

ANTIBACTERIAL ACTIVITY OF BEET EXTRACT (BETA VULGARIS L.) AGAINST GRAM-POSITIVE BACTERIA: SYSTEMATIC LITERATURE REVIEW

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Abstract

Increasing antibiotic resistance in Gram-positive bacteria is driving the development of alternative antibacterial agents based on natural ingredients. Beetroot (Beta vulgaris L.) is known to contain betalain and phenol that have antibacterial potential. This study aims to analyze the antibacterial activity of Beet extract against Gram-positive bacteria and evaluate the effect of concentrations, solvents, and testing methods based on the literature during the period 2016–2026. Systematic Literature Review was conducted using the PRISMA protocol through literature searches on Publish or Perish, Google Scholar, ScienceDirect, and DOAJ databases. A total of 20 articles that met the inclusion criteria were analyzed based on the value of the inhibition zone diameter (ZI) and the Minimum Inhibitory Concentration (MIC). Beet extract showed antibacterial activity against various Gram-positive bacteria, one of which was Staphylococcus aureus with an inhibition zone range of <5–25 mm and a MIC of 0.012–32 mg/mL. Ethanol is the most widely used solvent, while the agar well diffusion method tends to produce a larger inhibition zone than disc diffusion. Beet tuber extract has the potential to be developed as a natural antibacterial source against various Gram-positive bacteria.

Keywords: Beta vulgaris L. , Antibacterial , Gram-positive bacterial , Systematic Review .

A. Introduction

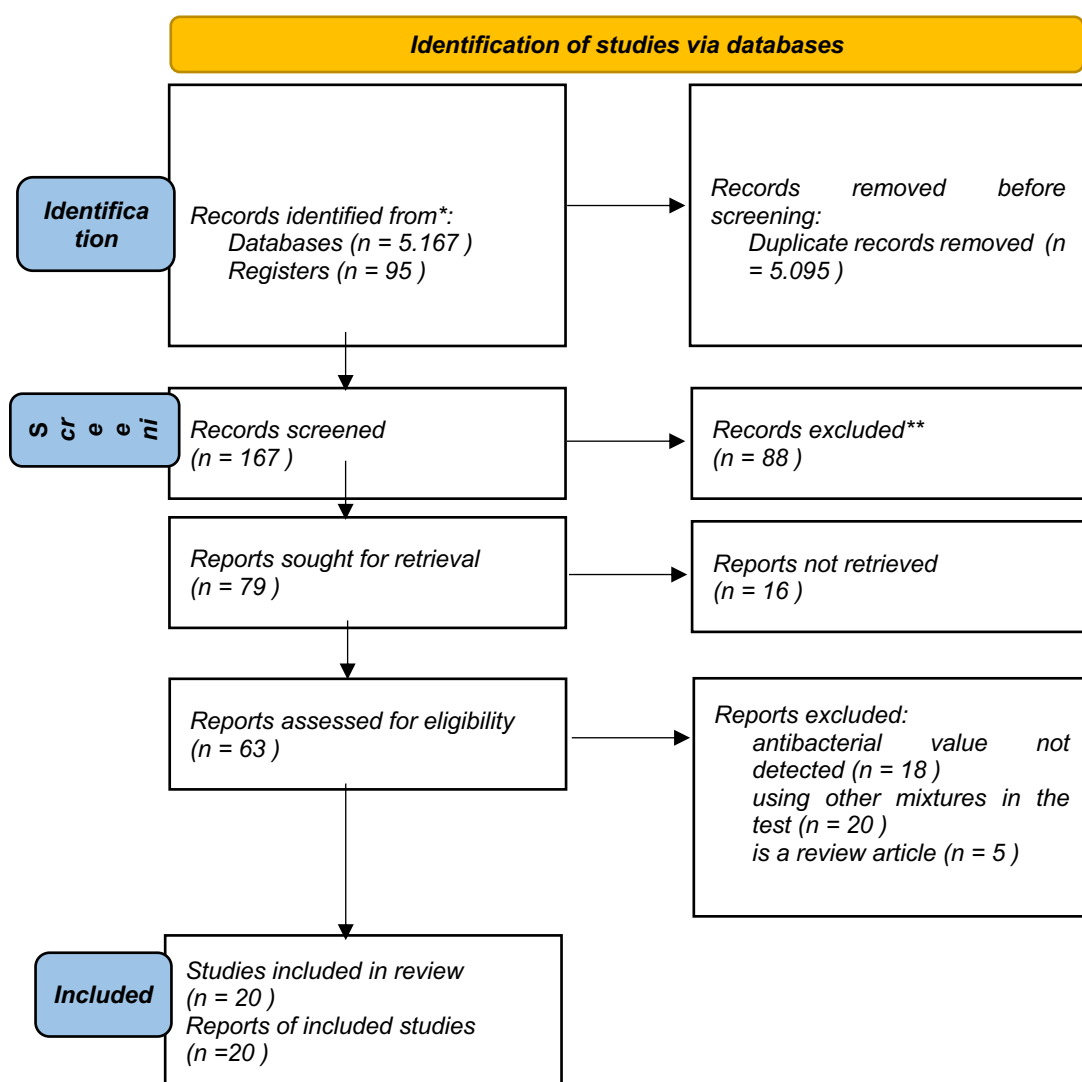
Infectious diseases caused by bacteria remain a significant public health challenge worldwide [1]. Gram-positive bacteria such as Staphylococcus aureus, including methicillin-resistant Staphylococcus aureus (MRSA) strains, are important pathogens that can cause a variety of clinical conditions, ranging from skin and soft tissue infections to serious systemic infections. [2], [3]. This problem is further complicated by the increasing resistance of antibiotics to conventional therapies [4], [5]. Therefore, efforts are needed to find alternative antibacterial agents, including those from natural sources (phytopharmaceuticals), as a potential approach to addressing this problem. Beetroot (Beta vulgaris L.) is known as a rich source of nutrients, modern phytochemical studies have shown that beetroot contains various bioactive compounds [6].

