Journal of
Babol University of Medical Sciences
JBUMS
e-ISSN: 2251-7170 p-ISSN: 1561-4107

Effect of Natural Dye Construction on Antibacterial Activity

K. Kadm Shanshal (MSc)[*1]

1.Department of Science, College of Basic Education, Mustansiriyah University, Baghdad, Iraq.

•**Corresponding Author: K. Kadm Shanshal (MSc)**
Address: Department of Science, College of Basic Education, Mustansiriyah University, Baghdad, Iraq.
Tel: +964 (770) 7768685. **E-mail:** Katreen.kadm@uomustansiriyah.edu.iq

Article Type

Research Paper

Received:
Jun 29th 2024
Revised:
Jul 22nd 2024
Accepted:
Aug 26th 2024

ABSTRACT

Background and Objective: Natural dyes have attracted much attention due to their antibacterial properties and various applications, especially in textiles, and also due to the environmental pollution of synthetic dyes. The aim of this study is to determine the antibacterial properties and effectiveness of natural dyes by analyzing common dyes against common bacterial strains.

Methods: In this laboratory study, sufficient extracts of natural dyes (Hibiscus Sabdariffa, Nigella sativa, and Lawsonia Inermis) were created for analysis. The dried plant components were pulverized, followed by adding 2g of the resulting dry plant powder of each plant to 100 ml of distilled water, and boiling for 15 minutes at 60◦C. The extract was then strained using a muslin cloth and filter paper. A standard Agar Well Diffusion method determined the initial effects on antibacterial properties.

Findings: The antibacterial activity of three aqueous plant extracts (Hibiscus Sabdariffa, Nigella sativa, Lawsonia Inermis) showed that each sample had a wide range of effects against bacteria from this experiment. Nigella sativa and Hibiscus Sabdariffa extracts had strong antibacterial activity against all bacteria in this study, while Lawsonia Inermis extract had weak antibacterial activity. It appeared from the well diffusion technique that Nigella sativa seeds inhibited E. coli and the inhibition zone was 20 mm and after diluting the concentration, the inhibition zones were 22 and 31 mm at 25 and 50%, respectively, with no inhibition zone at 75% concentration. Regarding Hibiscus Sabdariffa, the inhibition zone was 13 mm against staphylococcus aureus at 100%, and inhibition zone was 12 mm against klebsiell Sp., while the inhibition zone for Nigella sativa for the same bacteria was 11 mm. The Lawsonia Inermis extracts had no inhibition zone for all bacteria. In general, all extracts showed moderate antibacterial activity against bacteria and fungi. The extracts were characterized using Fourier transform infrared spectroscopy (FTIR), UV-Vis spectroscopy and atomic force microscopy (AFM).

Conclusion: The results of the study showed that plant extracts, due to their antibacterial properties against different species, can be effective in treating infections caused by resistant bacteria.

Keywords: *Green Technology, Hibiscus Sabdariffa, Nigella Sativa, Antibacterial Activity*.

Cite this article: Kadm Shanshal K. Effect of Natural Dye Construction on Antibacterial Activity. *Journal of Babol University of Medical Sciences*. 2025; 27: e40.

Introduction

Natural dyes, also known as plant extracts, have become increasingly popular due to the environmental pollution caused by synthetic dyes. Several countries have enforced environmental standards due to the harmful reactions caused by synthetic dyes; this has led to a growing interest in the use of natural dyes. The worldwide concern for eco-friendly and biodegradable dyes has further increased the demand for natural dyes, particularly in the cosmetic and food industries. Plants have served as a primary source of dyes since the beginning of human civilization (1). In Iraq, many plants have been used for both natural dyes and therapeutics for centuries.

The knowledge of plant usage was widespread in ancient civilizations, where plants were the primary therapeutic agents used by humans. Nowadays plants play a significant role in the world, and their potential to produce numerous benefits for society, especially for medicinal purposes, and approximately 50% of all drugs in clinical use derive from natural products. The medical power of plants is due to their photochemical, which can cause definite physiological actions in the human body to act against diseases and prevent them.

