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Article

Effect of Willow Bark Extract on The Activity of Gram-Negative Intestinal Bacteria: A Review

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Abstract: The increased growth of pathogenic Gram-negative bacteria such as Escherichia coli and Klebsiella pneumoniae contributes significantly to chronic gastrointestinal diseases like inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). These bacteria stimulate the immune system, releasing pro-inflammatory cytokines that cause additional inflammation and further tissue damage. The increase in antibiotic resistance of many Gram-negative bacteria makes treatment more difficult and calls for different approaches. Willow bark extract (WBE) from Salix species, in particular, has recently been noted to have the ability to inhibit pathogenic bacteria and modulate inflammation. WBE is rich in bioactive compounds such as salicin, flavonoids, and tannins, which are known to disrupt cell membrane of bacteria, inhibit biofilm formation and interfere with metabolic processes. Its anti-inflammatory activities, inhibiting NF-κB and oxidative stress, provide a more comprehensive way to manage gut health. This review was inspired by the gap for natural anti-microbial agents to explore the WBE mechanisms of action, applications for targeting Gram-negative bacteria, and restoring the gut microbiota diversity. On the other hand, WBE is limited by its variability in composition and extract, with little clinical validation and formulation challenges. Bridging these gaps would enable using WBE as a natural therapeutic for gastrointestinal and systemic disorders.

Keywords: Anti-microbial activity, Bioactive compounds, Gram-negative bacteria, Willow bark extract.

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1. Introduction

Studies have been conducted on the bark extracts from Willow trees of Salix species for their potential use in controlling pathogenic Gram negative bacteria as well as modulating inflammation to improve bowel health [1-2]. Inflammation in the gastrointestinal tract, including inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), is characteristically associated with the overgrowth of gram negative pathogens, particularly Escherichia coli and Klebsiella pneumoniae [3]. These pathogens are often responsible for immune reactions featuring proinflammatory cytokines such as TNFα, IL-6 and IL-1β, which when produced at high levels can lead to tissue inflammation and damage [4-5].

Willow bark extract anti-inflammatory activity is mostly attributed to the presence of bioactive constituents like salicin and flavonoids [6]. Salicin, in its glycoside form, undergoes hydrolysis to salicylic acid, which is inactivates COX enzymes. This will cause reduced release of prostaglandins and other factors that cause inflammation [7]. While her action is different from that of non-steroidal anti inflammatory drugs (NSAIDs), it is less synthetic and more nature-based. Flavonoids quercetin and apigenin enhance the anti-inflammatory effects by inhibiting the transcription factor NF-κB that is responsible for inflammatory gene expression [8]. Therefore, willow bark extract reduces inflammation via inhibition of NF-κB dependent synthesis of pro-inflammatory cytokines [9].

IBD entails chronic dysfunctions in the gastrointestinal tract's small intestine and colon. These patients may complain of abdominal pains, weight loss, and persistent diarrhea. Further inflammation is done by some pathogenic gram-negative bacteria because of immune response hyperactivation. IBD remains treated more completely by suppressing inflammation and bacterial growth through willow bark extract [10-11]. In patients suffering from IBD where inflammation is less severe, willow extract helps to restore the disturbed microbiotic balance [12].

Willow bark extract's therapeutic effects may also stem from the extract's antioxidant properties. The overabundance of reactive oxygen species (ROS) compared to antioxidant defenses causes oxidative stress which is the basis of inflammation within the gut [13]. Active components contained in willow bark extract such as flavonoids and tannins serve as scavengers of ROS and protect intestinal tissues from being oxidized. Thus, they embody the hallmark of gut inflammation and oxidation within the intestine [14].

2- Gram-negative intestinal Bacteria

As noted, these Gram-negative intestinal bacteria are a collection of single-celled organisms that live in the digestive system of humans and animals [15-17]. These microorganisms live within the intestines of humans and animals, possessing a characteristic outer membrane comprising lipopolysaccharides (LPS), a thin layer of peptidoglycan, and a cytoplasmic membrane [18]. This complex structure makes it difficult for most antibiotics and environmental stressors to destroy these organisms. This makes them important and dangerous, at the same time, to the gut microbiota. All Gram-negative microbiota, including Gram-negative organisms, contribute significantly to the well-being of the host by helping in the decomposition of biomolecules, production of some vitamins, and modulating the immune system [19]. Nevertheless, few Gram-negative microbes can infect or trigger disease once they move into different body regions or the balance of the gut microbiota is disturbed [20-21].

The most outstanding Gram-negative intestine bacteria are from the family Enterobacteriaceae, which includes genera like Escherichia, Klebsiella, Proteus and Salmonella [22]. These types of bacteria are anaerobic, which means that they do not require oxygen to live or develop, enabling them to exploit various regions within the gut [22-23]. For instance, Escherichia coli (E. coli) is a pathogenic microorganism that infects the human gut soon after birth and lives within the individual without causing disease. However, some strains of E. coli are pathogenic like enterohemorrhagic E. coli (EHEC) and enterotoxigenic E. coli (ETEC) which cause a variety of gastrointestinal disorders including diarrhea and hemorrhagic colitis [24].

The outer membrane of Gram-negative bacteria is critical for their self-defense against the immune system of the host. Lipopolysaccharides (LPS), one of the most important components of the membrane, when released into the blood stream can cause some of the most serious inflammatory responses [25]. This is especially difficult for the cases of sepsis where there is a movement of gram negative bacteria with their endotoxins from the gut to blood leading to widespread inflammation and multi organ failure. Poor diet, underlying diseases and even antibiotics can compromise the gut barrier that prevents this translocation leading to infection risks.

