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THE ANTIMICROBIAL POTENTIAL OF PLANT-BASED NATURAL DYES FOR TEXTILE DYEING: A SYSTEMATIC REVIEW USING PRISMA

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Abstract:

Dyeing plays a vital role in the textile industry, however, associated health and environmental issues have raised significant concerns regarding the types of dyes used. Among these, natural dyes, particularly those derived from plants, exhibit superior safety and environmental performance, making them a more sustainable alternative. Moreover, fabrics dyed with plant dyes can acquire diverse functional properties, including antimicrobial characteristics, attributed to various active ingredients present in plants during the dyeing process. With increasing environmental consciousness and the rising demand for functional fabrics, natural plant dyes have garnered growing attention. In our research, a systemic review of the antimicrobial properties of plant dyes in the textile field was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses method. A literature search was executed through the PubMed and Web of Science databases, from which 132 articles were selected. The results indicate that the overall number of publications in this field is on the rise, especially showing a significant increase in the past 7 years, demonstrating substantial research value and potential. Furthermore, this study conducted an analysis of the content included in the literature, summarizing the different standards and characteristics of antimicrobial testing, with a focus on revealing the antimicrobial mechanisms of plant dyes. It also discussed the mordants and other treatment methods that can effectively enhance the antimicrobial properties of plant dyeing. Building on this foundation, this review discusses the advantages, application potential, and future research directions of antimicrobial natural dyes derived from plants. Through this review, relevant researchers can gain a clearer understanding of the current state and development trends of plant-based natural dyes in terms of antimicrobial properties, thereby promoting further exploration in this field.

Keywords:

Natural dyeing, plant dyes, antimicrobial properties, textile materials, systematic review

1. Introduction

In textile production, dyeing stands as a crucial step wherein textiles undergo dyeing or printing to boost their fashion appeal [1]. Dyes can be categorized into two primary groups: natural and synthetic. Natural dyes are organic compounds derived from natural sources, and they have garnered increasing attention due to their inherent environmental friendliness and potential health benefits [2]. Natural dyes align with the growing emphasis on sustainability in the textile industry, providing a renewable alternative that supports both ecological balance and consumer well-being. Moreover, natural dyes can confer healthcare and protective functions to dyed textiles, with antimicrobial properties being one of the key attributes they offer. Textiles are considered suitable sources for the growth of fungi, bacteria, and other microorganisms, especially natural fibers, as they come from biological sources and have a high inherent moisture content [3,4]. Bacterial erosion of textiles can result in discoloration of dyes and pigments and alter the pH of fabrics. Additionally, it can cause skin irritation and unpleasant odors, and even lead to diseases such as asthma, skin allergies, or eczema [3,5]. In contrast, fungal infections have not been common in the past but have significantly increased in prevalence in recent decades, leading to diseases such as skin infections and a compromised immune system [6]. Integration of antimicrobial agents into the textile manufacturing process holds the potential to significantly mitigate health risks stemming from microorganism proliferation on garments, household items, and medical and hygiene goods [7]. Thus, natural dyes, possessing eco-friendliness and antimicrobial properties, are increasingly esteemed and represent a focal point for textile scientists' and coloring engineers' research endeavors.

Plants serve as the primary reservoir of natural dyes, with plant-derived dyes being utilized in fabric dyeing for centuries owing to their diverse health benefits and unique attributes [8]. Notably, plants are valued for their therapeutic attributes, attributed to the array of chemicals they harbor [9]. Studies have demonstrated that dyes extracted from numerous plant parts, like seeds, flowers, leaves, stems, and roots, exhibit antimicrobial properties [10]. As the need for natural dyes grows and natural dyeing methodologies evolve continuously, increased exploration is underway concerning the antimicrobial attributes of plant-derived natural dyes.

Several review articles have comprehensively examined plant-based natural dyes and their various aspects (Table 1). However, all the reviews were focused on dyes and did not consider the analysis of their antimicrobial properties. Despite covering

Table 1. A comparative analysis of related reviews

Title	Year of publication	Research topics	Main research content	Ref.
A review on source, chemistry, green synthesis and application of textile colorants	2020	Textile colorants	The sources, processes, chemistry, and properties of textile colorants containing natural dyes and synthetic dyes were critically described, with a discussion on the environmental challenges and sustainability of these dyes	[11]
A brief review on natural dyes, pigments: Recent advances and future perspectives	2023	Natural dyes	Summarized the chemical composition, production methods, dyeing mechanisms, and future prospects of natural dyes, primarily focusing on plant-derived ones	[12]
A recent (2009–2021) perspective on sustainable color and textile coloration using natural plant resources	2022	Natural plant dyes	Reviewed the research status of natural plant dyes from 2009 to 2021, covering dyeing methods, technologies, functional characteristics, types of mordants, and market potential across different sectors	[2]
Non-food applications of natural dyes extracted from agro-food residues: A critical review	2021	Natural plant dyes from agro-food residues	Summarized the characteristics and extraction technologies of dyes obtained from fruits and vegetables (anthocyanins, quinones, carotenoids), indicating food waste processing waste and agricultural losses as viable origins	[13]
Cleaner pathway for developing bioactive textile materials using natural dyes: A review	2023	Natural dyes and their functional performance	Summarized the effects of natural dyes on the surface modification of commonly used natural fibers and synthetic fibers, as well as their performance in areas such as antimicrobial properties, UV protection, and insect repellency	[14]
Phenolic compounds from by-products for functional textiles	2023	Natural plant dyes and their functional performance	Explored the effects of natural dyes on the surface modification of the textile and summarized the performances achieved with natural phenolic plant dyes, respectively	[15]
Sustainable plant-based bioactive materials for functional printed textiles	2021	Natural plant dyes and their functional performance	Extensively explored the historical background, applications, and functionalities of plant dyes in textile fabrics	[16]
Chromatic and medicinal properties of six natural textile dyes: A review of eucalyptus, weld, madder, annatto, indigo and woad	2023	Natural plant dyes and their functional performance	Conducted a literature review on six natural plant dyes, including eucalyptus, weld, madder, annatto, indigo and woad, focusing on their dyeing performance and medicinal properties in the textile field	[17]

the antimicrobial finishing of natural plant dyes, previous reviews lacked detailed analysis and explanation.

There have been relatively few comprehensive studies on the antimicrobial properties of natural dyes in the textile industry, as summarized in Table 2.

The above review discusses various aspects of natural dyeing, but our study is significantly different from existing reviews. First, the aforementioned review does not include studies conducted post-2020, while this review includes the latest research findings up to October 2024, which helps to clarify the latest developments in the field. Second, this review focuses on the antimicrobial potential, including inhibitory effects on bacteria and fungi. Based on existing research findings, the emphasis is placed on summarizing and analyzing antibacterial mechanisms. This is an important but relatively less studied area. Existing reviews are mostly focused on natural dyes rather than their antimicrobial properties, so this review can help relevant personnel to more directly and specifically learn about the relevant research findings on the antimicrobial properties of plant dyes. Third, as far as current knowledge extends, there exists no systematic review regarding the antimicrobial properties of plant dyes in the textile domain. Systematic reviews, in contrast to traditional literature reviews, offer comprehensive support to researchers by integrating existing information and furnishing data for decision-making via established methodologies [20]. Moreover, the standardized article selection and analysis procedures inherent in systematic literature reviews help scholars and researchers alleviate bias and improve the accuracy and credibility of conclusions [21].

Given the background outlined above, this study adopts a systematic review to investigate the most recent advancements in antimicrobial plant dyes research, identify research frontiers and hotspots, and summarize the following aspects: We first utilized knowledge graphs and the visualization tool CiteSpace to scientifically and intuitively depict the development path and trends in this field. Next, we summarize the different methods for antimicrobial testing, understanding that standardized testing methods ensure the comparability and reproducibility of results across different laboratories, thereby increasing research reliability. By elucidating standardized approaches, we can assist future researchers in selecting appropriate detection methods. Additionally, variations in experimental designs and methodologies may lead to differences in results. Analyzing antimicrobial testing methods can help ensure that the obtained data are scientifically valid, thus improving the credibility of conclusions. Third, we summarize the plant chemical substances responsible for the antimicrobial activity of plant dyes and their mechanisms of action. Through a deeper understanding of these chemical components and mechanisms, we can further explore the possibility of using plant dyes as alternatives to traditional synthetic antimicrobial agents. Finally, we also summarize the factors affecting the antimicrobial activity of plant dyes as well as other treatment methods to understand how to enhance the antibacterial ability of plant dyes, thereby providing more sustainable and healthy choices for the textile industry. This review can serve as a reference and guide for future practical and theoretical

Table 2. A comparative analysis of related reviews on antimicrobial properties of natural dyes

Title	Covered years	Research topics	Main research content	Ref.
Antimicrobial activity of natural dyes – A comprehensive review	2001–2019	Natural dyes	Summarized the main phytochemicals with antibacterial and antifungal activities, their modes of action, application methods, as well as market potential and challenges	[4]
Antimicrobial cellulosic textiles based on organic compounds	1998–2018	Organic antimicrobial reagents	Summarized the application of organic antimicrobial agents, including plant dyes, in the finishing of textile fabrics	[18]
Natural dyes and antimicrobials for green treatment of textiles	2001–2013	Natural dyes and antimicrobials	Explored the extraction and utilization of antimicrobial agents/natural dyes, along with performance examples and outcomes achieved in early textile processing	[19]

endeavors in this domain. To delineate the current study comprehensively, the following questions (RQs) are formulated:

RQ1. What is the overall trend in research on antimicrobial plant dyes in the textile field over time?

RQ2. What are the methods for testing the antimicrobial properties of textiles? What are the differences between them?

RQ3. Why do plant dyes have antimicrobial properties?

RQ4. What are the current mordants that effectively augment the antimicrobial efficacy of plant dyes? What are their mechanisms of action?

RQ5. What are the current treatment methods that effectively augment the antimicrobial efficacy of plant dyes? What are their mechanisms of action?

