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Antimicrobial Activity of Natural and Synthetic Dyes Used in Leather Dyeing: A Comparative Study

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Article

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ABSTRACT

Both natural and synthetic dyes have gained increasing attention in the leather dyeing process due to environmental and health considerations. This research was designed to determine and compare the antimicrobial efficacy of four natural dyes (Acacia catechu, Trema orientalis, Camellia sinensis, Swietenia macrophylla) with four synthetic dyes (brown BFR, brown NG, brown EDK, red G) to develop safer leather dyeing process. The above eight dyes were evaluated against eight prevalent pathogenic and opportunistic pathogenic bacteria Bacillus megaterium, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Salmonella sp., Escherichia coli, Shigella sp., Pseudomonas aeruginosa, and four fungi. Dyestuff from Acacia catechu, Trema orientalis, and Swietenia macrophylla was effective against the three Bacillus strains, and Staphylococcus aureus with an inhibition zone extending from 6.3 mm to 11 mm. Synthetic dye brown BFR was effective against the three Bacillus strains, and Staphylococcus aureus with zone diameter of 14.8 mm to 19.7 mm. Brown EDK and Red G showed activity against Bacillus megaterium and Bacillus cereus. Camellia sinensis and brown NG showed no antimicrobial efficacy. Also, all eight dyes showed no antifungal activity. For both types of dyes, the zone of inhibition increased approximately linearly as dye concentrations increased. Of the natural dyes, Acacia catechu showed the highest activity. Brown BFR showed the maximum zone diameter of the eight dyes tested. The results indicated that both natural and synthetic dyes were quite effective. Synthetic dyes had remarkable antibacterial properties compared to natural dyes. These dyes having antimicrobial activity can be used in a safer leather dyeing process.

KEYWORDS

natural dyes, synthetic dyes, antimicrobial activity, leather dyeing

INTRODUCTION

Plants are employed to produce various products including natural dyes which are utilized as colouring agents in the cosmetics, ink, leather, and textile industries [1]. In 1856, the discovery of a synthetic colouring substance known as ‘mauveine’ by a young English chemist William Perkins reduced the use of natural dyes which were mostly used in the leather industries until the mid of 19th century [2].

Typically, pathogenic and opportunistic pathogenic bacteria and fungi such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans* have been found on leathers, textiles, and many useable things [3]. In the leather industry, the activity of these microorganisms causes a devaluation of footwear and clothing products and also causes product deterioration in storage [4,5]. There is also a report of colonization of finished leather products such as footwear by bacteria and fungi which causes allergic reactions, skin lesions, and infections in immune-compromised people [6,7]. Therefore, leather products having antimicrobial activity is an important concern in the leather industry.

Antimicrobial agents are natural or synthetic substances that intercept the amplification of microorganisms by hindering the synthesis of lipids, proteins, or enzymes and killing the microbes by breaking the cell wall. Natural dyes are biodegradable, and also have antimicrobial properties [8].

Although used for centuries for both decorative and therapeutic purposes, the structures and protective qualities of natural dyes have only recently come to light [9]. Glycosides, tannins, flavonoids, phenols, saponins, and terpenoids are the phytochemicals that are said to be in charge of bacterial resistance [10]. The tannins, terpenes, and phytol of cutch (*Acacia catechu*) stop bacterial metabolism by deactivating functional cell protein and are active against *B. subtilis*, and *S. Aureus* [11,12]. Dyestuff from plants having antimicrobial effects extends the durability of the products [9]. Synthetic dyes are based on compounds such as quaternary ammonium salts, organosilicons, phenols, and organometallics [13]. Natural dyes have recently gained popularity over synthetic colourants among eco-conscious, health-conscious, and nature lovers [14].

The majority of past studies have documented the effectiveness of natural dyes in combating harmful microorganisms. However, no research has been done to date to compare the antimicrobial activity of natural and synthetic dyes. The goal of the present inquiry was to ascertain and compare the antimicrobial activities of natural and synthetic dyes against common pathogenic microorganisms which will enable the researcher and industrialists to gather a knowhow about the degree of antimicrobial efficacy of natural and synthetic dyes that are currently used in leather dyeing process.

EXPERIMENTAL

Sources of Natural and Synthetic Dyes

The natural dye powders of *Acacia catechu, Trema orientalis, Camellia sinensis, and Swietenia macrophylla*, and synthetic dye powders of brown BFR, brown NG, brown EDK, and red G were collected from Leather Research Institute, BCSIR, Nayarhat, Savar, Dhaka, Bangladesh. The botanic and native names, utilized portion, and the probable colours of the dyes utilized in this experiment were represented in Tables 1 and 2.