Natural dyes have garnered significant attention for their potential antibacterial properties, which can be leveraged in various applications, particularly in textiles. The natural anthraquinone dye purpurin, derived from madder plants, disrupts bacterial cell division by targeting the FtsZ assembly, exhibiting notable antibacterial effects (2). Similarly, natural dyes extracted from sea water bacteria, such as those found on Marina beach, show promising antibacterial activity against E. coli and S. aureus, with dyed fabrics retaining their color and antibacterial properties after washing (3). Studies on plant-based dyes, including extracts from Butea monosperma and Tagetes erecta, demonstrate effective antibacterial action against common pathogens like E. coli and S. aureus when used on cotton fabrics (4). Additionally, Melia composita leaves have been identified as a potent source of natural dye with substantial antibacterial efficacy, suitable for medical textiles (5). Furthermore, medicinal plant extracts such as thyme, clove, and lavender not only provide durable coloration but also impart antibacterial and antimicrobial properties to wool and cotton yarns (6).

The application of plant extracts that are recognized for their antimicrobial properties holds great importance in the field of medical treatments. Over the past few years, numerous research studies have been conducted in different countries to validate their efficacy. The antimicrobial properties of these plants stem from the compounds synthesized during their metabolic processes. These compounds contain active substances, such as phenolic compounds and tannins, which are well-known for their antimicrobial properties (7).

It has become increasingly important to discover novel antimicrobial agents to combat resistant microorganisms from diverse sources, particularly those derived from plants. Researchers from various fields are now exploring the potential of plants as an alternative source for existing drugs by investigating their antimicrobial properties.

Collectively, these studies underscore the dual benefits of natural dyes in offering sustainable color solutions while enhancing the antibacterial functionality of textiles, making them ideal for hygienic applications and protective clothing.

This study focuses on the preparation of extracts from Nigella sativa seed, Hibiscus Sabdariffa, and Lawsonia inermis. The extracts were analyzed to determine their properties and their effectiveness against certain types of both positive and negative bacteria (Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, Klebsiella sp., and Candida albicans). The study aims to assess the antibacterial effect of these extracts.

Methods

This research was approved by the ethics committee the College of Science Research Ethics Committee University of Baghdad (Reference Number: BCSMU/0923/0007Bon September 23, 2023), All plant material used in the experiment was synthesized using green technology (8). The collection included Nigella sativa seeds, Hibiscus sabdariffa, and Lawsonia inermis.

Nigella sativa, commonly referred to as black seeds, is an annual plant from the Ranunculaceae family, Genus Nigella, Species N.sativa. Its leaves come in various colors such as white, pink, yellow, pale blue, or pale purple, and it is found in southwest Asia, northern Africa, and southern Europe (Figure 1a). Hibiscus sabdariffa, known as Roselle or Karkadeh, is an herb native to West Africa and belongs to the Malvaceae family, Genus Hibiscus, Species H.sabdariffa. It grows up to 2-2.5 meters tall and has deep three to five-lobed leaves that range from green to red, with large blooms having a dark center (Figure 1b). Lawsonia inermis, commonly known as Henna, is from the Lythraceae family, Genus Lawsonia, Species L.inermis, and indigenous to North Africa and southwest Asia. The plant's leaves are elliptic to lanceolate in shape (Figure 1c).