2. Methods

The systematic review in this study was conducted following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), which includes identification, screening, qualification, and analysis. As an effective evaluation method, systematic reviews enable researchers to identify and screen relevant evidence pertaining to specific questions, evaluate and summarize the findings of the evaluations, and apply them in practice, regulations, and further research [23]. For the literature search, the Web of Science Core Collection database (accessed 10 October 2024) and the PubMed database (accessed 10 October 2024) were screened for information from the earliest study to the retrieval date, using the following search keywords: (“Natural dye” OR “Natural dyes” OR “Natural pigment” OR “Natural dyeing” OR “Natural dyestuff” OR “Natural colorants” OR “Plant dye” OR “Plant dyes” OR “Plant dyeing” OR “Plant dyestuff” OR “Plant pigment” OR “Plant colorants” OR “Herbal dye” OR “Herbal dyes” OR “Herbal dyeing” OR “Herbal dyestuff” OR “Herbal pigment” OR “Herbal colorants” OR “phyto dye” OR “phyto dyes” OR “phyto dyeing” OR “phyto dyestuff” OR “phyto pigment” OR “phyto colorants”) AND (Antibacterial OR Antimicrobial OR Antibacterial OR Antimicrobial OR Bactericide OR Bactericidal OR Microbicide OR Microbicidal) AND (Fashion OR Garment OR Clothes OR Fabric OR Textile*). The search scope included titles, abstracts, and keywords, and only English language articles were considered. The above search terms include three levels: plant dyes, antimicrobial properties, and the textile field. The specific reasons are as follows: first, “Natural dyes,” “Natural dyes,” “Plant dyes,” etc., which constitute the core of the research and cover various types of natural plant dyes. The terms “Herbal dye” and “Phytodye” emphasize different plant-based dyes, including herbal plants and other plant sources. This classification captures research on various plant-based dyes to ensure comprehensive literature coverage. Second, multiple terms, such as “Antibacterial,” “Antimicrobial,” and “Bactericide,” are used to cover different types and mechanisms of antimicrobial action, helping retrieve various relevant literature for a comprehensive understanding of the antimicrobial potential of natural plant dyes. Finally, terms such as “Fashion,” “Garment,” and “Textile” highlight the application of natural plant dyes in the textile and clothing industry. This level helps ensure that research related to specific application scenarios is found. In conclusion, through these selected keywords, the retrieval strategy can comprehensively and effectively capture literature related to the antibacterial potential of plant-based natural dyes and their applications in textile dyeing. This structured retrieval strategy meets the PRISMA guidelines’ requirements, contributing to ensuring the quality and reliability of our systematic review by providing effective support.

The search identified 594 articles, and then, the articles were screened to ensure relevance using the following criteria and process (Figure 1):

(1) A total of 68 records for reasons, such as comments, book chapters, letters, news, patents, conference papers, and reports, were excluded. A total of 526 papers advanced to the next round of screening.

(2) Additionally, 37 duplicate articles were removed, resulting in 489 articles for further screening.

(3) Based on the titles and abstracts, 116 records were excluded as their research content was unrelated to the antimicrobial properties of plant dyes in textiles. After this stage, a total of 373 papers proceeded to the next round of selection.

(4) Subsequently, the full texts of 373 articles were reviewed and evaluated to determine if they met the conditions defined in the review’s scope. In this step, a total of 241 articles were excluded for the following reasons: (a) unavailability of the full text and (b) lack of validation of the antimicrobial properties of the plant dyes on fabrics. (c) Lack of explanation of antimicrobial mechanism. Ultimately, this review included 132 studies.

In addition, CiteSpace (version 6.3.1) was utilized as a tool to conduct visual analysis of the 132 final included articles. Visual analysis included examination of keywords, and burst words, aiming to elucidate the research hotspots and future development trends in this field. CiteSpace is a software tool for literature visualization and analysis, presenting the structure, development patterns, and trend detection of research in easily understandable graphics and charts [24,25]. Therefore, it can be objectively and concisely used to analyze the knowledge structure, evolutionary patterns, and future directions in this field [24].

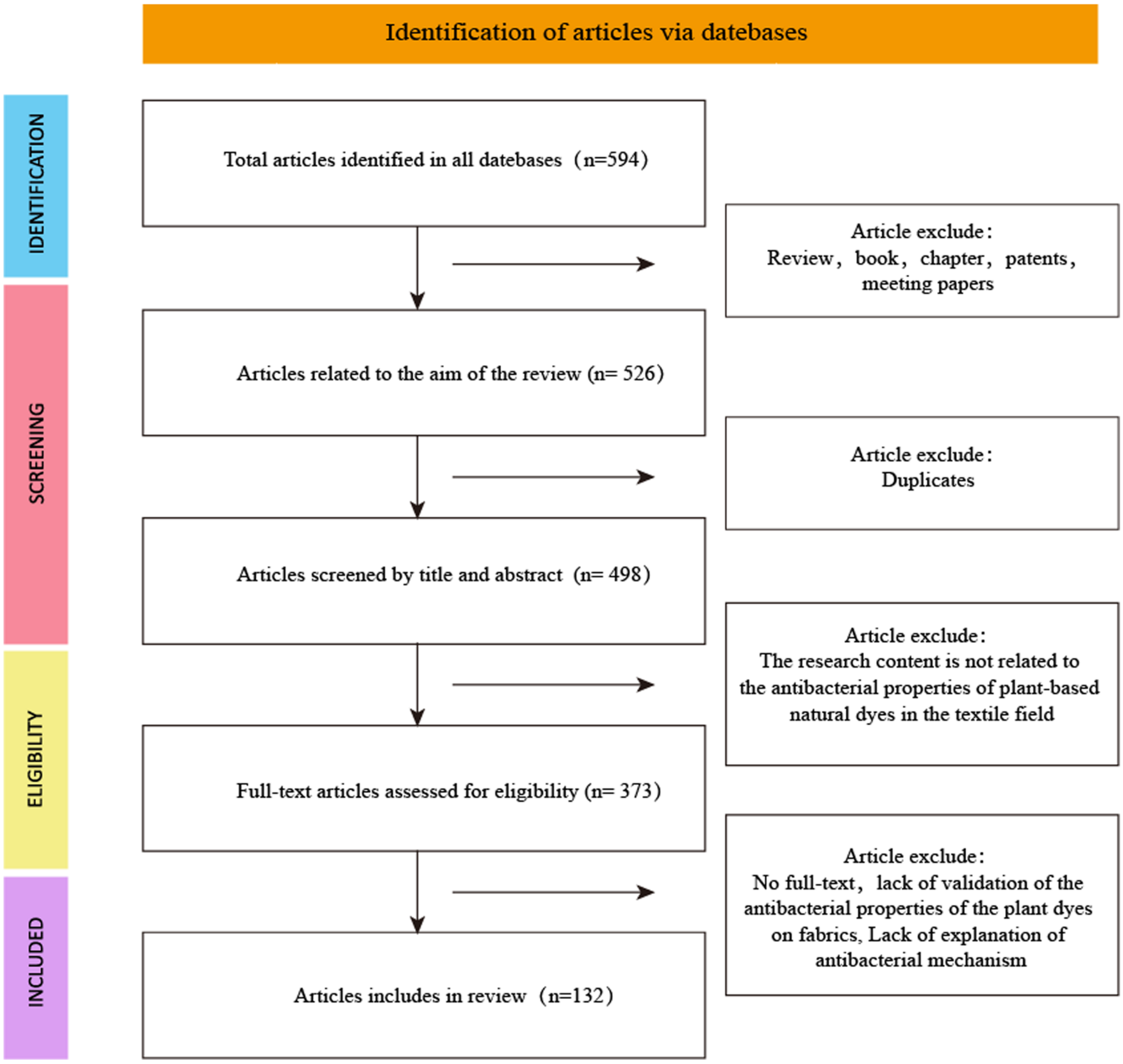


Figure 1. The flow diagram of the selective process of literature in accordance with PRISMA.

3. Results

3.1. Analysis of research trends

3.1.1. Number of publications

The quantity of research studies produced serves as a pivotal statistical indicator for gauging the advancement of a research field. As depicted in Figure 2, research on the antimicrobial properties of the textile plant dyes has shown a continuous upward trend since the publication of the first paper in 2004, spanning nearly two decades until 2024. Particularly since 2018, there has been a significant increase in the number of publications. From 2018 to 2024, there were a total of 100 publications, accounting for nearly 76% of the whole publications. By and large, the growing number of publications underscores the increasing significance and influence of this field, with a growing cohort of researchers dedicating themselves to related investigations.

3.1.2. Analysis of research hotspots and frontiers

Keywords serve as direct reflections of the research content within the literature, offering insights into the primary themes [27]. Figure 3 displays the keyword co-occurrence network of the literature, comprising 285 nodes representing keywords and 1,217 links, with a network density of 0.0301. According to Table 3, it can be observed that in addition to the keywords “natural dye,” “natural dyes,” “antibacterial activity,” and “antimicrobial activity,” other keywords such as “optimization,” “cotton,” “wool,” “silk,” “antioxidant,” “adsorption,” “fabrics,” “extract,” “UV protection,” “fastness,” “chitosan,” “textiles” are relatively more prominent. Among them, “cotton,” “wool,” “silk,” “fabrics,” and “textiles” indicate that the research centers on the textile industry, with a main concentration on natural fabrics. The frequent appearance of keywords like “antioxidant” and “UV protection” underscores a growing interest in multifaceted functional aspects of plant dyes with regard to improving the health and protective qualities of fabrics. Additionally, the

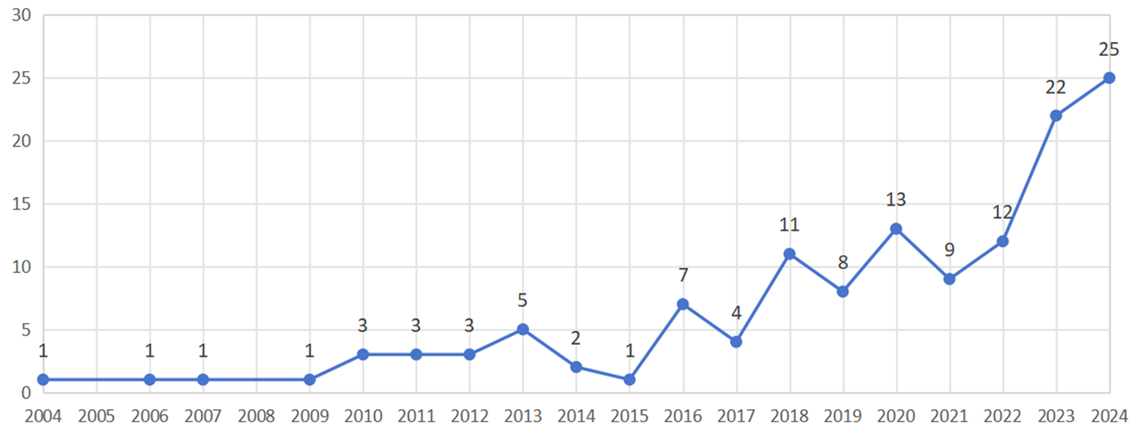


Figure 2. Annual number of publications.

