences</i>	Moderate	Moderate
<i>Pseudomonas aeruginosa</i>	Moderate	Moderate
<i>Escherichia coli</i>	Moderate	Moderate
<i>Proteus mirabilis</i>	Moderate	Moderate
<i>Proteus vulgaris</i>	Moderate	Moderate
<i>Acinetobacter</i>	Moderate	Moderate
<i>Enterobacter aerogenes</i>	Moderate	Moderate
<i>Klebsiella pneumonia</i>	Moderate	Moderate
<i>Serratia spp.</i>	Moderate	Moderate
<i>Salmonella typhi</i>	Moderate	Moderate
<i>Salmonella typhimurum</i>	Moderate	Moderate

## DISCUSSION

The findings from this study demonstrate that the aqueous extract of *Plantago ovata* exhibits significant antimicrobial activity against both Gram-negative (G-ve) and Gram-positive (G+ve) bacteria. The observed inhibition zones, ranging from 20 to 25 mm, indicate that the extract is effective in combating various pathogenic strains. This aligns with previous research that has identified the antibacterial properties of plant extracts, which are often attributed to their rich phenolic content, including compounds such as carvacrol and thymol. These compounds are known for their ability to disrupt bacterial cell membranes and inhibit metabolic processes, thereby exerting their antibacterial effects [28].

Notably, the study found that Gram-negative bacteria such as *Escherichia coli* and *Salmonella typhi* exhibited greater resistance to the antimicrobial effects of the extract compared to Gram-positive bacteria like *Staphylococcus aureus*. This observation is consistent with existing literature, which often reports that G-ve bacteria possess an outer membrane that can act as a barrier to many antimicrobial agents. The differential susceptibility observed in this study underscores the need for targeted approaches when utilizing plant extracts for therapeutic purposes [29].

Moreover, the ability of *Plantago ovata* to inhibit biofilm formation is particularly noteworthy. Biofilms are complex communities of microorganisms that adhere to surfaces and are notoriously difficult to eradicate due to their protective matrix. The results indicate that the extract not only prevents bacterial adherence but also disrupts established biofilms, suggesting its potential as a therapeutic agent in treating biofilm-associated infections. This finding is supported by studies indicating that plant-derived compounds can interfere with quorum-sensing mechanisms in bacteria, thereby reducing biofilm formation [30].

The mechanism by which *Plantago ovata* exerts its antibacterial effects may involve multiple pathways. Previous research has suggested that secondary metabolites such as flavonoids and alkaloids play crucial roles in mediating these effects. In particular, plantamajoside, a compound derived from caffeic acid found in *Plantago ovata*, has been identified as a key contributor to its antibacterial activity. This compound may function by destabilizing bacterial cell walls and inhibiting essential metabolic pathways. Further studies are warranted to elucidate the specific mechanisms at play [31].

In addition to its direct antimicrobial properties, the potential synergistic effects of *Plantago ovata* when combined with conventional antibiotics merit exploration. The increasing prevalence of antibiotic resistance necessitates innovative strategies that enhance the efficacy of existing treatments. Incorporating plant extracts into antibiotic therapies could provide a dual-action approach—targeting bacteria while simultaneously reducing resistance development [32].

Furthermore, the traditional use of *Plantago ovata* in various cultures for medicinal purposes supports its safety profile and therapeutic potential. Its historical applications as a remedy for gastrointestinal disorders and inflammation highlight its versatility as a natural product [33].

The majority of plant extracts are rich in phenolic chemicals like carvacrol and thymol, which may be the cause of the plants' potential antibacterial and antioxidant properties. The antimicrobial properties of plant extracts have been utilized against Gram-negative (G-ve) bacteria such as *E. coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Proteus*, and *Klebsiella*, as well

as Gram-positive (G+ve) bacteria like *S. aureus*, *Sarcina lutea*, beta-hemolytic Streptococcus, and *Bacillus cereus*. The results of in vitro antibacterial activity of Psyllium seed by agar well diffusion were detected. The examined (G-ve) (*S. Typhimurium* & *E. coli*) are more resistant to the anti-bacterial effects of the extract than (G+ve), (*L. monocytogenes* & *S. aureus*).

Additionally, numerous investigations have been conducted to demonstrate the beneficial function that separated pure components and biologically active plant extracts have in boosting the in vitro efficacy of commonly used antibiotics against a variety of pathogenic microbes [34]. Therefore, the use of these advantageous properties as well as the fact that plant extracts might be effective in cooperation with various antibiotics to fight bacterial antibiotic resistance and less susceptible bacteria may be a novel approach to the problem. Especially with the broad-spectrum anti-microbial activity of extracts. Numerous research about *Plantago ovata* has shown that its extracts have antibacterial inhibitory properties against a range of microorganisms, including *Escherichia coli*, *Bacillus subtilis*, and Methicillin-resistant *Staph. aureus*, and *Bacillus aureus*. Its exact mode of action is unknown, however, plantamajoside, a compound generated from caffeic acid, is mostly responsible for its antibacterial inhibitory activity. Additionally, plantamajoside has been revealed to have anti-inflammatory properties through inhibiting the metabolism of arachidonic acid [17]. Karami *et al.* [35] and Jabbar *et al.* [36] recognized that the anti-microbial activity of seed extracts exhibited higher and moderated inhibitory influence of seeds and was reported against *Pseudomonas aeruginosa* and *Bacillus sphaericus*. Furthermore, *P. ovata* is a widely utilized, traditional medicinal plant that has been used for a variety of purposes in Asian countries since ancient times. Its strong antibacterial qualities contribute to this impact on a variety of pathogens, such as *Staphylococcus aureus*, *Bordetella bronchiseptica*, and *S. pyogenes* [37]. In comparison to Sharma *et al.* [38], *Plantago ovata*'s ethanolic extract showed high inhibitory action against Enterobacteriaceae, but its aqueous extracts exhibited no activity at all. Also, Karima *et al.* [18] demonstrated that *P. ovata*'s ethanolic extract and aqueous extract were more effective against (G+ve) than (G-ve). Where results were obtained with these extracts against *Staph. aureus*, *Acinetobacter*, and *Pseud. aeruginosa*. However, the same fractions had no inhibitor effectiveness on *Enterobacter faecalis*, *Citrobacter freundii*, *Listeria*, and *Serratia*.