Figure 1. Nigella sativa (a), Hibiscus Sabdariffa (b), Lawsonia inermis (c)

Extract Preparation: For preparing plant extracts, the approach depends on which part of the plant is under review; different parts of the plants require different procedures. For Nigella sativa, we utilize its seed to prepare the extract, and for Hibiscus Sabdariffa and Lawsonia inermis, we utilize the flowers and leaves, respectively. The adopted method has been well handled and showed a high facility in managing the procedures and conditions. The process starts with ground dried plant material, then adding 2g of the dry plant powder of each plant to 150 ml of distilled water, heated to 60˚ C, and left at the same temperature for 30 minutes. The extract was filtered using the muslin cloth, and then by filter paper. The filtrate plant extracts were used for the bio-synthesis of NPs directly as soon as possible, as shown in Figure 2 a, b, c (Figure 2). Three different extracts were prepared; the white extract from Nigella sativa, the red extract from Hibiscus Sabdariffa (Roselle) and the brown extract from Lawsonia inermis (Henna).

Figure 2. Extracts of Nigella sativa (a), Hibiscus Sabdariffa (b), Lawsonia inermis (c)

Results

The structural, optical, and morphological properties of such listed samples were described by UV-visible spectroscopy, FTIR spectroscopy, and Atomic Force Microscopy (AFM). Then, the antibacterial activity of extract samples was investigated using the agar well diffusion method against different common bacteria.

Ultraviolet-Visible (UV-Vis) Spectroscopy Analysis: The UV-Vis absorption spectra, covering the wavelength range of 200 to 1100 nm, for the prepared plant extracts are shown in Figure 3. The spectra reveal significant absorption in the UV region (200-350 nm), with distinct absorbance peaks. The three dyes exhibit similar absorption peaks in the Ultraviolet (UV) region, with their intensity increasing and then decreasing over the visible wavelength region. Nigella sativa white extract displayed the highest absorbance at 210 nm, indicating a strong presence of UV-absorbing compounds. The red aqueous extract for Roselle (Hibiscus sabdariffa) showed two peaks at approximately 212 nm and 284 nm, typical of anthocyanin the red extracts from Hibiscus sabdariffa flowers. These absorption characteristics are attributed to the different functional groups on the anthocyanins and the colors of the extracts. Additionally, the henna brown extract (Lawsonia leaves) displayed weak absorption bands at 208 nm and 268 nm, due to the presence of π-π* and n-π* electron transitions in benzene and quinone (Figure 3).

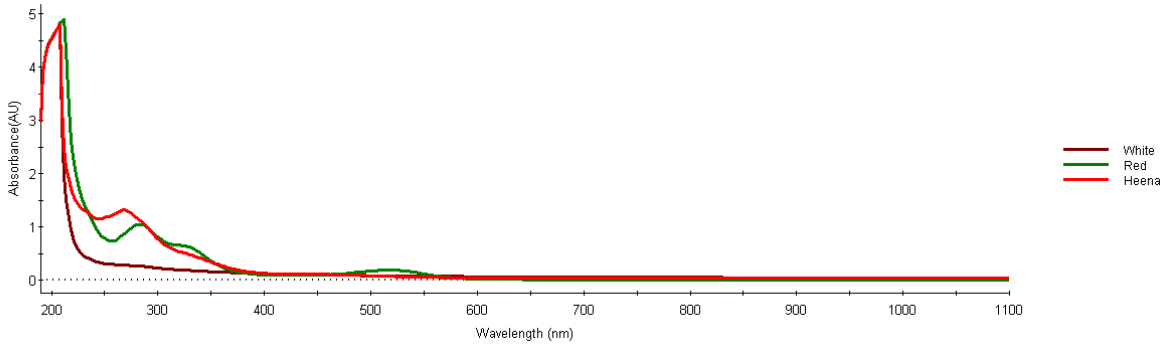

Figure 3. UV-Vis absorption spectrum of plant extracts prepared from Nigella sativa seeds (white), Hibiscus sabdariffa flowers (Red), and Lawsonia leaves (Henna)