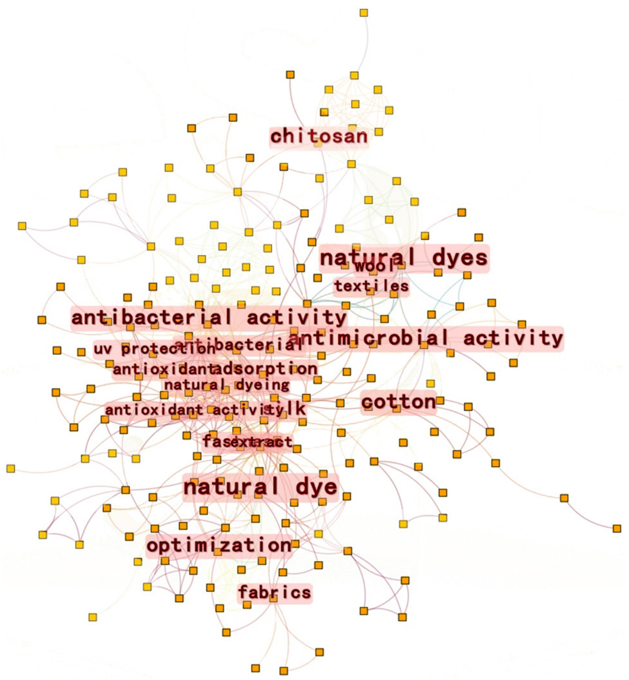


Figure 3. Co-occurrence network of keywords.

recurring presence of keywords such as “optimization”, “extract,” “adsorption,” “fastness,” and “chitosan” indicates ongoing advancements and refinements in plant dye extraction and dyeing techniques. While plant dyes offer safety and health advantages over synthetic dyes, challenges persist due to the complexity of extraction and dyeing processes, alongside issues of low color fastness. Particularly, the antimicrobial performance is closely correlated with the dye assimilation level onto the fiber. Therefore, it is indispensable to improve the binding dye capacity to the fiber, thus continuous technological advancements are essential to fully capitalize on the benefits of plant dyes for broader application and dissemination.

“Burst words” are high-frequency keywords and show the development of hot issues and related fields during some period [22]. The burst keywords are exhibited in this domain in 2004–2024 with the red line showing the burst period, as shown in Figure 4.

Based on recent keywords, it can be inferred that the research focus and future trends of plant dye antimicrobial properties have gradually shifted towards the following areas: (1) Antimicrobial mechanism research: anthocyanins belong to flavonoids, which have a wide range of biological activities and pharmacological properties. However, the antimicrobial mechanisms of different plant chemical components vary. Although there have been many studies published on the antimicrobial activity of plant dyes, the specific mechanism is still not fully understood [27]. Therefore, they can be better applied by further understanding the mechanism of plant dyes in inhibiting microbial growth. (2) Development of functional textiles: Currently, there is relatively more research on dyeing and antimicrobial treatment using cotton fabrics as carriers, because cotton is the most widely used natural textile fiber, with advantages such as soft touch and good water absorption, making it suitable for various types of textiles [3]. Especially in the field of medical textiles, antimicrobial cotton fabrics have tremendous application prospects [3]. Nevertheless, natural dyes have a lower affinity for cotton [2]. As a result, researchers are actively exploring new methods to solve this problem, like utilizing mordant dyeing, fabric modification, adjusting process parameters, etc. to improve the binding effect between natural dyes and cotton, and to find more environmentally friendly and sustainable dyeing techniques. In addition, in the future, it may be further expanded to develop new functional textiles by combining plant dyes with other biomaterials to achieve better antimicrobial performance and other functions and apply them in various fields. (3) Extraction technology improvement: Traditional plant dye extraction methods have some problems, such as being time-consuming and having low yield. Therefore, the optimization and innovation of extraction methods have become the current research hotspot, such as ultrasound-assisted extraction, which can improve the yield and biological activity of plant dyes. Future research may focus on developing new green extraction technologies, seeking more efficient, environmentally friendly, and economically viable extraction

Table 3. Keywords with a frequency ≥10

Frequency of appearance	Centrality	Year of first appearance	Keyword
42	0.11	2010	Natural dye
39	0.16	2006	Natural dyes
26	0.28	2011	Antibacterial activity
26	0.19	2007	Antimicrobial activity
25	0.11	2010	Optimization
25	0.14	2011	Cotton
19	0.03	2013	Wool
17	0.11	2011	Silk
15	0.11	2016	Antioxidant
15	0.14	2012	Antibacterial
15	0.13	2013	Adsorption
14	0.04	2010	Fabrics
13	0.17	2013	Extract
13	0.05	2015	UV protection
12	0.08	2012	Fastness
12	0.12	2013	Chitosan
10	0.06	2013	Natural dyeing
10	0.04	2014	Textiles

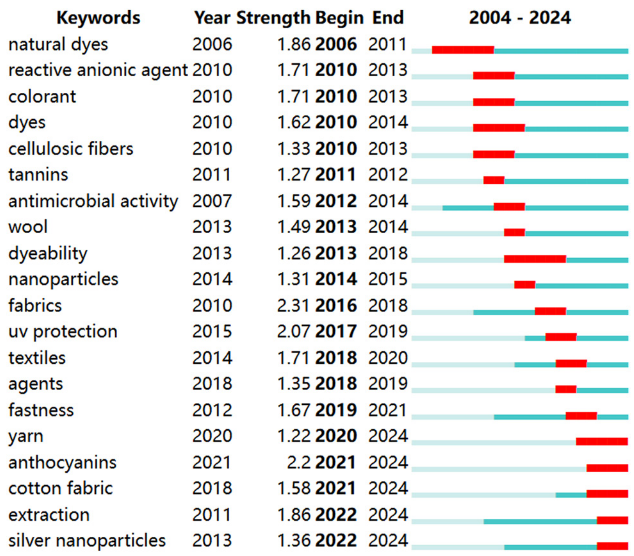


Figure 4. Keywords with the strongest citation bursts between 2004 and 2024.

processes. (4) Research on the combination with new technology: Silver nanoparticles have become a hot topic in research, demonstrating researchers' interest in combining traditional plant dyes with modern nanotechnology to enhance antimicrobial effects. Silver nanoparticles are increasingly being used for functional treatment of textiles due to their excellent antimicrobial properties. In the future, the research trend combining modern technology and green chemistry will bring more innovative possibilities for the application of plant dyes in the field of antimicrobials.

3.2. Antimicrobial testing methods

Antimicrobial tests are divided into qualitative and quantitative tests [27], each classified as follows.

3.2.1. Qualitative antimicrobial tests

Qualitative testing measures the size of the zone of inhibition (ZOI) formed on an agar medium, which helps to evaluate the antimicrobial activity of stained samples. Among the 132 articles included in this study, qualitative antimicrobial test methods such as AATCC 147 and ISO 20645 were employed. AATCC 147, also known as the parallel streak method, involves streaking the microbial culture across an agar plate to form parallel stripes. Samples are inoculated onto these stripes, and after 24 h, the breadth of the ZOI around the stripes is observed. Samples with antimicrobial activity show no microbial growth around them, whereas microbial growth can be observed under non-antimicrobial fabrics [28].

ISO 20645 is also referred to as the agar diffusion plate test. In this method, test strains are first evenly inoculated onto the surface of agar medium, and then textile samples are placed on the nutrient medium. Subsequently, they are incubated at 37°C for 18–24 h to observe microbial growth and compare the size of the clear zone around the sample with that of the control [29].

Both AATCC 147 and ISO 20645 qualitative testing methods evaluate antimicrobial properties based on microorganism proliferation in the contact area between agar and the experimental sample, with AATCC 147 assessing the width of inhibition zones and ISO 20645 measuring the diameter of clear zones.

The advantage of qualitative detection methods is that they are relatively simpler and faster to perform than quantitative methods, and the results are more intuitive. However, there are also certain limitations. First, these methods are applicable mainly to antimicrobial agents that can permeate fabrics and kill microorganisms. If the antimicrobial agent is "binding type" and does not permeate, this method may not be able to detect its antimicrobial activity. Second, it cannot provide specific quantified data on antimicrobial effectiveness [28].

3.2.2. Quantitative antimicrobial tests

Quantitative testing evaluates antimicrobial effectiveness by determining the percentage inhibition of microbial growth. Among the 132 articles, quantitative antimicrobial test methods include AATCC-100, ASTM E2149, and GB/T 20944.3.

The AATCC-100 method is also known as the suspension method [30]. In the experiment, the sample was placed in a container containing a microbial suspension to effectively adsorb the suspended microorganism and ensure that no residual liquid remained in the container. Then, a neutralizing solution was added, and the samples were incubated on a rotary shaker at 37°C ± 2°C for 24 h. Afterward, the sample was removed from the mixture and diluted before being inoculated onto agar media for further incubation at 37°C ± 2°C for 24 h. Finally, the antimicrobial effect was evaluated by counting colonies on agar plates. The antimicrobial effect was calculated by the following formula: $R = 100 \times (B - A)/B$. R represents the percentage reduction in microbial, A represents the colony counts in treated samples after inoculation and cultivation, and B represents the colony counts in samples without contact time [27,31]. Additionally, the microbial reduction rate can also be calculated by comparing colony counts between stained samples after microbial cultivation and unstained samples under identical incubation conditions. Specifically, when this formula is used, A refers to the colony counts in stained samples after 24 h of cultivation; B refers to the colony counts in unstained samples cultivated under similar conditions [28,32].