Table 1. Name of plants of natural dyestuff used in this research

Botanic name	Native name	Utilized portion	Probable colours
Acacia catechu	Cutch	Wood	Brown
Tremaorientalis	Indian Nettle/Nalita	Bark	Brown
Camellia sinensis	Tea	Used Tea extract	Brown
Swieteniamacrophylla	Mahogany	Seed pod	Tan

Table 2. Synthetic dyes used in this research (JAXACOR, Taiwan)

Dye name	Probable colours
Brown BFR	Reddish brown
Brown NG	Brown
Brown EDK	Dark brown
Red G	Red

Test Organisms and Culture Conditions

Cultures of eight bacteria (four Gram-positive and four Gram-negative) *Bacillus megaterium, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Salmonella sp., Escherichia coli, Shigella sp.,* and *Pseudomonas aeruginosa* and four fungi: *Aspergillus niger, Aspergillus fumigatus, Penicillium notatum,* and *Candida albicans* were used in the study. These microorganisms were collected from Leather Research Institute, BCSIR, Nayarhat, Savar, Dhaka, Bangladesh.

The organisms were preserved on nutrient agar (Himedia) and sabouraud dextrose agar (Himedia) at 4 °C. Stock cultures of bacteria and fungi were inoculated into 5 ml of nutrient broth and sabouraud dextrose broth to stimulate growth and incubated for 24 h at 37 °C and 30 °C, respectively.

Antimicrobial susceptibility test

The sensitivity of the microbial strains to the natural and synthetic dyes was searched using the well-diffusion method. 0.45 µm membrane filters were used to sterilize the dyestuff. The bacteria and fungi were inoculated into 10 ml nutrient broth and sabouraud dextrose broth and incubated for 24 h at 37 °C and 30 °C, respectively.

Equivalent layers of nutrient agar and sabouraud dextrose agar were made on sterilized Petri dishes. These agar plates were inoculated with standardized inoculums (10^8 cfu/ml) of each bacterial and fungal strain (2.1×10^3 cfu/ml) [15]. The wells on the agar plates were prepared using a cork borer of 5 mm in diameter. 100 µl of dye solution was loaded into each well. The plates were then incubated at 37 °C (bacteria) and 30 °C (fungi) for 24 h. After incubation, the inhibition zone diameter formed on the medium was measured in mm. Since distilled water (dH_2O) was utilized as an extraction solvent, it was used as a solvent control. Tetracycline antibiotic disc was used as a positive control. Triplicates of each experiment were conducted. The MIC (Minimum Inhibitory Concentration) values of the dye extracts that demonstrated antibacterial action were further examined.

Determination of Minimum Inhibitory Concentration (MIC) of the dyes

In the next set of experiments, the impact of dye concentration (0.1-6% w/v) on the antibacterial efficacy of a subset of dyes having antimicrobial effectiveness was evaluated. The smallest amount of dyestuff that prevents each microbe from growing visibly was established to be the MIC. Initially, eight concentrations (1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0% w/v) for natural dye and ten concentrations for synthetic dyes (0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0% w/v) were used.

Statistical analysis

All data were taken triplicate and presented as mean ± SE. Analysis of Variance (ANOVA) for different groups was executed utilizing the SPSS vs 25.0. To detect the significant differences ($P< 0.05$) between means, the Tukey HSD test was performed [16]. All graphs were prepared using GraphPad Prism 10.0.1.

RESULTS AND DISCUSSION

Antimicrobial activity of natural and synthetic dyes

The antimicrobial activity of four natural dyes against test organisms were given in Table 3. The data were presented as mean±SE. Of the 12 bacteria and fungi tested, natural dyestuff from *Acacia catechu, Trema orientalis*, and *Swietenia macrophylla* showed inhibition zone against *Bacillus megaterium, Bacillus cereus, Bacillus subtilis,* and *Staphylococcus aureus* but was not effective against *Shigella sp., Escherichia coli, Salmonella sp., Pseudomonas aeruginosa* and also these dyes showed no antifungal activity as indicated in table 3 [11]. The zone diameter ranges from 6.3 mm to 11.0 mm. Dyestuff of *Swietenia macrophylla* showed activity to *Bacillus subtilis* and *Acacia catechu* displayed activity against some gram-positive bacteria which is similar to the present findings [17]. Similar results were found in this study as *Acacia catechu* showed a small zone of inhibition of about 6.3 mm against *Staphylococcus aureus* but good activity to *Bacillus subtilis, Bacillus megaterium*, and *Bacillus cereus* with zone diameter of 11.0 mm, 11.0 mm, and 10.3 mm respectively [11,12]. However, no zone of inhibition was observed against any of the bacteria and fungi tested in the case of dyestuff from *Camellia sinensis*.