FTIR Spectra Analysis: The FT-IR analysis was performed on extracts from Nigella sativa seeds, Hibiscus sabdariffa flowers, and Lawsonia leaves, covering the infrared spectrum range of 400-4000 cm^{-1}. The results are shown in Figure 4, with subfigures 4a, 4b, and 4c representing the different samples. The FT-IR spectrum for Nigella sativa (Figure 4a) shows a broad and strong band at 3436 cm^{-1}, corresponding to the O-H stretching vibration in hydroxyl functional groups. Other notable peaks include 2068 cm^{-1}, 1635 cm^{-1}, and 685 cm^{-1}, which refer to alkanes, acids, and alkenes groups, respectively. In Hibiscus sabdariffa (Figure 4b), the strong peak at 3448 cm^{-1} is attributed to the O-H stretch vibration, indicating the presence of hydroxyl groups commonly found in various organic compounds. Peaks in the range of 1100-1071 cm^{-1} indicate the presence of specific anthocyanins, such as cyanidin-3-O-sambubioside and delphinidin 3-O-sambubioside, contributing to the plant's distinctive red coloration. Additionally, the region between 1610 and 1620 cm^{-1} is associated with carbon-carbon double bonds (C=C), implying the presence of unsaturated compounds containing double bonds. The FT-IR spectrum of Lawsonia inermis (Figure 4c) shows a peak at 3436 cm^{-1}, suggesting the existence of the hydroxyl (OH) functional group. Additionally, the presence of peaks at 1636 cm^{-1} indicates the presence of α and β-unsaturated carbonyl groups, demonstrating the stretching vibration of the carbon-oxygen double bond (C=O) (figure 4).

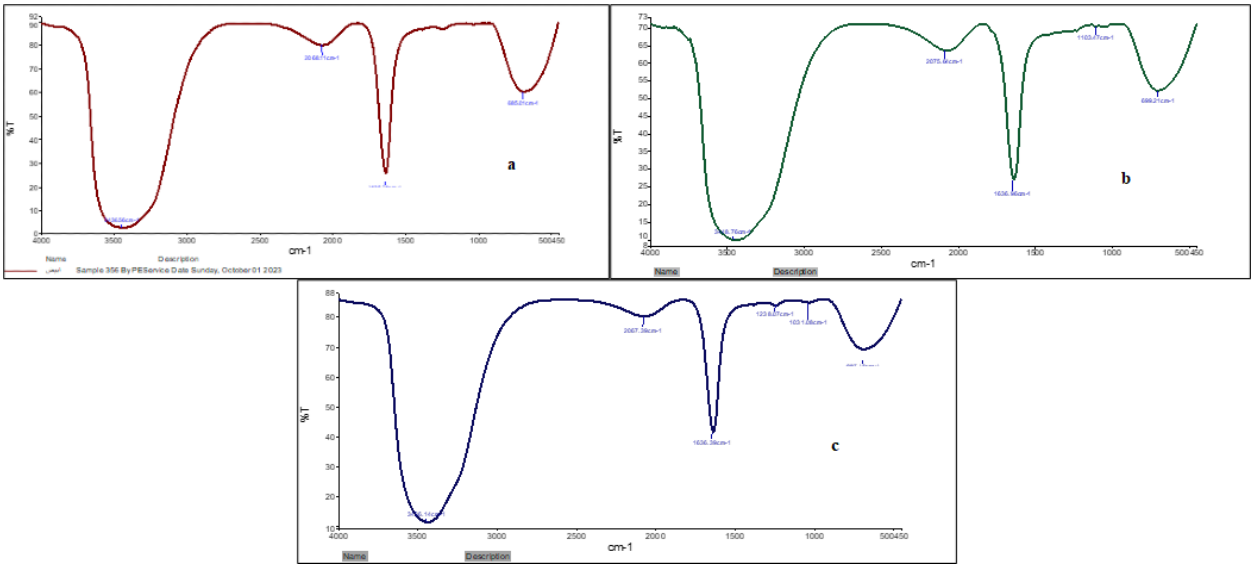

Figure 4. FT-IR spectra for plant extracts. **(4a) Nigella sativa extract, showing the O-H stretching vibration at 3436 cm^{-1} and additional peaks corresponding to alkanes, acids, and alkenes. (4b) Hibiscus sabdariffa extract, identifying hydroxyl groups at 3448 cm^{-1}, anthocyanins in the range of 1100-1071 cm^{-1}, and carbon-carbon double bonds between 1610 and 1620 cm^{-1}. (4c) Lawsonia inermis extract, indicating hydroxyl groups at 3436 cm^{-1} and α and β-unsaturated carbonyl groups at 1636 cm^{-1}.**