ASTM E2149 is a quantitative antimicrobial testing method conducted under dynamic contact conditions [33,34]. In this method, the test sample and control sample are separately placed in containers containing a certain concentration of microbial mixture. The mixture was then incubated in a shaker incubator at 35°C ± 2°C with agitation for 1 h ± 5 min. Subsequently, dilution and inoculation were performed on agar plates, followed by further incubation at 35°C ± 2°C for 24–48 h. After incubation, the colony counts on the culture dishes were calculated to determine the reduction rate of microorganisms [35,36]. The antimicrobial effect was calculated by the following formula: $R = 100 \times (B - A)/B$. R represents the percentage reduction in microbial, A represents the colony counts recovered from the treated stained fabric samples after inoculation, and B represents the colony counts recovered from the untreated control fabric samples after inoculation [37,38]. Alternatively, it can also be calculated by a "0" contact time method, where A refers to the colony counts of inoculated treated samples after cultivation and B refers to the colony counts of samples with 0 contact times [35]. GB/T 20944.3 and ASTM E2149 are based on similar types of testing methods, specifically the oscillation method. In this method, oscillation is carried out for a certain period of time (18–24 h) at a temperature of 24°C ± 1°C, followed by dilution and inoculation on agar plates and further cultivation at 37°C for 18–24 h. After incubation, the colony counts on culture dishes were calculated to determine the reduction rate of microorganisms. The antimicrobial effect was calculated by the following formula: $R = 100 \times (B - A)/B$. R represents the percentage reduction in microbial, and A and B represent visible colony counts from unstained samples and stained samples, respectively [39,40]. ASTM E2149 is an American standard, while GB/T 20944.3 is a Chinese standard. Besides the standards, differences exist in sample specifications, microbial concentrations, and inoculation quantities.

Compared with qualitative testing methods, quantitative detection methods have significant advantages in terms of accuracy of results. Specifically, they can provide specific antimicrobial performance data and quantify the antimicrobial effects of samples. They are suitable for most textiles, including both leachable and non-leachable antimicrobial fabrics. Additionally, these methods simulate the contact conditions of textiles in actual use. Therefore, more researchers choose quantitative testing methods to evaluate the antimicrobial properties of textiles. However, the testing process is time-consuming and expensive because of its complexity and requirement for multiple equipment and materials. In summary, while quantitative testing methods can provide accurate antimicrobial performance data, they are time-consuming and costly; however, qualitative testing methods are simple and fast but lack quantification ability with lower accuracy. Furthermore, according to a review of the literature, AATCC 100 is the most commonly used testing method.

3.2.3. Differences in the effects on different microbial strains

In all the studies, the bacterial species most commonly used for testing were *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)*. The main reasons for the use of these two bacteria as the most common test species are their wide presence in the environment and their potential harm to human health. *S. aureus* is a major cause of cross-infection in hospital, commercial, and household laundry practices. It causes skin and tissue infections, sepsis, endocarditis, and meningitis. *E. coli* can cause urinary tract infections, gastrointestinal infections, wound infections, and hospital-acquired infections [41]. Additionally, *E. coli* is a gram-negative bacterium (G −ve), whereas *S. aureus* is a gram-positive bacterium (G +ve). By simultaneously using these two bacterial species, the

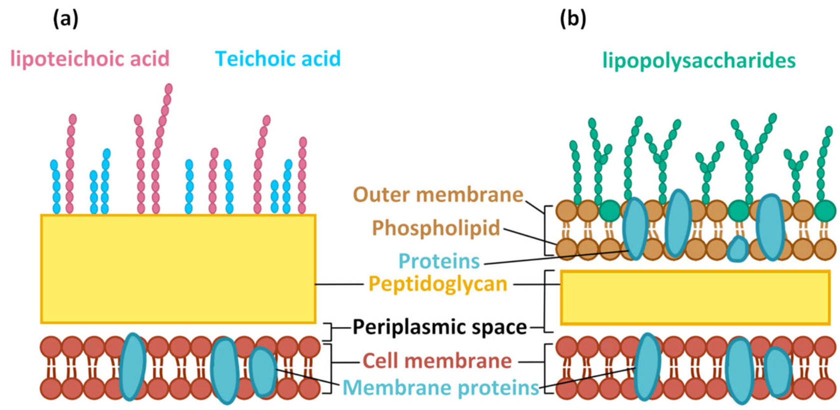


Figure 5. G +ve and G −ve cell wall structure comparison diagram. (a) G +ve. (b) G −ve.

effectiveness of antibacterial treatments on different types of bacteria can be evaluated to fully demonstrate the antibacterial capability of the materials.

According to the summary of literature, sensitivity to surface sterilization by the tested microorganisms can be ranked from high to low as follows: G +ve > G −ve. This difference is likely due to the single-layer structure of peptidoglycan in the cell wall of G +ve bacteria [42,43], compared to the multi-layered structure defined by lipoproteins, phospholipids, and lipopolysaccharides in the outer membrane of G −ve bacteria (Figure 5), which presents a potential barrier to foreign molecules [44–46].

However, some studies have shown better inhibitory effects on *E. coli* [32,47]. *S. aureus*, a gram-positive bacterium, has a thick peptidoglycan layer that often contains structures cross-linked by pentaglycine, increasing the stability and tolerance of its cell wall. On the other hand, *E. coli* is a gram-negative bacterium with a relatively thin peptidoglycan layer and does not possess this highly cross-linked structure [47]. In addition, compared with those of Gram-positive bacteria, the cell walls of gram-negative bacteria are more negatively charged and hydrophilic, resulting in stronger electrostatic interactions between cationic dyes and negatively charged cell walls and greater antibacterial activity [48]. Therefore, these structural differences make *E. coli* less permeable to certain substances but also more sensitive to certain antibacterial components in certain situations.

Compared with the validation of antibacterial effects, research on antifungal capacity is relatively scarce. The fungal strains involved primarily include two genera: Candida and Aspergillus. Among these, the inhibitory effects on Candida species, particularly *Candida albicans*, have been studied more extensively. *Candida albicans* is a common opportunistic pathogenic fungus that typically resides in the human body, especially in the digestive and reproductive systems [49]. When the immune system is compromised or its balance is disturbed, this fungus can lead to various health issues [6]. Currently, many studies have validated the inhibitory effects of various plant dyes against *Candida albicans* and other species within the Candida genus. In contrast, research on antifungal activity against Aspergillus species has yielded somewhat inconsistent results. In a study by Flax et al., dyes extracted from barberry, bittersweet, and wine berry did not demonstrate any inhibitory effects against *Aspergillus niger* [56]. Conversely, research conducted by Do et al. utilizing dyes extracted from madders revealed promising antifungal activity against white mold (*Aspergillus* spp.) [57]. Additionally, Mohamed et al. employed various agricultural waste materials (including onion outer skin, rose, eucalyptus, lemon, grape, and peach leaves) for dyeing and eco-printing silk fabrics. Antimicrobial testing revealed broad-spectrum antimicrobial activity against Gram-negative and Gram-positive bacteria, as well as Candida and Aspergillus fungi [54].

3.3. Antimicrobial mechanism of plant dyes

3.3.1. Chemistry of antimicrobial plant dyes

Natural plant dyes originate in numerous plant parts, including bark, roots, fruits, flowers, seeds, along with leaves. Their antimicrobial activity stems from the presence of active biological molecules, including phytochemicals. The phytochemicals can be divided into different categories; therefore, their modes of action will also correspondingly differ. The primary antimicrobial phytochemicals pertain to the group of phenolic syntheses [58,59], which structurally encompasses one or more hydroxyl groups (–OH groups) [58]. Polyphenolic compounds are a specific type of phenolic compound characterized by multiple hydroxyl groups [60]. Phenolic compounds can inhibit bacteria by disrupting the bacterial enzyme and biomembrane systems, thus affecting energy metabolism and selective substance assimilation [61]. The phenolic compounds can be further divided into various classes such as flavonoids, tannins, quinones, and curcuminoids, based on their different chemical structures [62].

Flavonoids, a diverse group of phenolic compounds, are widely distributed throughout the plant kingdom. Flavonoids include numerous subclasses, such as flavones, flavonols, and anthocyanins [62,63]. For example, Cyanidin, Delphindin, and Pelargonidin are flavonoids with an anthocyanin structure. Studies have identified that they are the main reasons for the good antimicrobial properties of the dyed fabric in *Hibiscus sabdariffa* [58]. Flavonoids, with their diverse biological activities [64], including antimicrobial properties, have long been a subject of intensive research and development efforts.

Plant tissues extensively contain tannins, complex polyphenolic compounds famous for their astringent taste [65]. They encompass two sub-classes: hydrolyzable tannins and condensed tannins [66,67]. Tannins exhibit a broad-spectrum antimicrobial effect against various pathogens, and owning to the adequate hydroxyl groups and other appropriate functional groups (i.e., carboxyl groups), they can formulate efficient robust complexes with protein chains and other macromolecules on special occasions. As a result, tannins are the most common natural mordants [68].

With high reactivity features, quinones are widely founded in nature, and they are aromatic rings showing two ketone substitutions [69]. Quinones can be classified into subclasses such as anthraquinones and naphthoquinones based on their structural features. Naphthoquinones, including lawsone from henna [62], juglone from walnut [65], shikonin from alkanet [70], and alkannin, have demonstrated antimicrobial effects. Widely distributed in nature, anthraquinones serve as important natural pigments, with Alizarin and purpurin from Madder being notable examples. Many research studies indicate that Madders exhibit significant antimicrobial activity when used for fabric dyeing [62,29].