Table 3. The antimicrobial efficacy of natural dyes against test organisms (inhibition zone, mm)

Test Organisms	Dye			
	Acacia catechu	*Trema orientalis*	*Camellia sinensis*	*Swietenia macrophylla*
Bacillus subtilis	11.0±0.6^{c}	9.0±0.0^{c}	-	7.7±0.3^{c}
Aspergillus fumigatus	-	-	-	-
Escherichia coli	-	-	-	-
Bacillus cereus	10.3±0.7^{c}	8.3±0.3^{c}	-	8.3±0.3^{c}
Pseudomonas aeruginosa	-	-	-	-
Aspergillus niger	-	-	-	-
Bacillus megaterium	11.0±0.6^{c}	9.7±0.3^{c}	-	9.7±0.3^{c}
Penicilliumnotatum	-	-	-	-
Salmonella sp.	-	-	-	-
Staphylococcus aureus	6.3±0.3^{c}	8.0±0.0^{c}	-	10.0±1.0^{c}
Candida albicans	-	-	-	-
Shigella sp.	-	-	-	-

The data are expressed as mean±SE and analyzed by ANOVA and Tukey HSD test. Means with different letters represent significant differences at P<0.05.

The antimicrobial activity of four synthetic dyes against test organisms was given in Table 4. The data were presented as mean±SE. Synthetic dye brown BFR showed activity against *Bacillus megaterium, Bacillus cereus, Bacillus subtilis,* and *Staphylococcus aureus* with zone diameters ranging from 14.8 mm to 19.7 mm but no zone of inhibition was observed against other bacteria and fungi tested. Brown EDK was effective against only *Bacillus subtilis, Bacillus cereus,* and *Bacillus megaterium* with zone diameters of 13.5 mm to 17.5 mm. Synthetic dye Red G was found to be active against *Bacillus megaterium* and showed little activity against *Bacillus cereus* of about 8.8 mm zone diameter. However, none of the dyes showed any antifungal activity.

Table 4. The antimicrobial activity of Synthetic dyes against test organisms (inhibition zone, mm)

Test Organisms	Dye			
	Brown BFR	Brown NG	Brown EDK	Red G
Bacillus subtilis	17.7±0.9^{a}	-	16.7±0.3^{b}	-
Aspergillus fumigatus	-	-	-	-
Escherichia coli	-	-	-	-
Bacillus cereus	18.0±1.0^{a}	-	13.5±0.8^{b}	8.8±0.6^{d}
Pseudomonas aeruginosa	-	-	-	-
Aspergillus niger	-	-	-	-
Bacillus megaterium	19.7±0.9^{a}	-	17.5±0.5^{b}	11.8±0.7^{d}
Penicilliumnotatum	-	-	-	-
Salmonella sp.	-	-	-	-

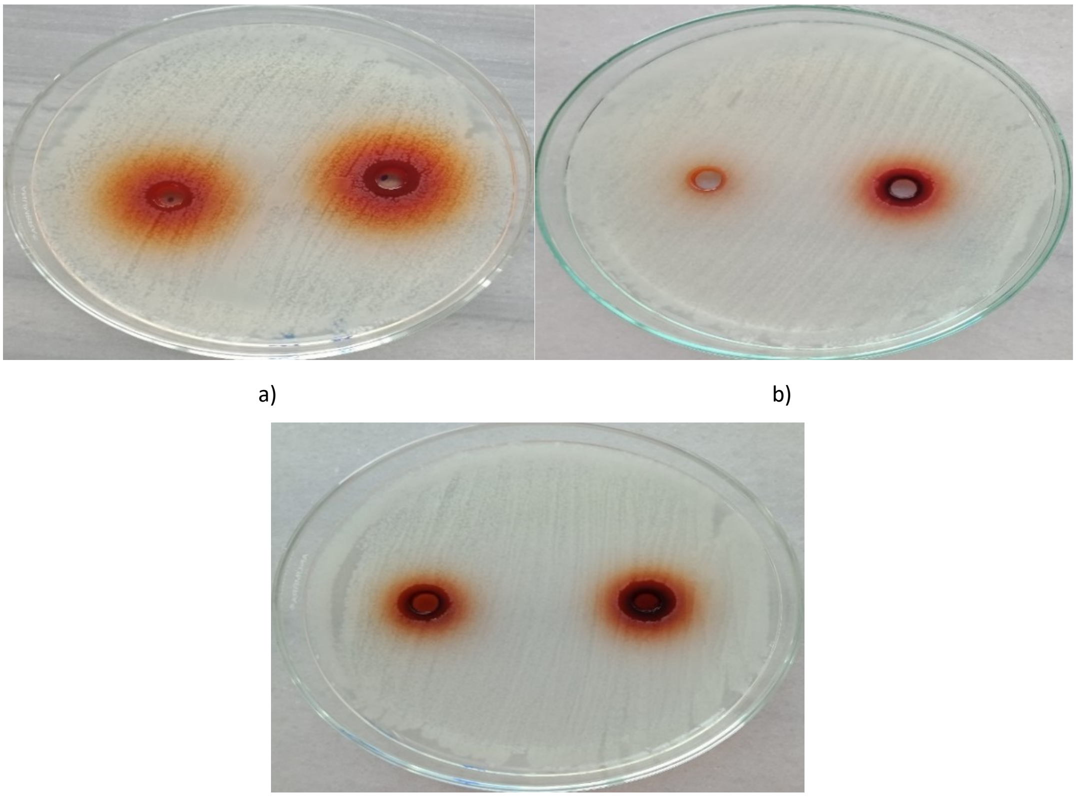
Test Organisms	Dye			
	Brown BFR	Brown NG	Brown EDK	Red G
Staphylococcus aureus	14.8±0.2[a]	-	-	-
Candida albicans	-	-	-	-
Shigella sp.	-	-	-	-