The main components that have been identified include betalains (betacyanin and betaxanthin), polyphenols, flavonoids, and saponins, which are known to have various biological activities, such as antioxidant, anti-inflammatory, and potential antimicrobial activity [7] Gram-positive bacteria have a cell wall structure dominated by a thick peptidoglycan layer which can be a potential target for antibacterial compounds [8] . The active compounds in beetroot extract are thought to work by disrupting the integrity of the cell wall or inhibiting bacterial intracellular metabolism. Various experimental studies have reported that beetroot extract is able to inhibit the growth of Gram-positive bacteria. However, the reported effectiveness shows quite significant variations, which are influenced by differences in concentration, extraction method, type of solvent, and test method used as well as the characteristics of the bacterial isolates tested [9], [10]. To date, data on the antibacterial potential of beetroot remains scattered across primary studies with varying parameters, limiting comprehensive conclusions. Therefore, a systematic evaluation is needed to integrate and synthesize the available scientific evidence. This systematic review aims to assess the antibacterial activity of Beta vulgaris L. extract, specifically against Gram-positive bacteria.

B. Research methods

Literature Search Strategy

This study is a Systematic Literature Review (SLR) compiled following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol. A comprehensive data search was conducted in major electronic databases, namely Publish Or Perish, Google Scholar, ScienceDirect, and DOAJ, to identify primary studies published within the last ten years (2016–2026). The initial data extraction process was carried out using Publish or Perish 8 software and websites from Google Scholar, ScienceDirect, and DOAJ to ensure the efficiency of meta-data searches. The search string used combines Boolean operators (AND, OR) with specific keywords: ("Beta vulgaris" OR "Beetroot") AND ("Antibacterial" OR "Antimicrobial" OR "Bioactivity") AND/OR ("MIC" OR "Inhibitor Zone"). For domestic literature, a combination of keywords was used: ("Beet" OR "Beta vulgaris") AND ("Antibacterial" OR "Antimicrobial" OR "Bioactivity") AND/OR ("Minimum Inhibition Zone").



*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).

**If automation tools were used, indicates how many records were excluded by a human and how many were excluded by automation tools.

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Figure 1. Literature search flowchart.