Atomic Force Microscope (AFM) Analysis: The AFM images in Figure 5 depict the surface topography of samples a, b, and c. Sample a show a relatively smooth surface with minimal roughness, sample b exhibits moderate roughness with distinct textural features, and sample c displays the highest roughness with pronounced surface peaks and valleys (Figure 5).

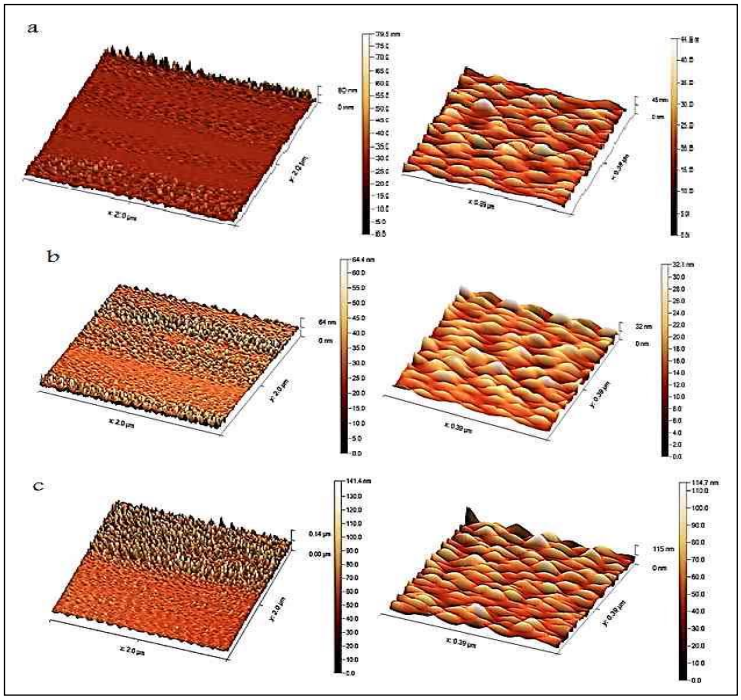

Figure 5. AFM of Nigella sativa(a), Hibiscus Sabdariffa (b), Lawsonia inermis (c)

Antibacterial activity: The antibacterial activity of extracts from various plants was determined using the Agar wells diffusion method against different bacteria. This method involves the following steps:

1. The bacteria were cultured in nutrient broth and compared with the McFarland tube at 37° C.
2. 0.1 ml of the previously cultured bacteria was taken and diffused on the surface of Nutrient agar, which was then left to dry at 37° C for 10 minutes.
3. After drying, 5mm wells were made in the agar plate.
4. The extracts were added to these wells and incubated at 37 degrees Celsius for 18 hours.
5. The antibacterial activity of the extracts was determined by the sample that produced a clear inhibition zone around the well.

The bacterial strains tested were both Gram-positive and Gram-negative. The results of the inhibition zones are shown in Table 1.

Our observations indicate that extracts derived from Nigella sativa seeds demonstrate considerable antibacterial efficacy against Escherichia coli, characterized by an inhibition zone measuring 20 mm in diameter. In contrast, the antibacterial activity against Staphylococcus and Klebsiella species is notably less pronounced, with inhibition zones of 11 mm. Additionally, these extracts show effectiveness against various other bacterial strains. Regarding extracts from the Roselle plant, they reveal significant activity against Candida albicans, with an inhibition zone of 11 mm, and against Staphylococcus aureus, which has an inhibition zone of 13 mm. Conversely, the leaves of Lawsonia inermis (Henna) display minimal antibacterial activity across all bacterial strains tested. Figure 8 illustrates the biological activity against different bacterial types (figure 6).