Curcuminoids, primarily composed of curcumin [71], are predominantly derived from the rhizomes of turmeric [72]. Research indicates that curcumin is environmentally safe, gentle on the skin, imparts a yellow hue, and demonstrates remarkable antioxidant and antimicrobial properties. However, due to its relatively small size and hydrophobic nature, curcumin molecules exhibit poor wash and rub resistance when applied to textiles [73]. The antimicrobial curcumin mechanism is commonly attributed to hydroxyl and methoxyl groups. However, the use of conventional colorfastness agents can diminish the availability of free hydroxyl groups, thereby compromising curcumin's antimicrobial efficacy [74]. Consequently, numerous studies have focused on developing techniques to enhance both the colorfastness and antimicrobial attributes of curcumin in textiles. One such method involves tannic acid fixation treatments, which have been shown to significantly improve dye retention while preserving curcumin's antimicrobial activity [73,75].

Besides the typical phenolic compounds, berberine, sourced from plants like barberry and *Coptis chinensis*, demonstrates notable antimicrobial properties. Berberine belongs to the alkaloid compound category and offers a diverse array of pharmacological activities, making it valuable for medical applications [76]. Additionally, other compounds such as glycosides [77], have exhibited antimicrobial effects. These bioactive compounds bind to fibers through a dying process, thereby endowing the fabric with antimicrobial properties.

3.3.2. Mechanisms of antibacterial activity

Table 4 is a compilation of the antibacterial mechanisms of different categorical phytochemicals mentioned in the literature.

In this table, the literature about the main antibacterial mechanism of different phytochemicals is summarized. Although antibacterial mechanisms show certain differences as they are based on different compounds, in general, they can be divided into the following several categories: cell membrane destruction, enzyme activity inhibition, elevated oxidative stress response, DNA damage, protein structure damage, and nutrient uptake site competition. The schematic diagram of

Table 4. Main phytochemicals with antibacterial mechanism

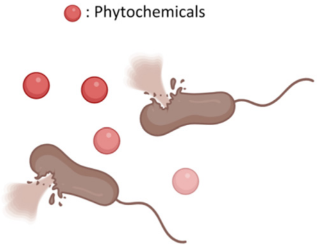
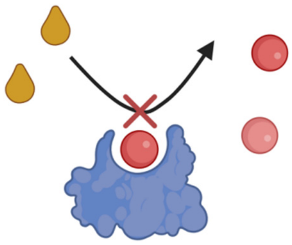
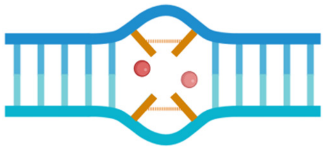
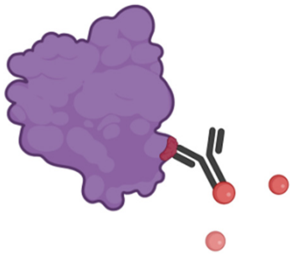
Phytochemicals		Antibacterial mechanism	Ref.
Phenols	Phenolic acids	Inhibiting nucleic acid synthesis hinders bacterial growth	[37]
	Polyphenols	Damaging the bacterial cell membrane and wall, which causes the leakage of cell contents. By combining with bacterial DNA and inhibiting the synthesis of ATP and DNA, it affects cellular activity. Inhibiting enzyme growth, affecting protein biosynthesis, and causing changes in metabolic processes. Additionally, producing more ROSs to trigger oxidative pressure in bacterial cells. These mechanisms work together to ultimately kill the bacteria	[38,60,78–87]
	Flavonoids	Disrupting extracellular proteins and binding to bacterial cell walls, while also inhibiting nucleic acid compound, suppressing cytoplasmic membrane function, hindering energy metabolism, inhibiting adhesion and biofilm formation, blocking pore proteins on cell membranes, altering membrane permeability, and attenuating the	[37,63,64,66,87–96]

(Continued)

Table 4: *Continued*

Phytochemicals		Antibacterial mechanism	Ref.
		pathogenicity, multiple mechanisms act in concert to achieve antibacterial effects	
	Tannins	Disrupting the integrity of the cell membrane and wall induce substance leakage. Complex formation with enzymes that promote bacterial growth and inactivating cellular proteins, thereby disrupting bacterial metabolism and growth. Inhibiting extracellular enzymes impacts the production of substrates essential for microbial growth. Multiple mechanisms working together achieve the antibacterial effect	[28,52,56,62,64–67,70,73,77,83,85,89,96–105]
	Quinones	Through redox reactions and complex formation with amino acids, quinones can inhibit bacterial growth by rendering the proteins on the bacterial cell wall non-functional. Quinones also prevent substrates from being utilized by microorganisms, thereby inhibiting cell growth. Additionally, naphthoquinones exhibit cytotoxicity and antibacterial activity by altering microbial DNA conformation; it is reported that anthraquinones possess antifungal potential as they trigger a pro-oxidant–antioxidant unbalance in fungal biofilms, which brings about decreased metabolic activities	[29,35,42,44,57,69]
	Curcumin	The antimicrobial curcumin mechanism is broadly considered to correlate with hydroxyl and methoxyl groups. Curcumin can disrupt the structure of membrane proteins. This makes the cell membrane permeable, and affects the RNA and DNA of microorganisms, ultimately leading to bacterial death	[62,71,72,74,106]
Berberine		Berberine, a quaternary ammonium composition, contains a positive charge on its N atom which can disrupt the negatively charged bacterium cell membrane by disturbing the charge balance of cell membranes. Other disadvantageous influences of quaternary ammonium compositions on the microbes include protein denaturation and disruption of cell structure	[50,56,76,107–114]
Glycosides		Suppress bacterial growth by inhibiting the generation of bacterial RNA	[77]
Saponins		Interacting with the phospholipid membrane of bacteria, leading to a reduction in cell wall combinations and ultimately resulting in bacterial cell death	[32]
Steroids		Interacting with the phospholipid membrane of bacteria, leading to a reduction in cell wall combinations and ultimately resulting in bacterial cell death	[32]

Table 5. Summary of antibacterial mechanisms

Antibacterial mechanism	Schematic drawing	Phytochemicals related to this mechanism
Cell membrane destruction: Lead to the leakage of its internal material, bacterial dysfunction and decreased viability		Polyphenols, Flavonoids, Tannins, Quiones, Curcumin, Berberine, saponins, steroids
Enzyme activity inhibition: Bacteria fail to perform normal metabolic and biochemical reactions		Polyphenols, Flavonoids, Tannins, Glycosides, Curcumin
Elevates oxidative stress response: Disrupt the physiological function and metabolic processes of bacteria		Polyphenols, Quiones
DNA damage: Bacteria fail to replicate and repair normally, resulting in their life blocked or even death		Phenolic acids, Polyphenols, Flavonoids, Quiones, Curcumin
Protein structure damage: The normal function and metabolic processes of bacteria can be affected, resulting in their failure to survive or reproduce		Polyphenols, Flavonoids, Tannins, Quiones, Curcumin, Berberine
Nutrient uptake site competition: Limit the essential nutrients needed for bacteria and interfere with their growth and reproduction		Flavonoids, Tannins, Quiones

antibacterial mechanisms is shown in Table 5. In addition, in the specific antibacterial process, multiple mechanisms often work together to finally kill the bacteria (Figure 6).

Finally, according to the available results, the exact mechanism of bacteria inhibition has not been fully confirmed. Meanwhile, in the actual antibacterial effect, multiple phytochemicals usually interact with each other [77]. In addition, existing research focus more on the resistance of plant dyes to bacteria, and antimicrobial experiments and analysis on fungi or other microorganisms are relatively few, requiring further research.

3.4. Effects of mordants on antimicrobial activity

Numerous benefits are associated with plant dyes over synthetic ones, including a broad spectrum of colors, reduced environmental impact from natural origins, and functional properties like antimicrobial effects on textiles. Nevertheless,

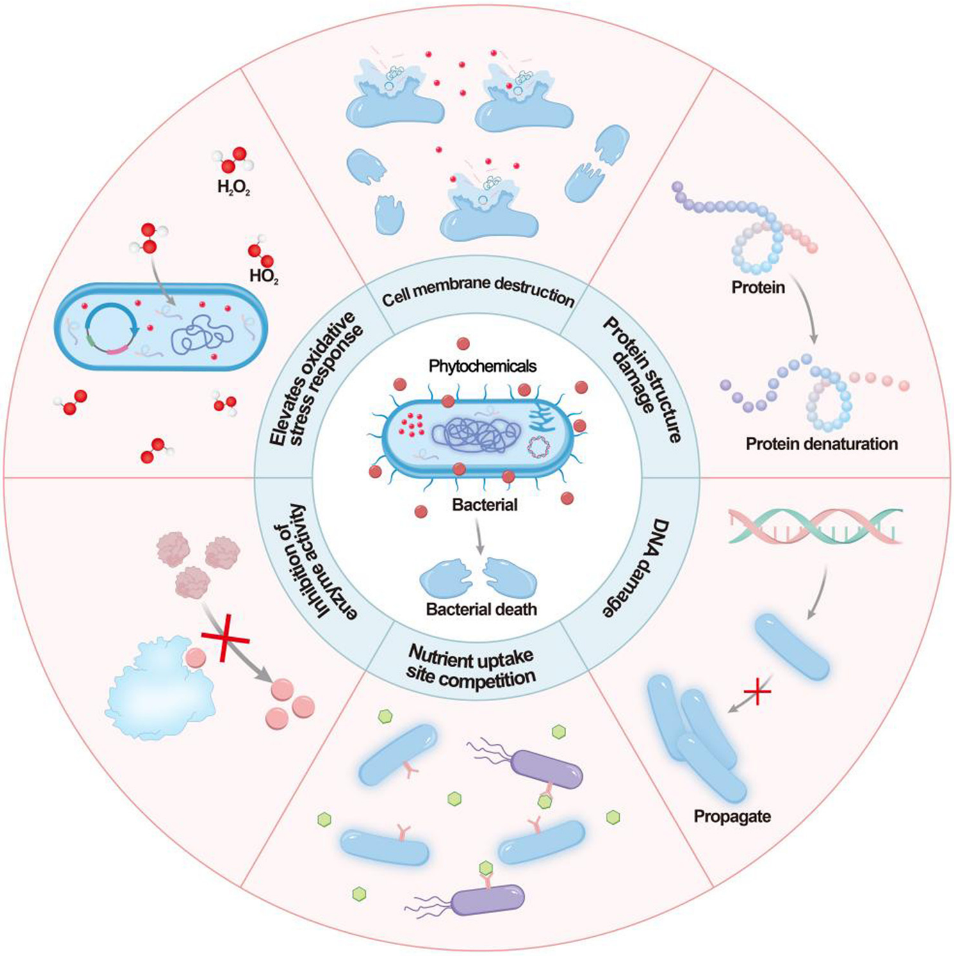


Figure 6. Mechanisms of antibacterial action.

challenges such as diminished colorfastness and resistance to washing and sunlight persist [114]. To address these issues, mordants are frequently used as auxiliary materials during the dyeing process. A mordant is employed to fix dyes on fabric by developing coordination complexes through the dyes and subsequently attaching them to the fabric [42]. Various studies have demonstrated that mordants improve the dyeing performance of natural dyes, and even significantly boost their antimicrobial efficacy [69,71,73,115].