The Antibiotic resistance of gram negative intestinal bacteria is of great concern. Multi-drug resistant (MDR) strains like extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae and carbapenem resistant Enterobacteriaceae (CRE) is a clear result of excessive and indiscriminate use of antibiotics [26]. These strains pose great danger to the public health since they reduce the chances of effective treatment and increase the risk of having an infection. This is far worsened by the fact that resistance is rapidly disseminated among bacteria due to horizontal gene transfer by plasmids [27].

But some strains of E. coli are pathogenic, like enterohemorrhagic E. coli (EHEC) and enterotoxigenic E. coli (ETEC), which are known to lead to several gastrointestinal disorders, including diarrhea and hindered colitis.

The outer membrane within Gram-negative bacteria serves very well for self-defence towards the host immune systems. A notable example of this membrane component is Lipopolysaccharides (LPS), which when unleashed into the blood circulation incite incredible inflammation [25]. This is the most severe problem in sepsis, where the migration of Gram- negative bacteria with their endotoxins from the gut into the bloodstream results in diffuse inflammatory responses along with multiple organ failure. Poor diets, pre-existing medical conditions, or even the use of some antibiotics may compromise the integrity of the gut barrier that is meant to be protective against translocation, and this increases the risk for infection.

The issue of antibiotic resistance in Gram-negative intestinal bacteria remains one of the most critical challenges to address. The evolution of multi-drug resistant (MDR) strains such as Enterobacteriaceae producing extended-spectrum beta-lactamase (ESBL) and carbapenem-resistant Enterobacteriaceae (CRE) is the direct effect of antibiotics being used too much and inappropriately being used [26]. These strains significantly damage public health because they severely limit the treatment options available and increase the chances of getting infected. This worsens the problem because resistance is quickly spread amongst bacterial communities due to horizontal gene transfer through plasmids[27].

3- Bioactive Compounds in Willow Bark Extract

Biowellness willow bark extract contains several bioactive phytochemicals, such as salicin, flavonoids, phenolic and tannins, which provide anti-inflammatory, anti-microbial, and antioxidant activities [28-30]. Some salicin compounds jointly fight pathogenic bacteria, inflammation, and gut health issues. The major active ingredient, salicin, gets metabolized to salicylic acid, and willow bark extract's flavonoids and tannins combined with salicin have an even stronger anti-microbial and anti-inflammatory activity, hence, making willow bark extract the extract with natural value for systemic and gastrointestinal disorders [29-31].

3.1 Salicin and Its Derivatives

Salicin is a glycoside compound present in willow bark and is identified as salicyl alcohol linked to a glucose unit with a β-glycosidic bond. Naturally, this structure leads to salicin's water solubility, aiding its absorption through the digestive system [30,32]. After consuming the compound, salicin is enzymatically hydrolyzed in the liver and intestines, where the glucose part is separated, resulting in salicyl alcohol. Further, this intermediate is oxidized to produce salicylic acid, the metabolite responsible for the pharmacological effects of salicin extract from willow bark [33].

Unlike conventional NSAIDs, naturally derived salicin and its derivatives are better tolerated by patients sensitive to synthetic medications. Salicylic acid, which has both an anti-inflammatory and analgesic activity, works by preventing the action of cyclooxygenase (COX) enzymes responsible for producing inflammatory substances such as prostaglandins [34]. This function is much like aspirin (acetylsalicylic acid), although salicin and its natural derivatives tend to be more tolerated than synthetics.

The salicin-to-salicylic acid transformation process illustrates the significance of metabolic activation in a drug's function. This biotransformation reinforces the argument that salicin could be converted into an active drug, a safer and more effective alternative in treating pain, inflammation, and fever [36].

3.2 Flavonoids And Phenolic Compounds

Quercetin, apigenin, and naringenin are flavonoids found in willow bark extract. In addition to constituting the extract, these polyphenolic compounds are known to have strong anti-microbial activity. They cause damage to the membrane of bacterial cells, stop enzymatic activity, and block DNA synthesis. Their capability to scavenge reactive oxygen species increases their anti-microbial action by reducing oxidative stress, which otherwise aids in bacterial survival and inflammation [37-38]. Other phenolic compounds also include phenolic acids and catechins and contribute to the anti-microbial actions of willow bark extract. These compounds bind to the proteins or cell walls of bacteria and inhibit their function and growth. In addition, these phenolic compounds can chelate some important metal ions required by bacteria, which further inhibit bacterial growth. These processes enable flavonoids and phenolic compounds to be strongly effective against many pathogenic bacteria, including Gram-negative ones such as Escherichia coli and Klebsiella pneumoniae [39-41].

Flavonoids and phenolic substances aid in immune modulation and anti-inflammation in addition to their anti-microbial activities. These compounds down-regulate the generation of pro-inflammatory cytokines and chemokines by blocking the action of an important inflammation-associated transcription factor known as NF-κB [42-43].

3.3 Tannins and Other Components

Tannins are polyphenolic compounds extracted from the bark of willow trees that can also be found in wine and tea. They are well-known for their astringent, antibacterial, and anti-inflammatory effects. These compounds exhibit anti-bacterial activity by binding to the proteins and cell walls of bacteria and consequently disintegrating them, which inhibits bacterial adhesion and proliferation. Further bacterial proliferation is inhibited, and bacterial enzymes become nonfunctional, thus forming less active compounds by bound tannins [44-45].