Table1. Antibacterial activity (Inhibition zones mm) of the plants

Bacteria	N. sativa (1)	Henna (2)	Roselle (3)
Genus: Staphylococcus Species: aureus (a)	No inhibition zone	No inhibition zone	13
Genus: Staphylococcus Species: epidermidis (b)	11	No inhibition zone	12
Genus: Escherichia Species: coli (c)	20	No inhibition zone	No inhibition zone
Genus: Klebsiella Species: sp. (d)	11	No inhibition zone	12
Genus: Candida Species: albicans (e)	No inhibition zone	No inhibition zone	11

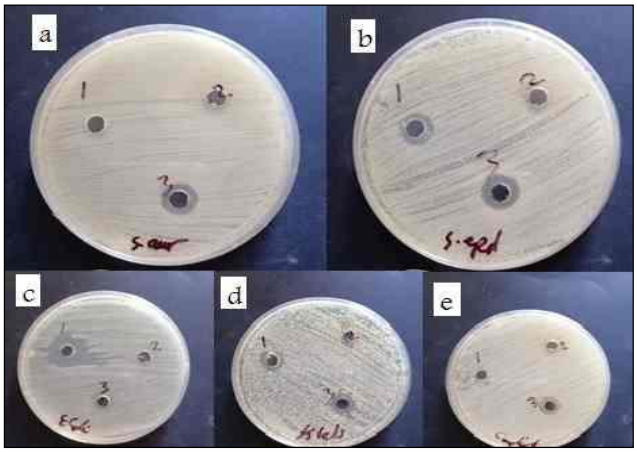

Figure 6. **Antibacterial effect of plant extracts**

In the same context, it has been found that the extract of Nigella possesses high antibacterial activity against E. coli. As a result, we diluted the concentration three times. When the concentration was at 25%, the inhibition zone increased to 22 mm. Similarly, when the concentration was at 50%, the inhibition zone increased to 31 mm. However, when the dilution was at 75%, there was no activity observed, as depicted in Figure 7, which is consistent with prior research (figure 7).

Figure 7. **The antibacterial of N. sativa for different concentrations**

Discussion

The present study investigated the structural, optical, and morphological properties of Nigella sativa, Hibiscus sabdariffa, and Lawsonia inermis extracts using UV-Vis spectroscopy, FTIR spectroscopy, and Atomic Force Microscopy (AFM). The UV-Vis spectra revealed significant UV absorption, with Nigella sativa showing the highest absorbance at 210 nm, Hibiscus sabdariffa displaying characteristic peaks at 212 nm and 284 nm, and Lawsonia inermis exhibiting weak absorption bands at 208 nm and 268 nm. FTIR analysis identified various functional groups, with Nigella sativa showing strong O-H stretching at 3436 cm^{-1}, Hibiscus sabdariffa displaying anthocyanins and unsaturated compounds, and Lawsonia inermis indicating hydroxyl and carbonyl groups. AFM analysis revealed that Lawsonia inermis had the highest surface roughness, followed by Hibiscus sabdariffa and Nigella sativa. The antibacterial activity, assessed using the agar well diffusion method, demonstrated that Nigella sativa extracts were most effective against Escherichia coli with a 20 mm inhibition zone, while Hibiscus sabdariffa showed significant activity against Candida albicans and Staphylococcus aureus. In contrast, Lawsonia inermis exhibited minimal antibacterial activity. Additionally, dilution experiments indicated that the antibacterial efficacy of Nigella sativa extracts against E. coli increased with concentration, with maximum inhibition observed at 50%.