3.4.1. Metal mordants

Metal mordants are among the most common mordants, as they can form metal complexes with dyes and fibers. These complexes consist of dye molecules, metal ions, and the functional groups of fibers, creating an insoluble compound [62]. The formation of this insoluble compound enhances the dye adhesion to the fiber, thereby enhancing color fastness. Thus, metal mordants enhance the antimicrobial properties of plant dyes on fabric by promoting their complexation and adsorption, and this results in the fabric maintaining good antimicrobial performance even after multiple washings and exposure to light [62]. Additionally, according to the concept of Overtone and Tweedy's chelation theory, the creation of a chelate complex between dye molecules and metal ions increases the liposolubility of the bacterial cell membrane, thereby enhancing the cell's permeability. This makes the bacterial cells more susceptible to the bioactive compounds present in the dyes [63,117,118].

In addition to their synergistic effects with dyes, metal ions themselves also exhibit good antimicrobial properties. Their antimicrobial mechanism primarily involve the bond of metal ions to proteins or the generation of reactive oxygen species (ROS). In the first scenario, metal ions bond covalently to the –SH groups in cellular enzymes, disrupting their function and modifying bacterial metabolism, leading ultimately to cellular demise [36,62,119–121]. The second scenario revolves around the pro-oxidant behavior of metal ions, whereby highly reactive oxygen radicals produced during the reaction damage and eventually disintegrate bacterial structures [58,62]. The antimicrobial effects of metal mordants have been well demonstrated, for example, in research conducted by Mirjalili and Karimi, turmeric was employed as a natural dye, and ferric sulfate, cupric sulfate, and potassium aluminum sulfate, as mordants for dyeing polyamide fabrics. The results showed a significant increase in the antimicrobial features of the dyed specimens mordanted with three metal mordants compared to the samples dyed without mordants [71].

However, some studies have also indicated that the use of metal mordants can diminish the antimicrobial activity of plant dyes. This can be interpreted by the structural modifications of colored compounds formed owing to the complexation of some dye hydroxyl groups through metal mordants, which subsequently reduce the availability of functional groups for interactions between the dye and microorganisms [45,70,120,122]. Nevertheless, in terms of durability, by virtue of the high chelating capacity of metal mordants, the dye molecules are more strongly fixed to functional groups on the fibers. Therefore, in terms of maintaining antimicrobial efficacy, fabrics mordanted with metal outperform fabrics dyed directly with plant dyes [45,120].

Metal mordants are crucial for improving dye effects and antimicrobial properties; however, they also pose potential hazards, such as causing skin allergies and environmental pollution [83,123]. To find more sustainable alternatives for plant dyeing, researchers have been exploring replacements for metal mordants with other materials.

3.4.2. Plant-based mordants

Biological mordants present superior biocompatibility with ecosystems and eliminate disposal concerns, making them a compelling alternative to traditional metal mordants. These strengths contain environmental sustainability, durability, biodegradability, and non-toxicity to humans, however, lack of allergenic responses. The benefits align with the worldwide trend in the textile industry toward eco-friendly practices [95]. As detailed in the appendix, the bio-mordants used in the literature can be categorized into plant-based mordants and chitosan. Plants serve as mordants primarily due to their rich phytochemical content, such as tannins, terpenoids, flavonoids, and so on. These compounds are rich in phenolic hydroxyl groups, making them create strong bonds between dye molecules and fabrics through hydrogen bonds and ionic interactions with the carboxyl and amino groups of fibers and hydroxyl groups of dye molecules, facilitating dyeing and color fixation [43,93,98,124]. Additionally, tannins impart carboxyl (–COOH) groups to fibers, enhancing dye absorption through increased ionic interactions between COO– anions and cationic dyes. The carboxyl groups in tannins can also esterify with the hydroxyl groups of fibers, resulting in more durable dye–fiber bonds. This naturally enhances the fabric's antimicrobial properties by enabling better dye adsorption [42].

This naturally enhances the fabric's antimicrobial efficiency by adsorbing more dye. Furthermore, as discussed in Section 3.3, these phytochemicals possess excellent antimicrobial properties on their own. When used as mordants, they can synergize with plant dyes, thereby boosting the overall antimicrobial effect [98]. For example, Singh et al. utilized the Tamarind seed coat, which is rich in tannins, as a biological mordant and the Kapok flower as the dye to color wool. The mordanted fabric exhibited improved antibacterial properties, which increased with higher concentrations of the mordant [75]. In addition, Somayeh Baseri's study using tamarind hulls as a bio-mordant and *Origanum vulgare* as a dye on cotton demonstrated a doubling in antibacterial activity compared to non-pretreated samples [95]. And another study involved adopting date seeds as a bio-mordant and *Trachyspermum coptiocum* (Zenian) as a dye for wool, which showed enhanced antibacterial properties after pretreating the wool yarn with date seeds [43]. Gallic acid is a natural polyphenol found in fruits, vegetables, and herbs that shows potential as a promising bio-mordant for cellulose materials. El-Zawahry and Gamal used 10% gallic acid combined with 2% gelatin for pre-mordanting cotton fabric, followed by dyeing with *Haematoxylum campechianum*, demonstrating high antibacterial activity against *Staphylococcus aureus* [55]. Safapour et al. employed gallic acid and ascorbic acid separately to mordant wool before dyeing with *Millettia laurentii* sawdust, revealing enhanced antimicrobial activity for both organic acids but better efficacy with gallic acid [47].

In addition, some plants contain different metal ions [124], such as various *Symplocos* species plants which are hyperaccumulators of aluminum (Al) compounds. These plant-based mordants with metal components accumulate high concentrations of aluminum compounds and form stable chemical bonds during the dyeing process, ensuring that the dye can firmly adhere to textiles [125,126]. Furthermore, these metal ions in these plants contribute to their antimicrobial properties [87].

3.4.3. Chitosan

Chitosan, a polysaccharide made from the copolymerization of glucosamine and *N*-acetyl-glucosamine, sources from the alkaline deacetylation of chitin, which comes from the exoskeletons of arthropods and crustaceans [127,128]. As a novel mordant, chitosan possesses multiple chemical properties that allow for multifunctional textile finishes and exhibits remarkable antimicrobial capabilities, making it a promising green and efficient biological material for textile modification [129].

On one hand, the amino groups ($-NH_2$) in chitosan can formulate hydrogen bonds through the hydroxyl groups (−OH) in the dye structure. Furthermore, the interaction between chitosan and fibers, through van der Waals forces and hydrogen bonds, provides sufficient binding strength ensuring that the dye–chitosan complexes are closely attached to the fabric surface, enhancing color fastness [57]. On the other hand, fibers pretreated with chitosan have protonated amino groups deposited on the surface of chitosan molecules, which can electrostatically bind to negatively charged dye molecules, increasing dye adsorption [69,93]. Therefore, chitosan demonstrates outstanding performance in increasing the color fastness of fabrics and dye absorption. Studies indicate that the combination of chitosan with natural plant dyes exhibits excellent antimicrobial effects [130,131].

In addition, chitosan itself also possesses antimicrobial properties. The antimicrobial effect of chitosan arises from the interplay between its positively charged structure and the negatively charged surface of microorganisms. This interaction leads to significant alterations and damage to the bacterial surface, inhibiting bacterial metabolism and ultimately resulting in bacterial death [69,132]. Specifically, the amino groups in chitosan attach to bacteria, thereby hindering their progress [133]. Furthermore, chitosan obstructs the uptake and utilization of external nutrients by bacteria and disrupts their internal metabolic pathways, preventing normal growth and reproduction [134–137]. The verification of chitosan's enhancement of antimicrobial properties has been extensive. For instance, a study by Tian et al., the extract of *V. bracteatum* Thunb. leaves, adopted as a natural dye, was treated with chitosan and $FeCl_3$ as mordants, and their effects were compared. The results indicated that chitosan was more effective than $FeCl_3$ in enhancing antibacterial performance, increasing it from 76.10 to 81.4% [39].

3.4.4. Novel environmental mordants

Additionally, the exploration of novel eco-friendly materials, such as choline chloride-ethylene glycol natural deep eutectic solvent (NADES) used as a mordant, is advancing in research from Zhejiang. This research has shown that textiles dyed with natural dyes and this innovative mordant exhibit excellent antibacterial, UV-resistant, antioxidant, and flame-retardant properties, alongside good fastness and structural integrity. Notably, the residual solvent demonstrates significant reusability, and the NADES reduces water usage, chemical reagent usage, energy consumption, along with cost, while enhancing the biodegradability of dye wastewater, suggesting a reduced environmental impact [138].

In Somayeh Baseri's study, whey protein isolate was utilized as a bio-mordant, pomegranate rind served as the dye for cotton dyeing. A comparison with cotton fabrics dyed solely revealed a higher antibacterial efficiency, maintaining efficacy even after multiple washings [89].