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Inclusion and Exclusion Criteria

To maintain the quality of the analysis, strict selection criteria were established. Inclusion criteria included:

1. Original experimental study (original research) testing beetroot (*Beta vulgaris L.*) extract against Gram-positive bacteria.
2. Articles that list quantitative parameters such as Zone of Inhibition (ZI) diameter or Minimum Inhibitory Concentration (MIC)

Exclusion criteria include:

1. Review articles , theses/dissertations not published in journals, and short reports.
2. Research that only uses plant parts other than tubers

Study Selection and Data Extraction

The selection process was carried out through four phases: identification, title and abstract screening, full - text eligibility assessment , and final inclusion. Data from eligible articles were extracted into a standardized table that included author identity, year of publication, extraction solvent type, target bacterial species, and antibacterial activity test results for further analysis and systematic presentation.

Data analysis

The collected data was analyzed descriptively and qualitatively to determine the correlation between solvent type, test method selection, and extract concentration with the resulting inhibitory effectiveness. Data synthesis focused on interpreting betalain and phenol compounds against Gram-positive bacteria to draw comprehensive conclusions.

C. Results and Discussion

Synthesis results from various literature indicate that beetroot (*Beta vulgaris L.*) extract has a broad spectrum of antibacterial activity against Gram-positive bacteria. Based on the collected data, there are variations in the Minimum Inhibitory Concentration (MIC) and the diameter of the zone of inhibition (ZI).

Table 1. Range of Inhibition Zone and MIC Values of Beetroot Extract against Gram-Positive Bacteria Reported in the Literature

Name of Bacteria	Number of studies	Extract Concentration Range	Inhibition Zone Range (mm)	MIC range (mg/mL)
Staphylococcus aureus	12	100 µg/mL – 16 mg/mL	<5 – 25	0.012 – 32
Methicillin-resistant Staphylococcus aureus (MRSA)	1	1.25 – 20 mg/mL	Nr	>20
Streptococcus mutans	1	12.5 -100 mg/ml	0.11 – 0.40	12.5
Streptococcus pyogenes	1	2 mg/mL	10	2
Bacillus cereus	7	0.5 – 45 mg/mL	4 – 25	0.5 – 45
Bacillus subtilis	3	0.012 – 2	10 – 20	0.012 – 2
Listeria monocytogenes	5	0.3 µg/mL – 40 mg/mL	11 – 15	2.5 – 40
Enterococcus faecalis	2	0.03 – 32 mg/mL	10 – 15	4-16 mg/mL
Micrococcus luteus	1	0.03 – 32 mg/mL	Nr	0.03 – 32
Staphylococcus epidermidis	2	Nr	<15	Nr
Bacillus pumilus	1	Nr	11 – 15	Nr

Nr = not reported

Table 2. Distribution of Extraction Solvents Reported in the Literature

Solvent	Number of studies
Ethanol (70% – 96%)	13
Methanol	5
Water	5
Acetone	1
Ethyl acetate	1
Chloroform	1

Note: Some studies used more than one solvent.

Table 3. Distribution of Antibacterial Test Methods

Test Method	Number of Studies
Agar well diffusion	9
Disc diffusion	6
Broth Microdilution	9
Agar dilution	2

Note: Some studies reported more than one parameter of antibacterial activity testing, including inhibition zone diameter and Minimum Inhibitory Concentration.

In Table 1. the inhibition test conducted by [11] and [12] shows a striking difference in the diameter of the inhibition zone in *Staphylococcus aureus* bacteria from beetroot ethanol extract. The results of the antibacterial test using the disc diffusion method at a concentration of 1,000µg/ml had a ZI value of 8±0.02mm, while the agar diffusion method with a concentration of 800µg/ml was able to produce a higher ZI value of 12.54mm. This shows that the selection of the microbiological test method has a significant influence on the visualization of the antibacterial activity of a plant phyto-constituent.

The inhibition zone values against *Streptococcus mutans* reported by [13] need to be interpreted with caution because the parameters used differ from the majority of other studies that report the inhibition zone diameter directly in millimeters. This study used the normalized width of inhibition zone (nw_{halo}) parameter, which is the ratio between the width of the clear zone and the disc diameter. Therefore, the reported values of 0.11–0.40 do not represent the actual inhibition zone diameter, but rather the result of mathematical normalization to the size of the test disc. Based on the equation used by the authors, a value (nw_{halo}) of 0.40 is equivalent to a total inhibition zone diameter of approximately 10.8 mm on a 6 mm diameter disc. Therefore, this value still indicates antibacterial activity against *Streptococcus mutans* and cannot be directly compared with the inhibition zone diameter data reported in millimeters in other studies.