The data are expressed as mean±SE and analyzed by ANOVA and Tukey HSD test. Means with different letters represent significant differences at P<0.05.

From table 3 and 4, it can be seen that both the natural and synthetic dyes significantly inhibited the growth of the gram-positive bacteria but no inhibitory effect was noticed on the gram-negative test bacteria (*Salmonella sp., Escherichia coli, Pseudomonas aeruginosa, Shigella sp*.). This may be due to the presence of LPS (lipopolysaccharide) on the cell membrane of the gram-negative bacteria which may have prevented the accumulation of the dyes [18,19]. It was reported that the presence of catechin, phlorotannin, saponins, flavonoids, glycosides, phenols, terpenoids, and quercitrin in the *Acacia catechu* dye imparts the antimicrobial activity against gram-positive bacteria [20]. Epigallocatechin-3-gallate (EGCG) polyphenols of *Acacia catechu* inhibit bacterial growth by crosslinking with different proteins of the peptidoglycan layer of cell walls of bacteria and damaging crosslinked peptides, cytoplasmic lipids, and proteins [21]. All eight tested dyes didn’t show any antifungal activity. This may be because the dyes could not penetrate the chitin cell membrane of fungi. *Staphylococcus aureus* is a pathogenic microorganism and this can cause serious infection to humans [22,23]. The report documented that *Bacillus subtilis* and *Bacillus cereus* group were cytotoxic and *Bacillus megaterium* is responsible for soft tissue infection as well as pleura infection [24-26]. The inhibition of the growth of these microbes on leather products through the use of these dyes is advantageous [27].

Determination of MIC of the dyes

The effectiveness of natural dyestuff from *Acacia catechu, Trema orientalis*, and *Swietenia macrophylla* at different dye concentrations (3% and 6%) was depicted in Figure 1. There was an increase in activity at 6% of concentration.

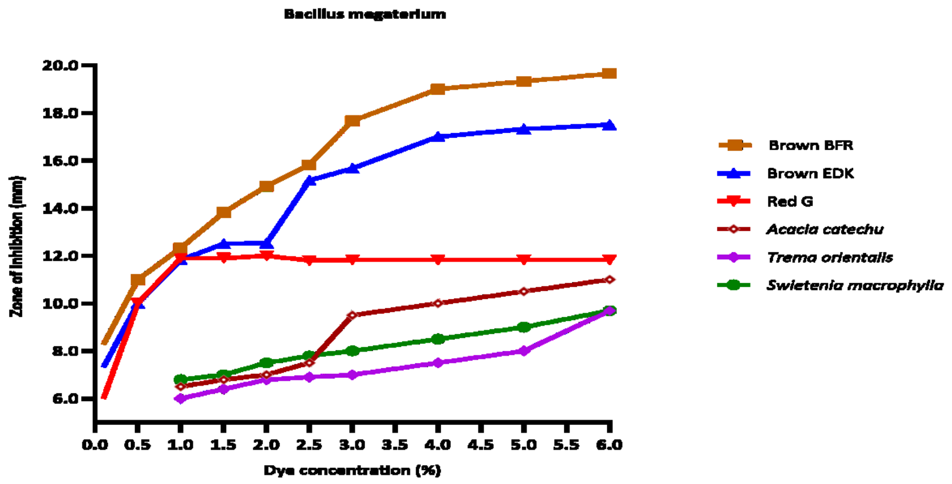


a) b)