Diverting from anti-microbial action, tannins are also well known for their anti-inflammatory effects. They block tissue damage through the neutralization of free radicals and the stabilization of cell membranes. Tannins also suppress the inflammatory response by inhibiting the release of pro-inflammatory mediators or cytokines such as histamine and prostaglandins [46]. They are particularly helpful in diseases such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), where both inflammation and bacterial overgrowth are central to disease progression [46-47].

Willow bark extract also contains catechins and phenolic acids, which help with the tannins' antioxidant and immune system effects. These three compounds synergistically work together to tackle bacterial overgrowth and inflammation [48]. Because tannins and other constituents work synergistically by targeting multiple pathways, willow bark extract is a comprehensive natural remedy for gastrointestinal and systemic health issues.

Table (1) summarises the most relevant bioactive components of the willow bark extract (WBE) and its anti-bacterial activity against a wide range of gram-negative bacteria. These compounds, which are of different chemical classes, such as phenolic glycosides, polyphenols, and flavonoids, possess myriad mechanisms restricting bacterial growth, adhesion, and viability.

Tabel 1. Bioactive Compounds in Willow Bark Extract and Their Anti-microbial Properties

Bioactive Compound	Chemical Class	Anti-microbial Properties	Target Bacteria
Salicin	Phenolic glycoside	Disintegrate bacterial cell membranes; obstruct the enzymatic activity of the bacteria.	*E. coli*, *P. aeruginosa*
Flavonoids	Polyphenols	Hinder bacterial adherence and biofilm	*K. pneumoniae*, *S. typhimurium*

		formation; neutralize free radicals.	
Tannins	Polyphenols	Bind to bacterial proteins and enzymes, halting their growth and metabolic activity.	*E. coli*, *P. mirabilis*
Phenolic acids	Phenolic compounds	Dismember cell membranes generate oxidative damage in microorganisms.	*P. aeruginosa*, *B. fragilis*
Catechins	Flavonoids	Restrict bacterial efflux pumps; increase the permeability of membranes.	*E. coli*, *Klebsiella spp.*

4- Anti-microbial Mechanisms of Willow Bark Extract

Various bioactive agents such as salicin, flavonoids, phenolic compounds, and tannins make Willow bark extract a potent anti-microbial agent. These components antagonistically act on bacterial cell membranes, biofilm formation, and metabolic processes. Moreover, the extract has action against both gram-positive and gram-negative bacteria, even though there is a difference in the action and the outcome between the two. Due to its anti-microbial effects on many microbes, the substance will be a beneficial natural agent for bacterial infections and gut health issues.

4.1 General Anti-microbial Activity

Willow bark extract has activity against a great spectrum of Gram-negative and Gram-positive pathogenic bacteria. This property is due to its composition of bioactive compounds, mainly flavonoids, phenolic acids, and tannins, which destroy the integrity of the bacterial cell membrane, inhibit enzymes, and stop DNA replication. Quercetin and apigenin are more well-known flavonoids that cause bacterial cell membrane destabilization and lysis; on the other hand, phenolic compounds block bacterial metabolism by chelating important metal ions [49-51].

The anti-microbial effects are further enhanced by tannins, which bind to protein substances and bacteria's cell walls, thus blocking adhesion and colonization [52]. Also, the extract's antioxidant properties alleviate oxidative stress that could otherwise assist in bacterial subsistence [53]. These complex mechanisms account for the efficacy of willow bark extract against Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus.

4.2 Mechanisms Against Gram-Negative Bacteria

4.2.1 Disruption of Cell Membranes and Walls

Willow bark extract acts against Gram-negative bacteria with outer membrane and cell wall disintegration. Outer membrane lipopolysaccharides (LPS) are cleaved by phenolic compounds and flavonoids, augmenting the impairment and leaking of cytoplasm [54]. Through binding to peptidoglycan and proteins, tannins make cell walls more porous and cause deterioration [55]. This facilitates the destruction of bacteria and increases the anti-microbial activity of the extract.

4.2.2 Biofilm Formation on WBE Efforts

The polysaccharide salicylates and polyphenolic compounds undergo biofilm inhibition through interference with adhesion, quorum sensing, and extracellular polymeric substance (EPS) production. A study [56] proposes that its active compounds may interrupt biofilm maturation by blocking central control circuits like LuxS/AI-2 quorum sensing of Escherichia coli and Salmonella spp. In addition, the extract has been cited to improve the ease with which bacterial biofilm structure goes through disintegration, which makes these bacterial cells more vulnerable to drugs. These findings offer promise for their being harnessed as a natural approach to mitigate biofilm-associated infections and antibiotic resistance among intestinal pathogens.

Order Salicylates and flavonoids acted on the gram-negative intestinal bacteria's metabolic enzymes by interfering with key bacterial enzymes and metabolic pathways [57]. For example, salicylates block the action of ATP-dependent enzymes to oxidative phosphorylation, gradually increasing bacterial energy and starving after severe restriction in available oxidative respiration. [58]. In addition to that, willow bark polyphenols make some of the common sugars and the cyclic compound of the Takedown and double cycle bring about some metabolic stress and reduce the growth rate of bacteria.

Furthermore, some willow bark compounds have been shown to reduce the activity of β-lactamase and efflux pumps in resistant strains of Escherichia coli and Salmonella spp., which results in increased susceptibility to antibiotics [59]. The extract also inhibits quorum sensing-associated enzymes, silencing bacteria's communication and expression of virulence factors.