Based on the equation:

$$nw_{halo} = \frac{(d_{iz} - d)/2}{d}$$

- nw_{halo} = normalized width of inhibition zone
- d_{iz} = total diameter of inhibition zone
- d = disc diameter

The Role of Betacyanin and Phenol as Major Antibacterial Agents

The bacterial cell wall plays a role in maintaining cell shape and enabling the cell to withstand osmotic pressure. The cell structure of Gram-positive bacteria is different from that of Gram-negative bacteria. Gram-positive bacteria have a cell wall consisting of a thick peptidoglycan layer that surrounds the cytoplasmic membrane but is highly permeable, thus facilitating the diffusion and penetration of active antibacterial compounds such as phenolics and betalains to reach intracellular targets. Gram-positive bacteria exhibit a layer of peptidoglycan strands that can reach sizes between 30 and 100 nm or even thicker, while Gram-negative bacteria have a layer of only a few nanometers. Gram-negative bacteria have much more selective permeability because they are protected by an outer membrane rich in lipopolysaccharides that acts as an efficient hydrophobic filter. As a result, Gram-positive bacteria

are much more susceptible to exposure to antibacterial agents than Gram-negative bacteria, which exhibit a higher level of natural resistance [14]. The antimicrobial activity of polyphenols and phenolic compounds is strongly influenced by the functional structural characteristics of their molecules, such as the length of the alkyl chain and the presence of hydroxyl groups [15]. [16] noted that the affinity of polyphenolic compounds such as catechins for the microbial lipid bilayer, as well as their antibacterial activity, increases with the increasing number of carbon atoms in the alkyl chain, with the strongest efficacy observed with alkyl chains consisting of 4–7 carbon atoms. In general, differences in cellular architecture between Gram-positive and Gram-negative bacteria lead to different manifestations of damage and inhibition mechanisms. The absence of an outer membrane in *S. aureus* allows phenolic compounds to diffuse directly through the cell wall. Once inside, these compounds trigger intracellular acidification (hyper-acidification) and cause irreversible damage to the ATPase pump, leading to cell death. Furthermore, polyphenol molecules such as epigallocatechin gallate (EGCG) have been shown to bind directly to the peptidoglycan layer, creating microscale grooves in the cell wall, and aggregate in the cell envelope to trigger cell lysis [17].

This structure-based inhibition mechanism is linear with the phyto-constituent potential of beetroot (*Beta vulgaris L.*). Based on research [18], beetroot methanol extract has a massive total phenolic content, reaching 150 mg of gallic acid equivalent per gram of extract. The abundance of this total phenolic content confirms the high density of active groups that can diffuse through the cell walls of Gram-positive bacteria to disrupt cellular homeostasis and energy pump systems, while strengthening the scientific basis for the use of beetroot ethanol extract as an effective antimicrobial agent. Betalains are water-soluble, nitrogen-containing pigments that accumulate in plant cell vacuoles, which are structurally classified into two main groups, namely the red-purple betacyanins and the yellow-orange betaxanthins [19]. In addition to their function as natural pigments, betalains exhibit significant antimicrobial activity against various macropathogens, including the bactericidal effects of betanin and isobetanin compounds against Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Wijesinghe & Choo, 2022). This inhibitory mechanism is strongly suspected to be driven by betalains' ability to disrupt the integrity of bacterial cell membranes leading to cell lysis, as well as their capacity to induce the accumulation of intracellular Reactive Oxygen Species (ROS) that trigger oxidative stress and microbial DNA damage (Wijesinghe & Choo, 2022). Furthermore, betalain components extracted from red beetroot then encapsulated with chitosan nanoparticles can inhibit biofilm formation in fungi such as *Candida albicans* by reducing the adhesion and viability of these fungal cells [21].