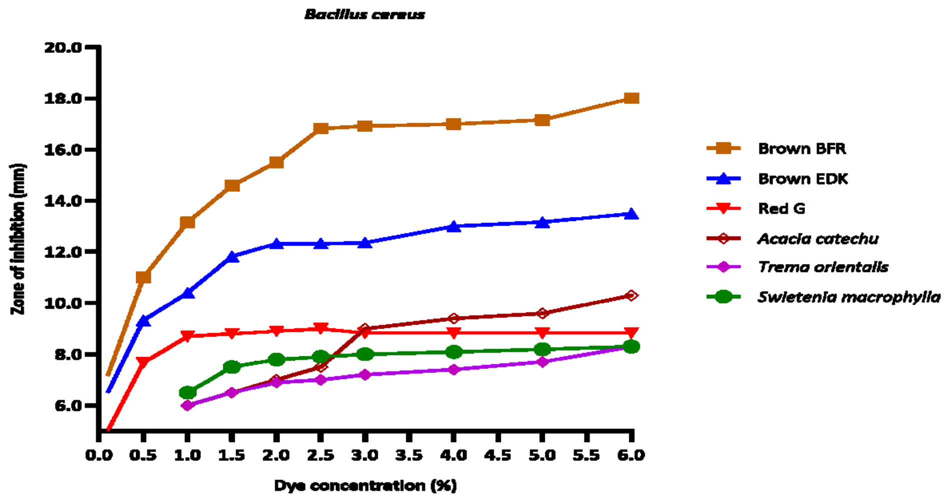
c)

Figure 1. Antibacterial efficacy of natural dyes (a) *Acacia catechu*;(b) *Trema orientalis*;(c) *Swietenia macrophylla* against *Bacillus megaterium* at different (A. 3%, B. 6%) dye concentrations

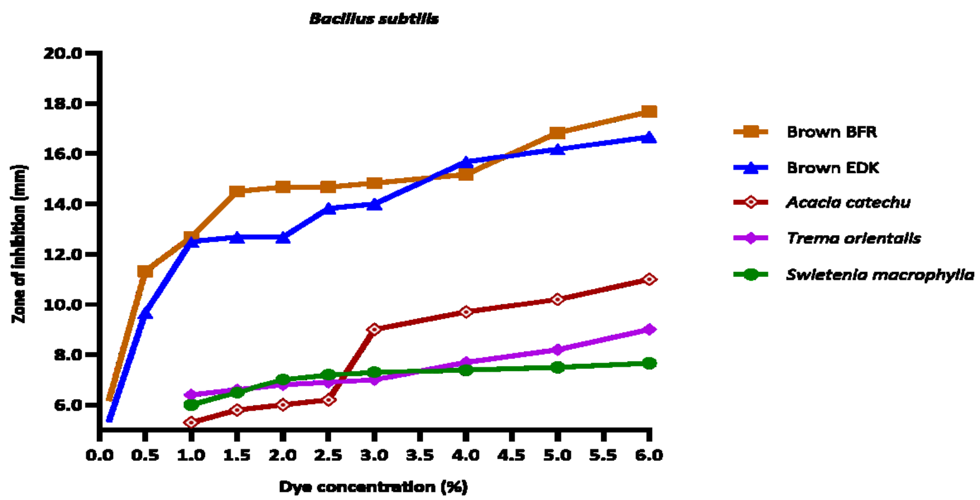
Figure 2 illustrates how dye concentration affects antibacterial action. The lowest dose of dye at which > 5 mm of zone diameter was detected was taken as the MIC of the dye against a particular microbe. It can be seen (Figure 2) that natural dyes *Acacia catechu*, *Trema orientalis,* and *Swietenia macrophylla* were completely effective against *Bacillus megaterium, Bacillus cereus, Bacillus subtilis,* and *Staphylococcus aureus* at the dye concentration of 1% and the antimicrobial activity of natural dye is also increasing linearly as the dye concentration increases. From Figures 2 and 3, it can be seen that synthetic dyes brown BFR, brown EDK and red G were effective at the dye concentration of 0.1% and the antimicrobial activity of brown BFR, and brown EDK is increasing linearly with an increase in dye concentration. In the case of *Bacillus megaterium* and *Bacillus cereus*, the activity of the synthetic dye red G increased up to 2-2.5% of concentration and after that no further increase in activity was observed. Red G, with an increase in dye concentration, may not be able to penetrate the bacterial cell wall completely like the other dyes. The dyes can be said to be very potent antibacterial substances as the MIC for natural dyes lies within 1% and the MIC of synthetic dyes lies in the region of 0.1%-0.5%. From Figure 2, it can also be seen that the natural and synthetic dyes showed better antibacterial activity from a concentration range of around 3% and 1%, respectively. So, using these dyes on leather from at least 3% (natural dyes) and 1% (synthetic dyes) to higher concentrations would give better antibacterial properties on leather. It is clear from the distinct zone of inhibition (Figures 1 and 3) that, instead of being bacteriostatic, the chosen dyes are bactericidal.



a)



b)