4.3 Comparison with Gram-Positive Bacteria

Willow bark extract selective grazing differs due to the structural and functional differences between Gram-positive and Gram-negative bacteria. Because gram-positive bacteria have a thick peptidoglycan layer and no outer membrane, they are generally more susceptible to the anti-microbial compounds in the extract [60]. The flavonoids and tannins in willow bark easily pass through the peptidoglycan layer and damage the bacterial cell membrane, resulting in cell death [61]. Phenolic compounds also aid this effect by covalently binding with teichoic acids integrated within the cell wall of gram-positive bacteria, which destroys their structural and functional integrity.

On the other hand, Gram-negative bacteria have an outer membrane with lipopolysaccharides (LPS), which serves as an additional barrier against anti-microbial agents. Willow bark extract affects this outer membrane through flavonoids and phenolic compounds by aiding LPS in becoming more permeable. Tannins are also involved to some degree by destabilizing the side of the cell wall and preventing biofilm formation [62]. Nonetheless, this outer membrane renders gram-negative bacteria more resistant than gram-positive species.

Willow bark extract is known to possess a broad range of mechanisms, such as membrane disruption, enzyme inhibition or interference with metabolic pathways [63], which enables it to work against both groups of bacteria. Because of its capacity to exploit multiple vulnerabilities within a bacteria, the chances of resistance development are minimal, making it a broad-spectrum anti-microbial agent of natural origin.

Table (2) included a comparison of willow bark extract and other natural anti-microbials with activity against gram-negative bacteria. This analysis compares the sources, anti-microbial activity, benefits and disadvantages of each compound, emphasizing the distinctive characteristics of willow bark extract, particularly its preferential anti-inflammatory activity, while recognizing the shortcomings, including variability in efficacy and lack of clinical demonstration.

Tabel 2. Comparison of Willow Bark Extract with Other Natural Anti-microbials Against Gram-Negative Bacteria

Natural Anti-microbial	Source	Activity Against Gram-Negative Bacteria	Advantages of Willow Bark Extract	Limitations of Willow Bark Extract
Willow bark extract	*Salix* species	Moderate to strong activity; selective inhibition.	Rich in salicin and flavonoids; anti-	Variable efficacy; limited clinical studies.

			inflammatory properties.	
Berberine	*Berberis* species	Strong activity disrupts bacterial membranes.	Well-studied, broad-spectrum activity.	It may disrupt beneficial gut bacteria.
Curcumin	Turmeric (*Curcuma longa*)	Moderate activity inhibits biofilm formation.	Anti-inflammatory and antioxidant properties.	Poor bioavailability; limited efficacy alone.
Garlic extract	*Allium sativum*	Strong activity; contains allicin.	Broad-spectrum activity; well-documented.	Strong odour; potential gastrointestinal side effects.
Honey	Bee products	Moderate activity; osmotic effect on bacteria.	Natural and safe; promotes wound healing.	Variable composition; limited activity against resistant strains.

5- Effects on Gram-Negative Intestinal Bacteria

5.1 Literature Review

Recent works have started investigating the anti-microbial activity of willow bark extract (WBE) on gram-negative intestinal bacteria and its bioactive elements, such as salicin, flavonoids, and phenolic acids. In [64], it is shown that WBE disrupts membranes of Escherichia coli and Salmonella enterica and inhibits their growth while maintaining beneficial gut microbiota. In the same manner, an investigation by [65] demonstrated that WBE inhibits quorum sensing and biofilm formation in Pseudomonas aeruginosa, which indicates its capacity to reduce pathogenicity. Supporting and complementary findings by [66] showed WBE also aids gut dysbiosis by selectively targeting gram-negative species while not harming Lactobacillus or Bifidobacterium populations.

Moreover, [67] found synergism between WBE and conventional antibiotics in treating multidrug-resistant Klebsiella pneumoniae. On the other hand, [68] raised concern concerning variability in the composition of WBE due to extraction methods, which might affect its anti-bacterial properties. Supporting this claim, [57] showed that the potency of WBE is dependent on the solvent used for extraction, with ethanol extracts being more potent than water extracts.

Also, recent findings by [69] explained the actions of WBE and how it targets lipopolysaccharide biosynthesis of gram-negative bacteria.

Other prominent contributions involve the work of [70], who recorded dose-dependent inflammatory marker decreases caused by [71] and studied WBE's prebiotic-like effects on microbial diversity. Overall, these studies highlight the potential therapeutic value of WBE, yet additional clinical research is needed to confirm its safety and benefits.

At the same time, new findings from [72] propose that WBE might mitigate antibiotic-induced diarrhoea by restoring the gut microbial community. This follows previous comments by [73], who reported cardiovascular improvement after WBE supplementation in animal models. Despite these developments, many unanswered questions remain regarding the long-term effects of dose amounts and frequency, as pointed out by [74] in his meta-analysis.

Additional studies deepen our comprehension of WBE's effect on gram-negative intestinal bacteria, expanding on these findings. One study's [75] focus was on the time-dependent kinetics of WBE’s anti-bacterial activity and concluded that its prolonged exposure increases the inhibition of both Proteus mirabilis and Serratia marcescens. In the same way, WBE's influence on bile acid metabolism and its capability of decreasing secondary bile acid synthesis by gram-negative bacteria leading to intestinal inflammation was demonstrated by [1].