These findings align with previous research which identified significant UV-Vis absorption due to anthocyanins and chlorogenic acid. UV-Vis measurements were utilized to monitor anthocyanin degradation, identifying both the initial polyphenols and their degradation products over time (9). Additionally, the UV-Vis spectra of Hibiscus sabdariffa extracts have shown stable absorption in the visible region, making it a suitable photosensitizer for dye-sensitized solar cells (10).

In our study, Lawsonia inermis showed weaker absorption bands at 208 nm and 268 nm, attributed to π-π^* and n-π^* electron transitions in benzene and quinone structures. This observation is supported by research highlighting the use of Lawsonia inermis extracts for assessing the viability of protoscolices from hydatid cysts, where UV-Vis spectroscopy confirmed the presence of lawsone, a red-orange pigment (11).

FTIR spectra of Hibiscus sabdariffa exhibit prominent peaks corresponding to hydroxyl (-OH), carbonyl (C=O), and aromatic C=C bonds. Biofabricated Fe nanoparticles using Hibiscus sabdariffa extract displayed -OH and C=O groups from polyphenols like anthocyanins (12), while other studies confirmed amine and hydroxyl groups for use as photosensitizers in dye-sensitized solar cells (13). Additionally, OCH and C-OH groups from sambubioside anthocyanins were identified (14). FTIR spectra of Nigella sativa show peaks for carbonyl (C=O) and hydroxyl (-OH) groups, reflecting its bioactive compounds like thymoquinone. These spectra revealed modifications linked to DNA damage and membrane status, crucial for anti-apoptotic properties (15). FTIR was also used to authenticate Nigella sativa seed oil, highlighting functional groups and seed oil purity (16). Lawsonia inermis (Henna) FTIR spectra exhibit peaks for quinone structures and benzene rings, indicating carbonyl (C=O) and hydroxyl (-OH) groups associated with lawsone, the primary dye agent. These findings support its use as a natural dye (17).

The antibacterial activity observed in our study aligns with previous research. Hibiscus sabdariffa showed notable inhibition of Staphylococcus aureus and Escherichia coli, consistent with studies in which methanolic extracts demonstrated strong antibacterial effects (18). Nigella sativa extracts, effective against Enterococcus fecalis and Pseudomonas aeruginosa, corroborate our findings on its broad-spectrum antibacterial properties (18). Similarly, Lawsonia inermis exhibited significant antibacterial activity, particularly in alcoholic extracts, supporting our results on its effectiveness against various pathogens (19). This comparison highlights the consistency of our findings with existing research on the antibacterial efficacy of these natural dyes.

Despite these promising results, our study faced several limitations. The variability in extraction methods could affect the consistency of antibacterial activity. While in vitro results are promising, in vivo studies are necessary to confirm the efficacy and safety of these natural dyes in practical applications. Lastly, the specific mechanisms of antibacterial action remain unclear and warrant further investigation to understand how these natural compounds interact with bacterial cells. Overall, our findings contribute to the growing body of evidence supporting the use of natural dyes as effective antibacterial agents and pave the way for further research to optimize their application in clinical and industrial settings.

Acknowledgment

This study was supported by Mustansiriyah University, Baghdad, Iraq.