Influence of Methodology and Solvent

Based on Table 2, ethanol is the most widely used solvent in studies of the antibacterial activity of beetroot. Ethanol extraction of natural products is effective due to its wide solubility range as a neutral solvent, capable of extracting a wide variety of compounds, including hydrophilic and lipophilic components. Its antimicrobial properties contribute to reducing the risk of contamination and maintaining the quality of the extract. Ethanol is technically relatively safe and economical, evaporating rapidly after extraction [22]. These characteristics make ethanol widely used to extract bioactive materials, especially those with high antioxidant activity. In addition to ethanol, several studies have also used methanol, water, ethyl acetate, and chloroform to evaluate the effect of different solvent polarities on the antibacterial activity of the resulting extract. In general, alcohol solvents have been reported to produce good antibacterial activity because they can extract various bioactive compounds from beetroot, including betalains and phenolic compounds. The combination of these compounds is thought to contribute to antibacterial activity through different but complementary mechanisms [19].

the agar well diffusion method in table 3 is thought to be related to the statement [23]. Meanwhile, Broth microdilution is able to produce quantitative parameters in the form of MIC values to assess the potential of beet extract concentrations to inhibit bacterial growth. Based on the literature [23] the choice of antibacterial test method between agar well diffusion and disc diffusion / Kirby -Bauer (disc diffusion) has a statistically significant effect ($p = 0.008$) on the diameter of the inhibition zone formed. Based on comparative visualization of experimental data, the well method produces an average inhibition zone 56.8% larger (13.254 mm vs. 8.454 mm) compared to the disc diffusion method at the same test substance concentration. This substantial difference can be attributed to diffusion dynamics factors and physical methodological characteristics. Wells can accommodate a much larger volume (200 μL in the study [23]) compared to the limited absorption capacity of paper disc diffusion (generally 20 μL). According to Fick's first law of diffusion, the diffusion rate is directly proportional to the concentration gradient; thus, a higher concentration of a compound in the well facilitates greater radial diffusion and consequently a larger zone of inhibition [24]. Beetroot (*Beta vulgaris L.*) viscous extract is rich in polyphenolic compounds, macrocyclic macromolecular pigments (such as betalains), and polysaccharides that increase the viscosity of the solution. In the

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disc diffusion method, the cellulose fibers of the paper act as a mechanical filter that traps large molecules or partially hydrophobic compounds (strong adsorption), thus inhibiting the efficient release of the active components. In contrast, the well method eliminates the steric hindrance of the filter paper through direct liquid contact (direct interface) between the viscous extract and the water of the agar matrix, ensuring that all dissolved active components can flush out and penetrate intact [24].

However, method selection can impact the assessment of antimicrobial activity differently depending on the compound class and bacterial species tested. These comparative studies collectively demonstrate that although the well diffusion method may offer advantages for screening plant extracts, particularly for viscous and multicomponent preparations, the disc diffusion method remains the gold standard for clinical antibiotic susceptibility testing due to its widespread standardization, regulatory acceptance, and interlaboratory reproducibility. Therefore, the choice of method should be guided by the specific research objectives and the nature of the test substance [24].

Study Limitations

It should be noted that the pooling of data from various previous studies in this article has the potential to introduce publication bias, given the extreme variability in inhibition zone ranges due to differing laboratory standardizations. This experimental bias is exacerbated by methodological discrepancies across the literature, with some studies implementing triplicate testing (three replicates) for statistical validity, while others did not replicate the testing clearly. Furthermore, data accuracy is limited due to several reference articles not fully including primary data preparation components, such as substance concentration specifications, and not including standard deviation values.

D. Conclusion

Based on 20 included studies, beetroot extract (*Beta vulgaris L.*) showed antibacterial activity against various Gram-positive bacteria, especially *Staphylococcus aureus*, with varying inhibition zone and MIC values between studies. This variation in activity was influenced by differences in extract concentration, solvent type, and testing method used. Ethanol was the most frequently used solvent in the extraction process, while agar well diffusion and broth microdilution methods were the most frequently reported testing methods. The antibacterial activity of beetroot extract is thought to be related to the content of bioactive compounds, especially betalains and phenols. Overall, beetroot extract has the potential to be developed as a natural antibacterial source against Gram-positive bacteria, although further research with more standardized methods is still needed to confirm its effectiveness and safety.

E. Thank-you note

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