Another important part was brought by [76], who researched WBE’s effects on gram-negative pathogen horizontal gene transfer and concluded that it greatly decreased the dissemination of plasmid-mediated antibiotic resistance. At the same time, [77] studied the impact of WBE on gut barrier function and showed its positive effect. WBE upregulates tight junction protein expression, thereby lowering the chance of bacterial translocation in states of inflammation. Also, [38] compared WBE and synthetic salicylates and reported that the natural extract was more active against gram-negative bacteria owing to a more complex phytochemical composition. Additionally, [78] reported on the orally active components of WBE active constituents and pointed out the need for better formulation approaches aimed at systemic delivery.

Furthermore, the study of [79] showed that WBE’s interaction with dietary fibre results in a greater anti-bacterial effect when used with prebiotics such as inulin.

Newer studies by [80] emphasized WBE's promise in helping fight Acinetobacter baumannii biofilm-related infections through the breakdown of extracellular polymeric substances. Lastly, [81] advanced our understanding of the impact of WBE on gut microbiota resilience by WBE’s prescription, suggesting it fosters the recovery of microbial diversity following antibiotic disturbance. In combination, these studies emphasize the multi-purpose function of WBE in controlling gram-negative intestinal bacteria and, simultaneously deepen the understanding of its use.

Tabel 3. Summary of Studies on the Anti-microbial Activity of Willow Bark Extract Against Gram-Negative Intestinal Bacteria

Study	Key Findings	Target Bacteria/Condition	Mechanism Of Action
[64]	Significant inhibition of bacterial growth; preservation of beneficial gut microbiota.	Escherichia coli, Salmonella enterica	Disruption of bacterial cell membranes.
[65]	Reduction in quorum sensing and biofilm formation.	Pseudomonas aeruginosa	Interference with bacterial communication pathways.
[66]	Modulation of gut dysbiosis; selective targeting of gram-negative bacteria.	Gram-negative species (E. coli, etc.)	Selective anti-microbial activity without harming beneficial bacteria (Lactobacillus, Bifidobacterium).
[67]	Synergistic effects with antibiotics against multidrug-resistant strains.	Klebsiella pneumoniae	Enhanced efficacy of conventional antibiotics.
[68]	Variability in anti-bacterial activity due to extraction methods.	General gram-negative bacteria	Influence of extraction solvents

			on composition and potency.
[57]	Superior inhibition by ethanol-based extracts compared to water-based ones.	General gram-negative bacteria	Solvent polarity affects extract potency.
[69]	Molecular mechanism involving interference with lipopolysaccharide biosynthesis.	Gram-negative bacteria	Disruption of lipopolysaccharide production.
[70]	Dose-dependent reduction in inflammatory markers induced by Enterobacteriaceae.	Enterobacteriaceae family	Anti-inflammatory properties.
[71]	Prebiotic-like effects promoting microbial diversity.	Gut microbiota	Enhancement of beneficial microbial populations.
[72]	Mitigation of antibiotic-associated diarrhea.	General gut microbiota	Restoration of microbial balance.
[73]	Improved gastrointestinal health outcomes in animal models.	Animal gut microbiota	Supplementation improved gut health metrics.
[74]	Gaps in long-term effects and optimal dosing regimens.	General gram-negative bacteria	Meta-analysis highlighting research gaps.
[75]	Time-dependent kinetics enhancing inhibitory effects.	Proteus mirabilis,Serratia marcescens	Prolonged exposure increases anti-bacterial activity.
[1]	Modulation of bile acid metabolism to reduce intestinal inflammation.	Gram-negative bacteria	Reduction in secondary bile acid production.
[76]	Reduction in plasmid-mediated antibiotic resistance dissemination.	Gram-negative pathogens	Inhibition of horizontal gene transfer.
[77]	Enhancement of gut barrier integrity.	General gut microbiota	Increased tight junction protein expression.
[38]	Broader-spectrum activity compared to synthetic salicylates.	Gram-negative bacteria	Complex phytochemical profile of WBE.
[78]	Importance of formulation strategies for systemic delivery.	General gram-negative bacteria	Oral bioavailability of active compounds.
[79]	Enhanced anti-bacterial activity when co-administered with prebiotics.	Gram-negative bacteria	Interaction with dietary fibres (e.g., inulin).

[80]	Disruption of biofilm-associated infections.	Acinetobacter baumannii	Disruption of extracellular polymeric substances.
[81]	Promotion of microbial diversity recovery after antibiotic perturbation.	Gut microbiota	Ecological resilience of gut microbiota.

2. Materials and Methods

The methodology for this study is using a qualitative literature and experimental work, review to assess the antimicrobial properties of willow bark extract (WBE) toward Gram-negative intestine bacteria. The study takes an integrative methodology, incorporating data from the microbiological, phytochemical contents, and clinical studies. These data were collected from peer-reviewed scientific articles, experimental studies, and clinical trials to demonstrate the efficacy of WBE's bioactive constituents, salicin, flavonoids and tannins. Criteria for the selection of studies published in the literature were to understand WBE's mechanisms of action, e.g., bacterial membrane disruption, inhibition of biofilm formation, modulation of inflammatory responses. Experimental studies that reported on the antimicrobial activity of WBE against Escherichia coli,Klebsiella pneumoniae, and Pseudomonas aeruginosa were given priority. Besides, in vivo studies testing WBE's influence on gut microbiota and inflammation suppression were evaluated to properly address its more extensive augmented therapy. Comparative evaluations were performed to to evaluate WBE efficacy versus the standard antibiotics and natural antimicrobial substances. The study also included evidence on the mechanisms bacteria responsible for resistance to assess the long-term sustainability of WBE as a treatment option or combination therapy. Variability in WBE's composition because of the application of different extraction means and of solvent kind was involved in accounting for discrepancy in antimicrobial action. Lastly, the methodological framework was set up to integrate lapses in established understanding through combining evidence on WBE's potential for gastrointestinal health management, getting insight into its clinical utility while recognising the tension for further empirical justification that comes in from controlled human studies.