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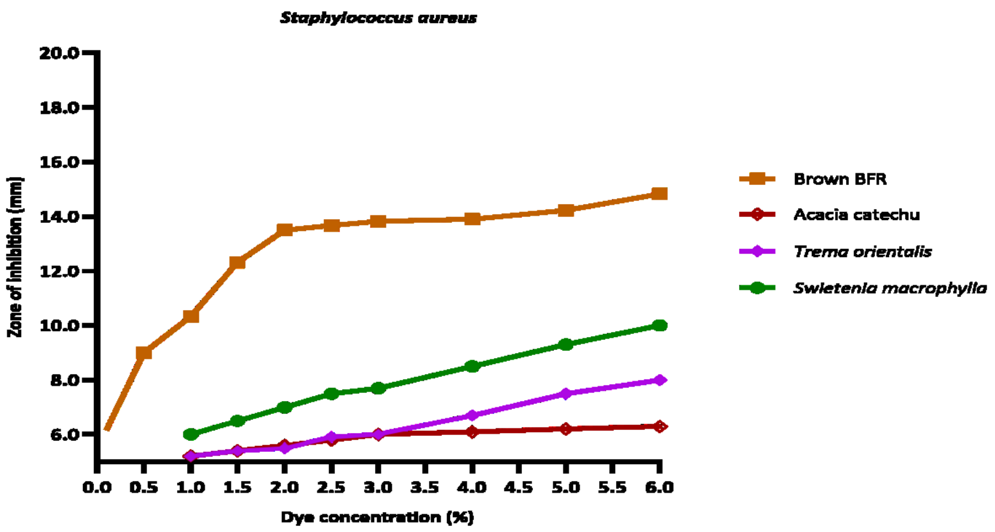
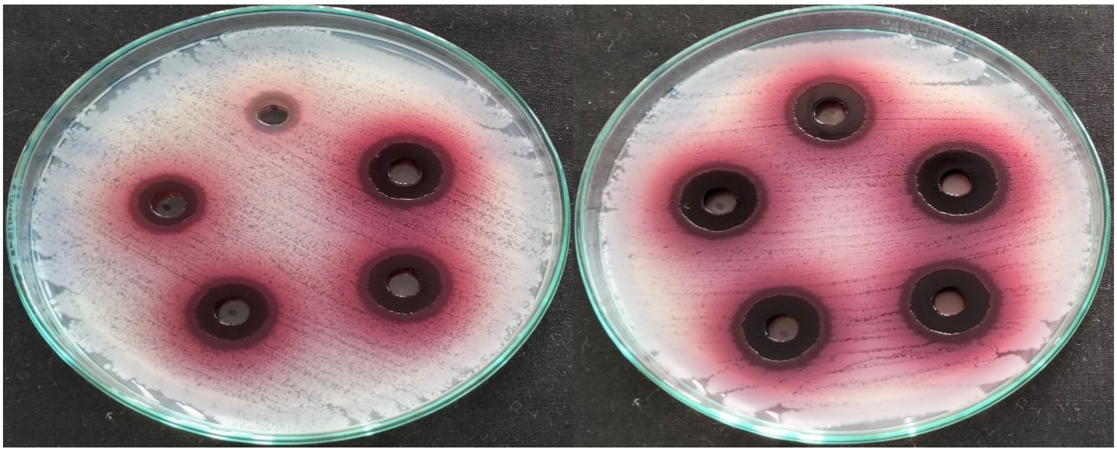
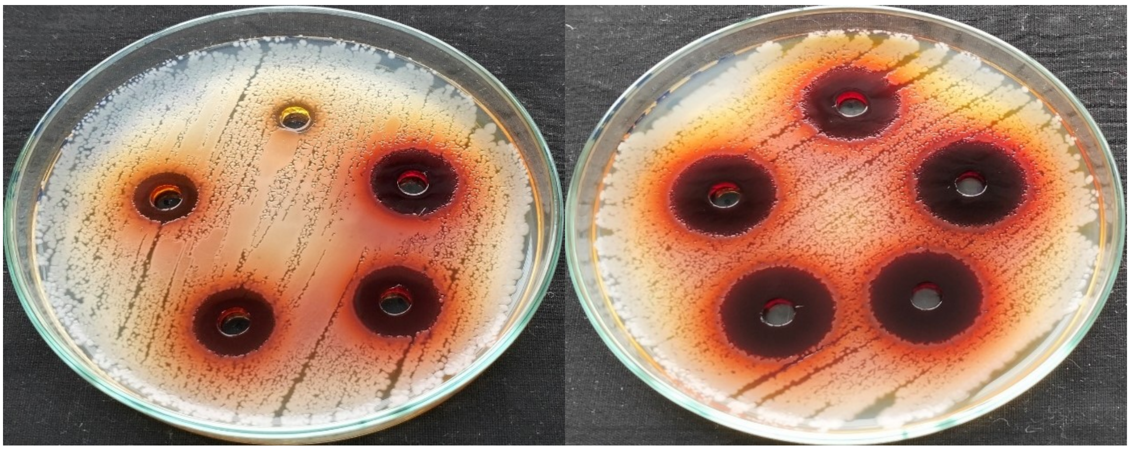


d)