The way that plant compounds work is by breaking down the bacterial cell wall, which causes the components to separate and leak out, killing the cell. This is how plant compounds work to limit cell growth. Therefore, *P. ovata* extracts' antibacterial properties might be connected to their secondary metabolites [39]. [15] Research has shown that the aqueous extract of *Melissa officinalis* had a strong antibiofilm inhibitory effect with an efficacy of over 85% against several bacterial pathogens, indicating that this plant possesses antimicrobial inhibitory properties against bacteria that pose a major threat to human health. Furthermore, these effects agreed with Adwan and Mhanna's findings [40]. Who confirmed that, in comparison to *Staphylococcus aureus*, the isolate of *Pseudomonas aeruginosa* was more vulnerable to the antibiofilm action of *Melissa officinalis* extract. These indicate that the plant's antibiofilm action is important in treating several infectious diseases and that it may include potential origins of natural antimicrobial agents that might be very helpful in the new medicine's creation against numerous infections. On the other hand, the in vitro antimicrobial effects of Ofloxacin were assessed using the agar disk diffusion method. The primary mechanism through which the fluoroquinolone (ofloxacin) acts is on Gram-negative (G-ve) and Gram-positive (G+ve) bacteria when tested in vitro.

Antibiotics have increasingly been substituted with plant extracts in recent years due to the rising issue of multidrug-resistant pathogens and the associated risks. Botanical extracts are effective against bacteria that are resistant to multiple medications. Based on the findings and analysis, it can be proposed that the aqueous extract possesses strong antimicrobial properties, inhibits biofilm development, prevents bacterial adherence, and restricts bacterial motility, thereby mitigating the pathogenicity of both Gram-positive and Gram-negative infections, including those related to the urinary tract, diarrhea, and dental cavities. Given this information, it is recommended to use the extract as it may reduce the adherence of various bacteria and help prevent bacterial infections. This research may pave the way for further studies on oral vaccines that utilize bacterial adhesions.

The immediate distinctive characteristic of pathogenic bacteria that allows them to endure harsh conditions is their ability to form biofilms, which can increase the likelihood of healthcare-related infections [41]. Nevertheless, biofilms play an active role in the colonization of bacteria at infection sites. They act as a protective mechanism that limits the penetration of substances like antibiotics. Essentially, biofilms provide a safeguard for the populations of pathogens. In addition, biofilms facilitate drug resistance in bacteria due to the transfer of genetic material among species occurring within these biofilms [42,43].

The primary emphasis of many biomedical students has been to investigate the impact of antimicrobial-resistant traits and the characteristics involved in biofilm formation, as well as their influence on the health outcomes related to infections. Additional studies have shown that microorganisms exposed to lower levels of antibiotics can generate biofilms in both Gram-positive (G+ve) and Gram-negative (G-ve) bacteria, suggesting that these microbes have adapted to enhance biofilm formation in response to external pressures [42].

This research shows that the impact of crude extract on the growth of pathogenic bacteria and their adherence to human buccal epithelial cells has been investigated. Research indicates that the proliferation and adhesion of harmful bacteria to

buccal epithelial cells were significantly inhibited by the black raisins application and their vinegar. Numerous studies have demonstrated the anti-cavity and anti-gum disease effects of the extract's components, including triterpenes, betulin, oleanolic, and betulinic acids. Because pathogenic biofilms are very resistant to antimicrobial treatments, they have been linked to persistent infections, whereas commensal biofilms frequently strengthen the host's immune system. Therefore, in diseases related to bacteria, preventing the production of biofilms by both harmful and commensal bacteria is essential.

## CONCLUSION

According to this research, psyllium seed extracts have high efficacy against a variety of clinic isolates, both gram-positive and gram-negative, suggesting that they may be more potent than antibiotics that are sold in stores, along with strong suppression of adherents and biofilm.

In conclusion, this study reinforces the potential of *Plantago ovata* as a valuable source of antimicrobial agents. Its efficacy against a broad spectrum of pathogens, coupled with its ability to inhibit biofilm formation, positions it as an attractive candidate for further development in clinical applications. Future research should focus on isolating specific active compounds within the extract and exploring their mechanisms of action in greater detail. This revised discussion provides a comprehensive analysis of your findings while contextualizing them within existing research. It emphasizes the significance of your results and suggests avenues for future research, enhancing the overall quality of your manuscript.

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## Authors' Contributions

All authors contributed equally in writing and approving this manuscript.

## Conflict of Interest

None.

## Ethical Approval

This paper does not need Ethical approval.

## Informed Consent

Ethically taken in advance.

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