3. Results

6- Challenges and Limitations

6.1 Variability in Efficacy

This compilation of studies in Table (3) offers a detailed overview of the most recent research, which scrutinizes the anti-microbial properties of willow bark extract (WBE) towards gram-negative intestinal bacteria. This compilation of studies accords WBE recognition as a possible natural anti-microbial agent, explains its mode of action, and describes its potential impact on gut health and gut microbiota.

The anti-microbial activity of willow bark extract (WBE) is dependent on the method of extraction, the concentration applied, and the bacterial species involved. The degree of effectiveness observed can be because of the differences in polarity of the solvent used in the extraction. For instance, extracts using ethanol as a base are more active in anti-bacterial properties than water-based extracts which shows how important the extraction process is [57]. Moreover, the amount of WBE administered is important because larger amounts tend to create stronger inhibitory effects, but can adversely affect the beneficial gut microbiota. In addition, the extent to which different strains of bacteria are able to be affected differs greatly, and some Gram-negative bacteria are resistant because of their outer membrane. These variations help explain the need to develop suitable methods of extraction and define dose levels for specific circumstances. These methods need to be developed in order to aid the use of WBE as a therapeutic agent.

The lack in consistency of WBE's anti-microbial efficacy presents an additional hurdle toward its proper application in the clinical setting. The presence of different strains of bacteria exacerbate the situation further because some pathogens may have some level of resistance. For example, Pseudomonas aeruginosa and Klebsiella pneumoniae are known to be resistant to some very potent naturally occurring anti-microbials, because of their impermeable outer membrane and efficient efflux system [68]. Such obstacles require careful planning of research to define optimum extraction parameters and bacterial localization sites of WBE's promise while avoiding the pitfalls of compromising safety and specificity.

6.2 Bacterial Resistance Mechanisms

Willow bark extract (WBE) can alter bile acid metabolism by decreasing the amount of secondary bile acids produced and consequently lessening inflammation of the intestines [1]. In addition, WBE enables the balance of microflora to be maintained, which preserves the integrity of the gut lining and, thereby, promotes overall gastrointestinal health. For example, S. aureus is a predominant Gram-negative pathogen and is resistant owing to the strong efflux mechanism many multi-drug resistant strains of E. coli and K. pneumoniae employ against the effects of flavonoids and phenolic acids. The outer membrane contains an LPS layer, which acts as a wall guarding hydrophobic substances from easily crossing into the cell. These mechanisms show the challenge of aiming for Gram-negative bacteria and that new approaches must be developed to tackle resistance.

Despite WBE's multifactorial anti-microbial features, bacterial attacks frequently undermine their effectiveness. Also, the existence of porins, which are protein channels that control the passage of molecules, further reduces the chances for larger bioactive compounds to be permeable. The issue is aggravated by the fact that horizontal gene transfer facilitates the quick spreading of resistance genes within populations of bacteria [27]. Combination therapies of WBE and standard antibiotics seek to address these problems and appear to induce increased bacterial susceptibility. Again, more effort is required to determine how WBE can be applied in a way that will bypass or inhibit resistance mechanisms.

6.3 Absence of Clinical Proof

While anti-microbial and anti-inflammatory activities of willow bark extract (WBE) have been reported in preclinical studies, there is still a considerable absence of clinical evidence on its use in humans. Most studies have been performed in vitro or in vivo using animal models, which may not accurately represent human physiological responses. In the absence of human trials, there is still much uncertainty regarding the best dosing strategies, the effect of prolonged use, and the side effects that may occur. This absence of clinical proof undermines the application of WBE for broad clinical practice, especially for multifactorial diseases such as inflammatory bowel disease (IBD) and antibiotic-induced diarrhoea.

The lack of coherent formulations makes assessing WBE’s clinical utility hard. The variability of extract composition stems from the differences in source materials, extraction methods, and study grade [68]. In order to close this gap, focused clinical trials are required to show how WBE can best be utilized. These studies will be conducted in different populations, with the consideration of other therapies. WBE need not be used to fight infections and promote gut health purely empirically without proper justification. These knowledge gaps must be addressed to enable effective evidence-based practice integration of WBE while ensuring safety in its application against Gram-negative bacterial infections.

6.4 Contributions

This review focuses on the impact of willow bark extract (WBE) on Gram-negative intestinal bacteria and its implications for infection control and gut health. The overall contributions of this work are:

1. **Classify Over Bioactive Compounds:** This review details bioactive compounds in WBE, including salicin, flavonoids, phenolic acids, and tannins, which are described as having anti-microbial, anti-inflammatory, and antioxidant actions [28-31]. This helps to appreciate the intricate ways WBE exerts its therapeutic effects.
2. **Mechanistic Insights into Anti-microbial Activity:** The review describes the mechanisms of WBE disruption of Gram-negative bacteria as cell membrane damage, biofilm inhibition, bacterial enzyme interference, and modulation of various metabolic pathways [49-56]. This information suggests the therapy of WBE as a natural anti-microbial agent.
3. **Comparison with Other Natural Anti-microbials:** A comparative analysis of WBE with other natural anti-microbials, such as berberine, curcumin, garlic extract, and honey, highlights its unique advantages, including selective inhibition of pathogens and anti-inflammatory benefits, while also acknowledging its limitations (Table 2).
4. **Applications in Gut Health and Infection Control:** The review outlines the diverse applications of WBE, such as targeting pathogenic bacteria, inhibiting biofilm formation, enhancing antibiotic efficacy, modulating gut microbiota, and reducing inflammation [Table 4]. These applications demonstrate WBE's versatility in addressing gastrointestinal and systemic conditions.
5. **Identification of Challenges and Limitations:** The review critically evaluates the challenges associated with WBE, including variability in efficacy due to extraction methods, limited clinical evidence, and bacterial resistance mechanisms [57, 68]. Addressing these gaps is essential for advancing its therapeutic use.
6. **Synthesis of Recent Studies:** By summarising findings from recent studies, the review consolidates current knowledge on WBE's anti-microbial activity against Gram-negative bacteria, providing a foundation for future research (Table 3).
7. **Highlighting Future Research Directions:** The review emphasizes the need for standardized extraction protocols, large-scale clinical trials, and innovative delivery systems to optimize WBE's therapeutic potential [74, 78]. These directions can guide researchers and clinicians in developing evidence-based applications.
8. **Integration of Multidisciplinary Perspectives:** By integrating microbiology, pharmacology, and nutrition science, the review offers a holistic understanding of WBE's role in managing Gram-negative bacterial infections and promoting gut health [12, 47].

7- Potential Applications

7.1 Gut Health

Willow bark extract (WBE) holds significant potential for improving gut health by selectively targeting pathogenic Gram-negative bacteria without disrupting the commensal microbiota. Pathogens such as Escherichia coli , Klebsiella pneumoniae , and Pseudomonas aeruginosa are known to exacerbate gastrointestinal conditions like inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). WBE's bioactive constituents, such as salicin, flavonoids, and tannins, have proven selective anti-microbial activity by acting against pathogenetic bacteria and sparing benevolent bacteria like Lactobacillus and Bifidobacterium [66]. This ability is very important in avoiding dysbiosis, one of the effects of broad-spectrum antibiotics. Moreover, research has indicated that WBE modifies bile acid metabolism by inhibiting the synthesis of secondary bile acids, which reduces intestinal inflammation [1]. WBE serves to mitigate the imbalance of microflora, which helps in maintaining the gut barrier and hence promotes overall health of the gastrointestinal system.

The precision by which WBE targets specific bacterial populations makes it easier to consider its use as a natural remedy for dysbiosis of the gut. For instance, WBE has been documented to reduce the dominance of some pathogenic bacteria, like Proteus mirabilis and Serratia marcescens, which are associated with persistent infection and inflammation [75]. In addition, its prebiotic-like effects are noted to be linked with remarkable increase of microbial diversity [71]. These features enable WBE to be effective in counteracting dysbiosis, particularly under conditions of compromised gut health.

7.2 Antibiotic Adjuvant

Willow bark extract (WBE) not only enhances the activity of conventional antibiotics, but it is also noted for its ability to function as an antibiotic adjuvant against multidrug-resistant (MDR) Gram-negative bacteria. Efflux pumps and transfer of plasmids have made treatment effective for a multitude of people impossible due to resistance emergence [27]. WBE helps tackle these problems by inhibiting the activities of some bacterial enzymes, such as β-lactamase, and efflux pumps, thus increasing sensitivity of the bacteria to the drugs [59]. For this reason, greater bacterial clearance has been observed with WBE in the treatment of Klebsiella pneumoniae and Escherichia coli infections [67]. Such approaches improve treatment outcomes while minimizing the likelihood of developing resistance to antibiotics.

Additionally, the disruption of biofilms is facilitated by WBE which greatly increases the functionality of WBE as an antibiotic adjuvant. Chronic infections are largely due to the existence of biofilms, which render bacteria anti-microbial resistant. WBE has the ability to hydrolyze biofilm structures because of quorum sensing and extracellular polymeric substances, making the bacteria more responsive to antibiotic treatment [65].

There are still issues with standardizing doses and combinations for clinical application. These obstacles must be solved through intensive research to exploit WBE as a supplementary therapy and an innovative solution to mitigate antibiotic resistance while enhancing patient care.

7.3 Effects on Inflammation

Willow bark extract has been shown to directly contribute to gut health benefits by helping manage diseases such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). The chronic inflammation within the human gut is often sustained by the excessive production of pro-inflammatory cytokines like tumour necrosis factor (TNF-α), Interleukin 6 (IL-6) and Interleukin 1 beta (IL-1β), which is a consequence of pathogenic Gram-negative bacteria in addition the their lipopolysaccharide [4]. WBE reduces inflammation by blocking oxidizing NF-k B and lowering oxidative damage via antioxidant activity [43]. WBE reduces tissue damage caused by inflammation by hindering the undermining processes and further helping gut barrier properties. This assists in aiding the balance of the gut microbiota population.

The modulation of bile acid metabolism by WBE also contributes to inflammation reduction. While certain Gram-negative bacteria can lead to the production of secondary bile acids that increase intestinal inflammation, WBE curbs these acids' production, thus making the intestinal environment easier for beneficial microbes to thrive in [1]. WBE facilitates gut health through a single approach due to its anti-inflammatory action and enhancement of the resilience of microbiota. It is important to note, however, that dissociation anti-inflammation mechanisms from direct anti-microbial action is not straightforward. Those processes must be studied further in order to improve the therapeutic effectiveness of WBE without increasing the adverse effects of inflammatory bowel disease.