Figure 2. Effect of natural dye (*Acacia catechu, Trema orientalis* and *Swietenia macrophyll*a) and synthetic dye (brown BFR, brown EDK and red G concentration on the activity of bacteria. (a) *Bacillus megaterium*; (b) *Bacillus cereus;* (c) *Bacillus subtilis*; (d) *Staphylococcus aureus*. The activity of all six dyes increased with an increase in dye concentration



a)



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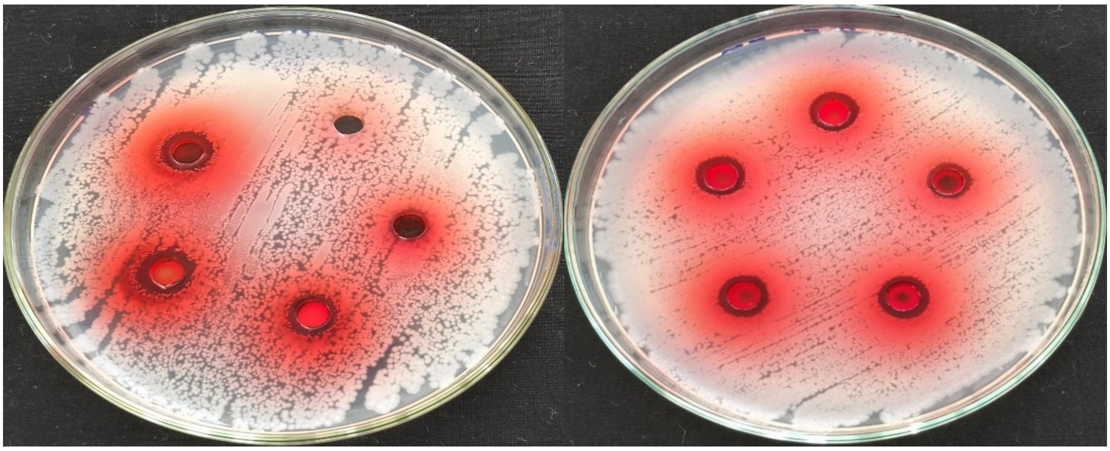

c)

Figure 3. Effect of three synthetic dyes concentration on the activity of the bacterium *Bacillus cereus*. (a) Brown BFR; (b) Brown EDK; (c) Red G (A: 0.1%, B: 0.5%, C: 1%, D: 1.5%, E: 2%, F:2.5%, G: 3%, H: 4%, I: 5%, J: 6% of dye concentration)

Figure 4 illustrates a comparison of the antimicrobial activity of natural and synthetic dyes having activity from the previous observation. From Figure 4, it can be seen that the Synthetic dye brown BFR showed the highest activity against the four bacteria (*Bacillus megaterium, Bacillus cereus, Bacillus subtilis, and Staphylococcus aureus*). Brown EDK also showed better activity compared to natural dyes against the three *Bacillus* strains. Of the natural dye, *Acacia catechu* dyestuff showed the highest activity against the three *Bacillus* strains but low activity against *Staphylococcus aureus*. Of the natural dyes, *Swietenia macrophylla* showed the highest activity against *Staphylococcus aureus*.