Chitosan is employed in combination with dendrimers to create new environmentally friendly composite bio-mordants, such as chitosan-poly(amidoamine) dendrimer hybrid (Ch-PAMAM) [139] and chitosan–polypropylene imine dendrimer hybrid (CS-PPI) [33]. Pre-treating textiles with chitosan dendrimer hybrid not only introduces new functionalities to the textiles but also generates new functional groups on textiles, thereby enhancing the assimilation sites of dye molecules on fibers, improving dye uptake and antibacterial activity of the textiles [139].

In summary, mordants utilized in plant dyeing fall into four categories: metal mordants, plant-based mordants, chitosan, and novel environmental mordants. Metal mordants are widely used due to their economic and simple application but can lead to metal pollution. In contrast, plant-based mordants, chitosan, and novel environmental mordants offer environmental benefits and show strong potential as replacements for metal mordants, presenting significant developmental value.

3.5. Treatments to enhance antimicrobial properties of plant-dyed fabrics

Besides mordants, there are several treatments that can effectively enhance the color fastness and various properties of natural plant dyes. Some of these treatments have significant effects on enhancing antimicrobial properties. The main treatment methods mentioned in the literature that can enhance antimicrobial properties are summarized as follows.

3.5.1. Enzyme treatment

Enzymes can change the surface properties of fibers without damaging them, improving hydrophilicity and thus better absorbing natural antimicrobial extracts. Moreover, due to their biodegradable nature, enzymes are considered environmentally friendly for use in textile processing [91]. Garg et al. used laccase enzyme to polymerize phenolic compounds from *Camellia sinensis* directly on wool. The enzyme-catalyzed polymerization enhanced the wash resistance between dye and fiber, and this improvement guaranteed a significant quantity of phenolic polymer. This provided more antibacterial activities [61]. Vajpayee et al. used cellulase enzymes for wet processing of lotus fabric, resulting in a more durable antibacterial effect on the material. Even after three wash cycles, the fabric maintained its antibacterial properties, while untreated lotus fabric lost its antibacterial effectiveness [91]. Samant et al. applied three enzymes (xylanase, neutral cellulase, and acid cellulase) to improve the dyeing and functional properties of cotton fabrics. Accordingly, it is indicated that the total three enzymes can effectively enhance antibacterial activity, with xylanase showing the most outstanding effect [102].

In addition, enzymes can be used to catalyze the polymerization of dyes. In the study by Baek et al., horseradish peroxidase was used to catalyze the synthesis of antibacterial gallic acid-*g*-chitosan derivatives, which were then used for dyeing textiles. This enzymatic catalysis method simplified the pre-treatment process of chitosan and significantly enhanced the antibacterial performance of dyed fabrics [140].

3.5.2. Crosslinking agent treatment

Citric acid, a natural compound with three carboxy groups [76], acts as a multi-carboxylic cross-linking agent that responds with the hydroxyl groups of cellulosic chains in cotton, forming ester linkages that cross-link with the fiber [35,108]. This treatment not only improves the physical performance of the fabric but also enhances its antimicrobial properties. The antimicrobial activity of citric acid is ascribed to the decrease in pH value and the destruction of enzyme activity. Another possible reason could be that carboxylic acid groups of CA-treated cotton can chelate with metal ions in cell walls, potentially preventing the assimilation of key nutrients and triggering destroy and cell death [108]. In addition, when dyeing with cationic dye berberine, CA can increase the capacity of the negative charge of the fabric. This is useful to adsorb a large amount of berberine to enhance the exhaustion of the dye, thereby enhancing the antibacterial activity [76].

Habib et al. used citric acid cross-linking to permanently combine curcumin and nano-silver onto the fabric surface, preparing textiles with excellent antibacterial activity and wash fastness. This is because the hydroxyl groups of the antibacterial curcumin dye and the hydroxyl groups of the cellulose fabric form ester bonds through citric acid, resulting in better bonding. Moreover, after citric acid treatment, the carboxylic acid functional groups on the surface of the naturally dyed fabric can develop coordination bonds through the silver ions on the surface of nano silver. This coordination stabilizes the nano silver particles, preventing their aggregation, ensuring their uniform dispersion in the substrate, and improving their adhesion to the textiles, thus enhancing durability and antibacterial effects [72].

However, there are studies suggesting that citric acid cross-linking with chitosan might negatively impact antibacterial properties. Mirshahi et al. used citric acid as a cross-linking agent to fix chitosan on cotton fabrics. During the cross-linking process, the carboxyl groups in citric acid can respond with the hydroxyl or amino groups of chitosan, formulating ester or amide bonds. Since amino groups are the source of chitosan's positive charge, reacting with citric acid can reduce antibacterial performance. Nonetheless, increasing the concentration of chitosan can enhance antibacterial properties [35].

In addition to citric acid as a crosslinking agent, Sadeghi-Kiakhani et al. used cyanuric chloride as a cross-linking agent to graft chitosan onto cotton structures. Compared to the samples processed through chitosan alone, samples processed through the Chitosan–Cyanuric Chloride hybrid showed enhanced antibacterial efficiency and durability, owing to the development of robust covalent bonds with the cotton fabric [141].

3.5.3. Fabric modification

In the treatment of fabric modification, the most important is cationic modification. Cationic modification technology involves chemically treating fabrics to introduce cationic groups on the fiber surface before dyeing with natural dyes. This improves the bonding ability between dyes, mordants, and fibers, thereby enhancing dyeing effects and antimicrobial activity [142]. For example, Alebeid et al. modified cotton with 2,3-epoxypropyltrimethylammonium chloride (EP3MAC), which reacted with the hydroxyl groups in cotton fibers to form cationic sites on the fiber surface. These cationic sites reacted with the negatively charged carboxyl groups in Lawsonia dye, allowing the dye to directly bind to the cotton fibers without adding electrolytes. This modification enhanced the affinity of cotton fibers for the dye, improving dye absorption efficiency and thereby helping to enhance antibacterial effects [107]. In a study by Gedik et al., cotton fabrics were firstly changed with cationic agents and subsequently combined with mordants and natural dyes to achieve optimal antibacterial activity [142]. In their study, El-Sedikd et al. modified cotton fabrics using 3-chloro-2-hydroxypropyltrimethylammonium chloride and then dyed them with curcumin. Compared to untreated fabrics and solely cationized fabrics, the cationic dyed fabrics exhibited significantly enhanced antibacterial ability [106].

In addition, Szadkowski et al. modified bamboo fibers using 3-aminopropyltriethoxysilane, followed by binding with lawsone dye. The aminosilane activated surface of the bamboo fibers has functional groups that react actively with organic dyes (lawsone), allowing for the formation of chemical bonds between the surface and silane as well as lawsone. This treatment method enables the modified compound to anchor onto the surface, enhancing the antibacterial activity of lawsone [34].

In the study by Zhu et al., zinc ions were used to modify the surface of Angora wool using high-voltage electrospray technology, in order to enhance its binding ability with natural dyes. Subsequently, tannic acid was employed as a mordant, which not only achieved complete coordination of the metal ions but also imparted extremely high antibacterial performance to the fabric (with a bacteria reduction rate exceeding 90%) due to the synergistic effect of dye, metal ions, and mordant [144].

3.5.4. Plasma treatment

The reason why plasma treatment technology can improve antimicrobial effectiveness is mainly through modifying the fabric surface, improving the affinity between natural dyes and fibers, and enhancing the dyeing rate, so that the treated fiber surface can adsorb more antimicrobial dye molecules [111,145]. In addition, plasma treatment enhances the sample wettability by etching the hydrophobic surface layer of the fibers, enhancing the attachment of media dyes or other auxiliaries to the fiber surface and promoting their absorption [36]. Thus, compared to plasma treatment alone, its combination with other materials can further enhance antimicrobial activity.

Haji et al. employed atmospheric plasma treatment to alter the surface properties of nylon 6 fabric, dyed with berberis extract, using copper sulfate as a mordant to compare antibacterial effects. Results showed that untreated dyed samples did not exhibit antibacterial activity, samples mordanted through copper sulfate alone exhibited a low antibacterial activity, plasma-treated dyed samples exhibited moderate antibacterial activity, and plasma-treated samples mordanted through copper sulfate exhibited high antibacterial activity [111]. In another study by the same author, low-temperature oxygen plasma was adopted to improve wool fibers' assimilation of β-cyclodextrin (β-CD) and dyeing with berberine. Due to β-CD's ability to increase wool fibers' adsorption of antibacterial dye (berberine) and its composite effect with the dye, dyed samples exhibited a 99.9% decrease rate for *E. coli* and *S. aureus* [113].

3.5.5. Ultrasonic-assisted treatment

Ultrasonic-assisted treatment can be used for the extraction of dyes and dyeing processes. Compared to traditional water-based extraction methods, ultrasonic-assisted extraction is more effective and faster. This is because the high power (20–100 kHz) of ultrasound can be utilized to enhance and intensify the extraction process. After ultrasound irradiation, microbubbles grow and vibrate rapidly, even violently bursting under high pressure. These microbubbles disrupt the solid surface and create microchannels and pores. Additionally, by disrupting cell walls, heat and mass transfer are facilitated in the liquid phase near particles through these channels [146]. Due to improved extraction efficiency, the concentration of antimicrobial active components in the final extract also increases [147], thereby enhancing its antimicrobial effect. Furthermore, many compounds in plants are sensitive to high temperatures, which may lead to their degradation with prolonged boiling [148]. Ultrasonic extraction typically does not require higher temperatures [149], helping protect thermosensitive active components in plants from being destroyed during the extraction process.

During the dyeing process, the use of ultrasound assistance can improve the utilization rate of dyes. The cavitation effects of ultrasound enhance the diffusion and penetration of dyes by disrupting aggregated dye particles, allowing dyes to penetrate deeper into the fibers and increase their binding capacity [96,150]. Therefore, ultrasonic dyeing allows fabrics to absorb more antimicrobial ingredients in dyes compared to traditional dyeing methods, thereby enhancing their antimicrobial effectiveness.