References

1.Yadav S, Tiwari KS, Gupta C, Tiwari MK, Khan A, Sonkar SP. A brief review on natural dyes, pigments: Recent advances and future perspectives. Results Chem. 2023;5:100733.
2.Mahanty S, Rathinasamy K. The natural anthraquinone dye purpurin exerts antibacterial activity by perturbing the FtsZ assembly. Bioorg Med Chem. 2021;50:116463.
3.Syaifudin A, Sutrisno S. Antibacterial Activity and Potential Of Natural Textile Dyes From Sea Water Bacteria. Al-Hayat: J Biol Appl Biol. 2022;5(1):33-46.
4.Ansari TN, Iqbal S. Antibacterial efficiency of naturally occurring dyes and mordants. Proc Indian Natl Sci Acad. 2021;87:408-19.
5.Pal A. Chemical investigation on selected indigenous plants for natural dyes and dyeing characteristics [PhD Thesis]. Dehradun, Uttarakhand, India: Forest Research Institute (Deemed) University; 2017.
6.Ahmadi Z, Gholami Houjeghan F. Assessment of Antibacterial, Antimicrobial, and Colorimetric Properties of Cotton and Woolen Yarns Dyed with Some Plants Extracts. Text Leather Rev. 2022;5:463-83.
7.Sk S, Mia R, Haque MA, Shamim AM. Review on extraction and application of natural dyes. Text Leather Rev. 2021;4(4):218-33.
8.Faris BS, Abd AN, Hamza BH. Synthesis of Tin Oxide Nanoparticles by Celery Leaves Extract for Antibacterial Activity. Iraq J Appl Phys. 2024;20(38):638-42.
9.Achir N, Sinela A, Mertz C, Fulcrand H, Dornier M. Monitoring anthocyanin degradation in Hibiscus sabdariffa extracts with multi-curve resolution on spectral measurement during storage. Food Chem. 2019;271:536-42.
10.Willoughby AA, Soge AA, Dairo OF, Olukanni OD, Durugbo EU, Michael WS, et al. Fabrication and Characterization of a Dye-Sensitized Solar Cell using Natural Dye Extract of Rosella (Hibiscus sabdariffa L.) as Photosensitizer. J Niger Soc Phys Sci. 2021;3(4):287-91.
11.Elowni EE, Ahmad MF, Abdelnabi GH, Badawi RM. Potential Application of Hibiscus sabdariffa L. (Malvaceae) Aqueous Extract for Assessment of Viability of Protoscolices from Hydatid Cysts. Open Access Library J. 2020;7(6):e6398.
12.Alshehri A, Malik MA, Khan Z, Al-Thabaitia SA, Hasanb N. Biofabrication of Fe nanoparticles in aqueous extract of Hibiscus sabdariffa with enhanced photocatalytic activities. RSC Adv. 2017;7:25149-59.
13.Pathak C, Surana K, Shukla VK, Singh PK. Fabrication and characterization of dye sensitized solar cell using natural dyes. Mater Today Proc. 2019;12(3):665-70.
14.Rojas-Valencia OG, Regules-Carrasco M, Hernández-Fuentes J, Reza-San Germán CM, Estrada-Flores M, Villagarcía-Chávez E. Synthesis of blue emissive carbon quantum dots from Hibiscus Sabdariffa flower: Surface functionalization analysis by FT-IR spectroscopy. Materialia. 2021;19:101182.
15.Mihoubi W, Sahli E, Gargouri A, Amiel C. FTIR spectroscopy of whole cells for the monitoring of yeast apoptosis mediated by p53 over-expression and its suppression by Nigella sativa extracts. PLoS One. 2017;12(7):e0180680.
16.Nurrulhidayah AF, Man YC, Al-Kahtani HA, Rohman A. Application of FTIR spectroscopy coupled with chemometrics for authentication of Nigella sativa seed oil. J Spectrosc. 2011;25(5):243-50.
17.Priyadharsini P, David ST. Natural dyes and their FT-IR spectroscopy studies. Int J Appl Bioeng. 2014;8(2):16.
18.Al-fawwaz AT, Al-Khaza'leh KA. Antibacterial and antifungal effect of some natural extracts and their potential use as photosensitizers. Eur Sci J. 2016;12(147):10-9044.
19.Rimawi MA, Masri MA, Husein N, Al-Hinnawi AN, Masimi OA, Sabrah L. Natural antimicrobial activity of Lawsonia inermis and Indigo tinctoria against clinically isolated microorganisms. Int J Pharm Pharm Sci. 2018;10(1):191.