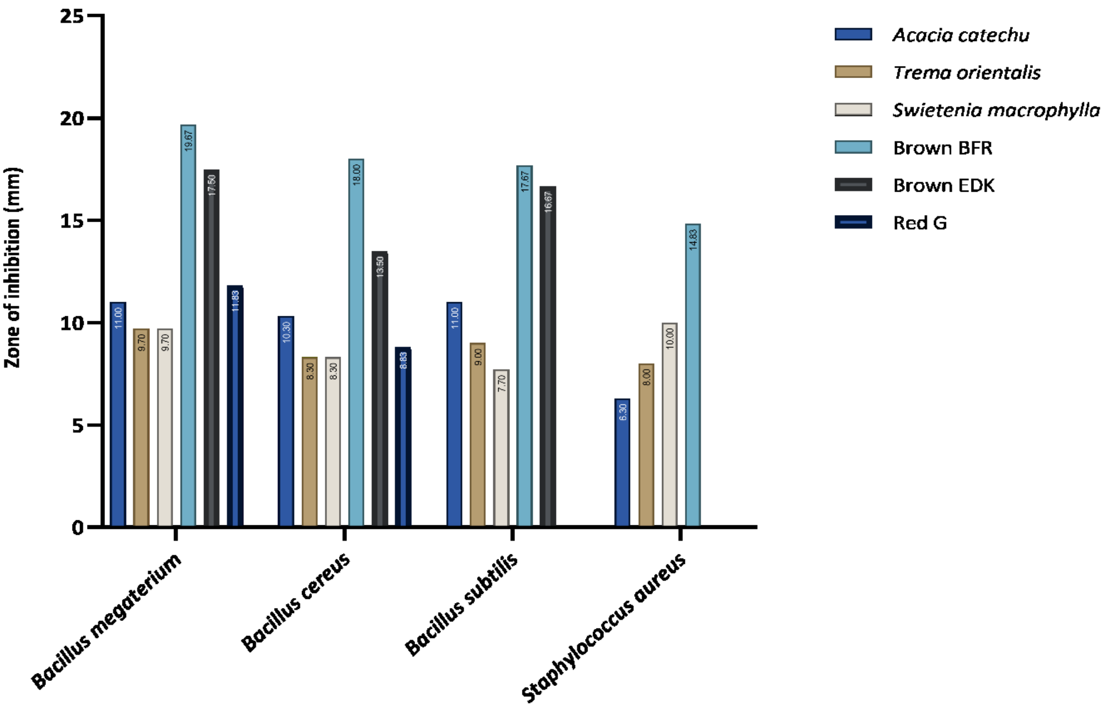

Figure 4. Comparison of antimicrobial activity of natural dyes *Acacia catechu, Trema orientalis, Swietenia macrophylla* and synthetic dyes brown BFR, brown EDK, red G against *Bacillus megaterium, Bacillus cereus, Bacillus subtilis*, and *Staphylococcus aureus*

Table 5 represents the antibacterial rate (%) of six natural and synthetic dyes against test organisms using the zone diameter of tetracycline antibiotic disc as control. The positive control tetracycline antibiotic disc showed an inhibition zone of 14 mm to *Bacillus cereus*, 17 mm to *Bacillus subtilis*, 33 mm to *Bacillus megaterium*, and 16 mm to *Staphylococcus aureus*. The antibacterial rates were calculated according to the formulae (1).

$$\frac{Inhibition\ zone\ diameter\ in\ treatment\ group}{Inhibition\ zone\ diameter\ in\ control\ group} \times 100\% \tag{1}$$

From Table 5, it can be seen that the synthetic dye Brown BFR showed the highest percentage of activity to the four gram-positive bacteria both in terms of antibacterial rate (59.7-128.6%) and spectrum of inhibition (number of bacteria inhibited) and the next highest antibacterial rate was exhibited by Brown EDK (53.0-98.2%). In terms of the spectrum of inhibition, the three natural dyes (*Acacia catechu, Trema orientalis,* and *Swietenia macrophylla*) showed good activity and the antibacterial rate ranges from 29.4 to 73.6%.

Table 5. The antimicrobial rate of six natural and Synthetic dyes against test organisms

Test Organisms	Inhibition rates (%)					
	Acacia catechu	*Trema orientalis*	*Swietenia macrophylla*	Brown BFR	Brown EDK	Red G
Bacillus subtilis	64.7	52.9	45.3	104.1	98.2	-
Bacillus cereus	73.6	59.3	59.3	128.6	96.4	62.9
Bacillus megaterium	33.3	29.4	29.4	59.7	53.0	35.8
Staphylococcus aureus	39.4	50.0	62.5	92.5	-	-

The activity of the same dye varies to diverse type of bacteria as seen in Table 4 and this demonstrates that the dye's chemistry and the structural components of different bacteria significantly affects its antibacterial action. Although both natural and synthetic dyes have antibacterial properties, their properties vary substantially. This is an intriguing finding and further research into how dye structure affects antibacterial properties is necessary.

This study comes with some limitations. The phytochemical and chemical analysis of natural and synthetic dyes is necessary to carry out to determine the dye structure, in particular the existence of functional groups on it, which is responsible for antimicrobial activity, to support the findings. Additionally, the antimicrobial activity of dyed leather of both dyes could have been determined to reach a robust conclusion on the subject matter.

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CONCLUSION

The antimicrobial activity of natural and synthetic dye samples against twelve common pathogenic microorganisms was determined and compared in this study. The results indicated that along with those of the natural dyes, synthetic dyes possessed good antibacterial properties. The antibacterial rate and activity of synthetic dyes were better compared to natural dyes. Different types of pathogenic and opportunistic pathogenic microorganisms on leather products cause product deterioration as well as allergic reactions, and infections in immune-compromised people. As these dyes possess antibacterial activity, the use of these dyes in the leather industry can impart microbial resistance to leather products and can prevent problems related to leather goods.

Author Contributions

Conceptualization – Parveen S; methodology – Akter T and Sarker SS; formal analysis – Akter T and Sarker SS; investigation – Akter T; resources – Parveen S and Sujan SMA; writing-original draft preparation – Akter T; writing-review and editing – Parveen S, Akter T and Sujan SMA; visualization – Parveen S and Akter T; supervision – Parveen S. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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