3.5.6. Dye modification

In the research of Alebeid et al., *Acacia nilotica* pods were applied to extract Henna dye to enhance the dye color intensity as well as the affinity between the dye and fibers. Results show that the wool fabric dyed with modified dyes has excellent antibacterial performance [151].

Rehan et al. prepared azo dyes by coupling modification of peanut red skin extract with *p*-nitroaniline. The prepared dyes were better bound to the fabric surface through electrostatic interactions and non-electrostatic interactions with functional groups on the fabric, exhibiting excellent antibacterial performance [152].

3.5.7. Optimization of dying process

In addition to the more extensively studied methods mentioned above, the literature also highlights other approaches to effectively improve the antibacterial features of plant-dyed fabrics. For example, as found by Ibrahim et al., a treatment sequence of salt treatment → alkali treatment → oxidation treatment was adopted to fulfill *in situ* deposition of metal mordant oxides, and then natural dyeing. This method demonstrated excellent antibacterial properties and wash fastness [44].

Additionally, some plant chemicals exhibit different antibacterial activities at different pH levels, primarily due to structural changes in the compounds responsible for antibacterial activity. Ren's research revealed that tea-dyed wool fabrics exhibited the highest antibacterial efficacy at a dye bath pH of 5.5, mainly due to structural changes in catechins, the primary antibacterial substances in tea. When the dye bath is acidic or alkaline, particularly under alkaline conditions, the oxidation and polymerization of tea polyphenols reduce the amount of –OH groups, significantly decreasing antibacterial activity [78]. Ren et al. also proposed an *in situ* polymerization dyeing method for cellulose fibers based on the oxidation and polymerization of catechins, achieving brown coloration, good color fastness, and antibacterial characteristics for cotton structures without chemical mordants [83].

In summary, the aforementioned methods primarily enhance antibacterial efficacy by improving the adsorption rate of dyes or mordants on fabrics, allowing the antimicrobial components to function more effectively. Additionally, *in situ* synthesis involving silver nanoparticles and metal oxide nanoparticles can effectively improve fabric antimicrobial properties. However, this is mainly based on the fact that these nanoparticles themselves act as antimicrobial agents [53,153,154].

4. Discussion

4.1. Advantages of antimicrobial plant dyes

Functional characteristics like antimicrobial properties to textiles is a special advantage of natural plant dyes over synthetic dyes [155]. At the same time, the rapid development of health and hygiene-related issues has raised the need for antimicrobial textiles [156,157]. During the last few decades, a series of composite antimicrobial chemicals, like metal salts, triclosan, and quaternary ammonium-based products, have been formulated and broadly utilized as finishing agents to manufacture antimicrobial textiles. Nonetheless, these products possess remarkable limitations, including toxic adverse effects, wastewater issues, and water contamination. Reducing the loading of composite antibacterial agents using natural antimicrobial dyes can offer a valid method to reduce water and energy usage, thereby integrating dyeing and finishing processes [158,159].

4.2. Potential of using plant dyes in industrial applications

As the demand for functional textiles continues to grow, plant dyes with antimicrobial properties demonstrate significant potential for development and application [160]. One of the primary areas of application is medical functional products, with a promising outlook in the field of biomedical textiles and medical care applications [63]. For instance, hospital bedding, operating gowns, medical masks, nurse uniforms, and wound dressing mats can benefit from these textiles, offering protection to medical staff and patients and thereby reducing the risk of microbial infections [76,106,158]. Research by Shahid-ul-Islam illustrates that wool fabric processed with *Tectona grandis* leaf extract exerts antibacterial effects and maintains stability through washability, making them highly suitable for medical care and hygiene applications [120]. According to the research results of El-Sedik et al., cotton fabrics treated with cationization and curcumin coloring exhibit significantly reduced bacterial growth characteristics. Based on these findings, plant-dyed textiles with antibacterial properties may be suitable for various applications that require antibacterial performance, especially in medical environments where there is a significant risk of pathogenic bacterial infection, such as wound healing situations [106].

Vulnerable populations such as infants, children, and the elderly are particularly susceptible to microbial infections. Therefore, materials with antimicrobial properties provide additional protection. Plant-dyed textiles possess antimicrobial properties and, owing to their natural dye characteristics, further mitigate the risk of skin irritation and allergic reactions compared to synthetic dyes. Thus, antimicrobial plant dyes hold substantial promise in the development of products for infants and the elderly [62].

Furthermore, increased sweating during exercise creates an environment conducive to microbial growth on clothing, leading not only to unpleasant odors but also to potential skin infections or other health issues. Therefore, antimicrobial plant dyes offer broad prospects in sports textiles [28,65]. For example, as demonstrated by Marikani et al., the cotton fabrics that are dyed with the extracts from *Bixa orellana* seeds, are well-suited for sportswear due to their exceptional antibacterial performance and durability, essential for frequent washing and use [59].

Finally, textiles in public settings such as hotels, frequently in contact with the human body (e.g., bed sheets, towels), are prone to accumulating bacteria and other microorganisms. Antimicrobial textiles effectively inhibit the proliferation of these microorganisms, thereby reducing the risk of cross-infection and enhancing the health and safety standards for guests. Hence, antimicrobial plant dyes, as a substitution to synthetic dyes, offer an emerging perspective for formulating infection-resistant clothing in public places like hotels [49].

4.3. Future prospects

The future trends and opportunities include the following aspects: First, with the continuous development of technology, more environmentally friendly and efficient natural dyeing production practices and materials are being continuously developed. In future research, it is possible to conduct relevant research on the latest technology and materials, and explore in depth the specific operational methods of each stage in the dyeing process. In the field of dye extraction, it is possible to achieve effective utilization of raw material resources and reduce the negative impact on the environment by optimizing the extraction process or using new technologies. In terms of fabric treatment, new types of mordants and other effective processes to enhance the fabric's ability to absorb plant dyes can be explored. These methods can enhance the interaction between dyes and fabrics, while ensuring color intensity and fastness, and enhancing the antimicrobial effect. In addition, there are many aspects worth further research in the dyeing stage. For example, how to adjust temperature, pH value, and other conditions to obtain ideal color and excellent antimicrobial performance [161]. In addition, how to reduce costs and avoid residual pollutants are all issues that need attention and experimental verification to ensure that the entire production process meets environmental standards [162]. In addition, plant dyes can also be used in conjunction with emerging technologies such as the *in situ* synthesis of nanoparticles using plant dyes, which can result in antimicrobial agents with higher efficacy and durability [163,164].

Second, in addition to the existing plant dyes with verified antimicrobial properties, new plant sources can also be explored. To ensure the stability of dye acquisition, attention can be turned to those plants that are widely cultivated or naturally grown and have abundant resources and are easy to obtain. In addition, there is a huge value in utilizing a large amount of by-products and waste generated in the agricultural industry chain [165]. If it is possible to extract dyes from these waste materials, it can not only effectively reduce the pollution caused by agricultural waste to the environment, but also create more economic benefits.

Third, existing research has confirmed that many plant dyes have strong antimicrobial abilities. The active ingredients contained in these plant dyes can interfere with key life processes of bacteria, such as cell wall structure, protein synthesis, and DNA replication, in order to achieve the effect of killing or inhibiting bacterial growth and reproduction. However, more specific mechanisms need to be further explored. In the future, we can delve into the active ingredients of various plant dyes and the mechanisms of interaction with different types of microorganisms, and seek more effective methods to utilize these antimicrobial properties.

Finally, another important aspect is to pay attention to consumer behavior and education, understand consumers' views and needs for natural dyed textiles, and formulate relevant strategies to help consumers better understand the advantages of natural dyes in terms of antimicrobial performance. This will facilitate the promotion of related products and increase the market share of natural dyed textiles. In conclusion, the research and application of antimicrobial plant dyes have tremendous value, and their demand is expected to continue increasing as consumer awareness of health and environmental protection improves [166].

4.4. Research limitations

This study also acknowledges certain limitations. The systematic review was confined to the WOS and PubMed databases; thus, excluding many valuable studies found in other databases. Future research should include a wider array of databases for a more comprehensive analysis of research trends and outcomes. Second, the scope of this systematic review was limited to scholarly journals, and subsequent research may profit by allowing for other released works, like business journals, books, book chapters, reviews, and industry reports. Additionally, this review used CiteSpace software as the tool for visualizing citations. Different tools or software have their own advantages and may produce varying results, suggesting the need for comparative studies. to increase the reliability of the results.

5. Conclusions

This study conducted a systematic review of the antimicrobial properties of plant dyes in the textile sector to enhance awareness of eco-friendly yet natural dyes and serve as a reference for further practical and theoretical research in this area. Generally, publications on the antimicrobial properties of plant dyes in the textile field are consistently increasing, indicating a rising level of interest in this area with high research value.

Through a systematic review of 132 studies, our research outlined the standards and methods involved in antimicrobial testing, the main compounds and mechanisms used for the antimicrobial features of plant dyes, the types of mordants, and other treatments that can enhance the antimicrobial features of natural plant dyes. In light of the summary of the literature, the AATCC100 standard in quantitative detection is currently the most widely used antimicrobial testing standard. Phenolic compounds in plants play a major role in antimicrobial performance, however, the bad wash fastness and light fastness of plant dyes remain an area worthy of further research. The application of various mordants helps improve the antimicrobial activity and color fastness of natural dyes in a way, with metal mordants and bio-mordants (mainly plant-based mordants and chitosan) being the most commonly used types. With the increasing environmental awareness and technological advancements, bio-mordants are receiving more attention. In addition, treatments such as enzyme treatment, crosslinking agent treatment, fabric modification, plasma treatment, ultrasonic assistance, and dye modification also play significant roles in enhancing the antimicrobial properties of plant dyes by increasing the adsorption of dyes or mordants on the fabric, allowing the antimicrobial components to function more effectively and thus improve the overall antimicrobial efficacy.

With the continuous rise in environmental consciousness and the increasing demand for functional textiles, the field of plant dye antimicrobial properties holds significant research value. This systematic review aids researchers in gaining a clearer understanding of the existing research outcomes and offers valuable insights and references for future investigations into natural dyeing.

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