Willow bark extract, as summarized in Table (4), seems to impact health of the intestines and infections with gram-negative bacteria in a more systemic way. The multifaceted ability of willow bark extract lies in the ability to target pathogenic microorganisms, biofilm inhibitors, increase effectiveness of recombined gut microflora antibiotics and inflammation.

Tabel 4. Potential Applications of Willow Bark Extract in Gut Health and Infection Control

Application	Description	Relevant Gram-Negative Bacteria	Challenges
Targeting Pathogenic Bacteria	Selective inhibition of pathogens like *E. coli* and *P. aeruginosa*.	*E. coli*, *P. aeruginosa*, *K pneumoniae*	Ensuring selectivity to avoid harming beneficial bacteria.
Biofilm Inhibition	Preventing biofilm formation in chronic infections.	*P. aeruginosa*, *Proteus spp.*	Overcoming resistance mechanisms in biofilm-forming bacteria.
Antibiotic Adjuvant	Enhancing the efficacy of antibiotics against resistant strains.	Multidrug-resistant *E. coli*, *Klebsiella*	Standardizing doses and combinations for clinical use.
Gut Microbiota Modulation	Promoting a healthy balance of gut bacteria by reducing pathogenic overgrowth.	*Bacteroides*, *E. coli*	Ensuring minimal disruption to commensal bacteria.
Anti-Inflammatory Effects	Reducing gut inflammation, indirectly supporting microbiota balance.	N/A (indirect effect)	Separating anti-inflammatory effects from anti-microbial activity.

4. Discussion

Willow bark extract (WBE) has emerged as a promising natural remedy for Gram-negative intestinal bacteria and associated gastrointestinal ailments. Salicin, flavonoids, phenolic acids, and tannins, among others, are bioactive compounds that have various anti-microbial actions—destruction of bacterial cell membranes, biofilm inhibition, and metabolic disruption. This makes WBE potent against pathogens like Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. Despite these prospects, there remains considerable difficulty in standardizing composition to afford consistent effectiveness. Differing extraction techniques and the polarity of the solvent are shown to greatly affect the potency of WBE, as studies indicated various forms of ethanol resulted in superior activity than water [57, 68]. Resolving these discrepancies poses a challenge in mitigating WBE as a reliable anti-microbial agent.

One of the most notable applications of WBE is its ability to target Gram-negative pathogenic bacteria while preserving beneficial gut microbiota. This selectivity is important in avoiding dysbiosis, a frequent result of broad-spectrum antibiotics [66]. Furthermore, WBE's anti-inflammatory effects add to its usefulness in treating chronic conditions like inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). WBE applies various means of reducing inflammation, such as inhibiting NF-κB and oxidative stress, thus fostering a comprehensive scope of gut health care [70]. Still, complex mechanisms must be addressed to disentangle anti-inflammatory effects from anti-microbial activity and optimize its therapeutic application.

Another noteworthy function of WBE is its use as an antibiotic adjunct, improving the action of common antibiotics on multiresistant strains. Synergistic effects of WBE and antibiotics, especially against resistant Enterobacteriaceae, have been confirmed [67]. This is very important because of the rising issue of antibiotic resistance because of horizontal gene transfer and plasmid-based resistance gene diffusion [27]. The ability of WBE to diminish plasmid transfer among Gram-negative pathogens is a promising way to tackle the challenge of public health danger due to antibiotic resistance [76]. However, standard dosage and combination for clinical manipulation are critical and need further testing.

Even with the benefits, WBE has limitations in efficacy and clinical validation. While its potential is shown in preclinical studies, no large-scale human trials can ensure safety and efficacy. Furthermore, the oral bioavailability of WBE's active compounds is another issue that calls for better formulation approaches to enhance systemic delivery. To address these gaps, interdisciplinary efforts integrating pharmacology, nanotechnology, and WBE’s clinical research are essential to realize its therapeutic promises.

In addition, the impact of WBE on gut microbiota resilience is another important consideration. Recent studies indicate that WBE may help recover microbial diversity following antibiotic disturbance, thus preventing antibiotic-induced diarrhea. This prebiotic-like action indicates its usefulness in helping to rebalance gut microbiota and risks of minimal disruption to commensal bacteria. Nonetheless, more studies are needed to explain the impact of WBE on gut ecology with time, along with its interaction with dietary fibres, which may further increase anti-bacterial activity.

5. Conclusion

Willow bark extract (WBE) holds great promise as a natural anti-microbial substance against Gram-negative intestinal bacteria. Its bioactive components, such as salicin, flavonoids, and tannins, have multiple mechanisms, including disruption of bacterial membranes, inhibition of biofilm formation, and inflammation modulation. These features make WBE a strong candidate for dealing with gut dysbiosis, antibiotic resistance, and chronic inflammatory conditions such as IBD and IBS. Nonetheless, variability in extract composition, clinical validation, and formulation challenges must be overcome to optimize therapeutic usage.

More research should be directed towards developing protocol standardization for extraction methods and large-scale clinical studies that establish the boundaries of the application of WBE. Its interactions with dietary fibres and other natural compounds could be studied to augment their effectiveness, while delivery systems could maximize bioavailability. Furthermore, research on WBE's long-term and ecological effects on gut microbiota is crucial for ensuring safety and sustainability.

Filling in these gaps would allow WBE to be used as a supplement for antibiotic resistance and enhancing gut health, and with proper research, it can be integrated into standard medicine.

Conflicts Of Interest

No Conflicts of Interest

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