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Evaluation of Berberine Chloride as a New Antibacterial Agent Against Gram-Positive Bacteria for Medical Textiles

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Abstract
Berberine, an isoquinoline alkaloid, is reported to possess excellent antibacterial properties. Berberine is found in the roots of Coptis chinensis and the stems of phellodendron. In the current study, the efficacy of berberine chloride as a new antibacterial agent on textile substrates against Staphylococcus aureus and Enterococcus faecalis was investigated. In particular, the relationship between bacterial inhibition and the concentration of berberine chloride needed to exhibit effective bacterial action was studied. Results showed berberine chloride to be an effective antibacterial agent at a minimum inhibitory concentration of 0.2% in the solution. On textile substrates such as 100% polyester, 100% nylon and 50% cotton-50% polyester blend, there was a 62-76% reduction in bacterial counts. The effectiveness of antibacterial action was retained after laundering and exposure to light.

Key words: *berberine, textile substrates, antibacterial agent, Staphylococcus aureus, Enterococcus faecalis.*

Introduction

Nosocomial or hospital-acquired infections occur in at least 5% of hospitalised patients [1]. According to 2007 data from the U.S. Centers for Disease Control, nosocomial infections account for an estimated 1.7 million infections and 99,000 associated deaths each year [2]. Among the causes of death, nosocomial infections rank fourth, preceded only by heart disease, cancer and strokes [3]. Hospital-acquired infections add an estimated $4.5 to $5.7 billion per year to the cost of patient care [4]. *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* were reported to be among the most frequent microorganisms responsible for nosocomial outbreaks [5]. Nosocomial infections are a result of the transmission of pathogens and bacteria from patients to healthcare workers and vice-versa. The garments of healthcare workers are an important aspect of the environment that can easily become contaminated. For example, staphylococci are spread by the movement of contaminated articles, such as bedclothes, bed curtains, and the protective clothing of nurses. A hospital outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) was also directly linked to a stretcher and hand-held shower [6]. Additionally, it was reported that about one-half of all surgical procedures resulted in an accident where at least one medical worker was contaminated with blood [7]. A widespread contamination of enterococci has also been found in the wet sites of hospitals and is commonly considered to spread by person to person contact.

Of late, synthetic fibres and their blends with natural fibres are increasingly being used in hospitals. It has been proven that bacteria can grow and survive on these fabrics for more than ninety days, contributing to the transmission of diseases [8]. Although it has been found that protective clothing, such as surgical gowns, scrub suits and lab coats reduces the risk of exposure by acting as a barrier to infectious agents, they are still inadequate in preventing the transmission of disease itself [9, 10]. A better level of protection is obtained by using antibacterial finished textile substrates [11]. In this regard, even though a wide variety of antibacterial agents are available, the search for new antibacterial agents with a positive bias towards the environment continues at a rapid pace. One potential source of novel antibacterial agents is plants. Plants are known to produce secondary metabolites for protecting themselves against microbial attack, usually by killing or resisting the microbes [12]. Among the various plant-derived compounds with antibacterial activity, berberine chloride is noteworthy because it is thought to be active against the methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant enterococci (VRE) [13].

The purpose of this research was to evaluate berberine chloride as an antibacterial agent on textile substrates against *Staphylococcus aureus* and *Enterococcus faecalis*. The durability of the antibacterial activity of the treated substrates against laundering and light exposure was also examined. The justification for the use of berberine chloride is that organic compounds containing a quaternary ammonium salt structure strongly show antibacterial functions. As shown in ***Figure 1***, berberine contains a positively charged nitrogen atom in its chemical structure. The structure of the quaternary ammonium salt in berberine molecules potentially destroys the cell membrane of bacteria. The positive charges in berberine molecules could destroy the negatively charged cell membrane of bacteria due to disturbing the charge balances of the cell membrane [14]. Therefore, berberine treatments can be utilised in the functional finishing process for antibacterial properties.

Experimental

Materials

Substrates

Polyester, nylon and a 50% polyester-50% cotton blend were the substrates used for this study, selected due to their wide use in medical environments. The substrates were characterised with re-

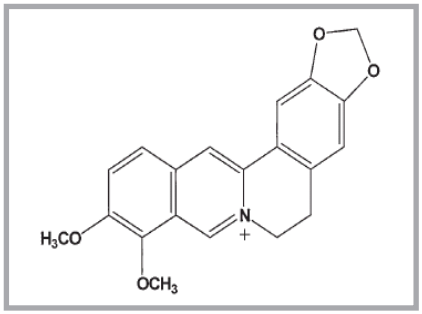

Figure 1. *Structure of berberine.*

Table 1. Fabric characteristics.

Substrate	Weight, g/m^2	Thickness, mm	Construction
Polyester	335	0.813	Twill Weave
Nylon	73	0.280	Knit Tricot
50%Polyester-50%Cotton Blend	140	0.305	Plain Weave

spect to their weight and thickness, the data of which is shown in ***Table 1***.

Berberine chloride

Berberine chloride was obtained in powder form from Sigma-Aldrich Co., USA.

Test organisms

Staphylococcus aureus (ATCC® 6538, MicroBioLogics) and *Enterococcus faecalis* (ATCC® 51299, MicroBioLogics) were the two microorganisms used in this study. They are among the two most common bacterial isolates found in hospital environments and have been identified as the major cause of cross infections among patients. Both the organisms are gram-positive bacteria. Standard microbiological procedures were employed to maintain cultures of the bacteria in a laboratory.

Methods

Primary screening

The agar disc diffusion assay method was used for primary screening of antibacterial activity and also to determine the Minimum Inhibitory Concentration (MIC) of the agent required to exhibit antibacterial activity. A solid culture medium was prepared using nutrient agar poured into petri dishes and inoculated with the bacteria. Six hundred µL of 0.1, 0.2, 0.3 and 0.4% antibacterial agent concentrations were pipetted onto sterilised Whatman filter paper discs and placed on individual agar plates. The plates were then incubated for 24 hours at 37 °C in an incubator. At the end of this period, the plates were examined for bacterial growth, and the size of the inhibition zone around each disc was measured.

Berberine chloride application

Representative samples from the three substrates were treated with berberine chloride in an Ahiba Nuance ECO-B infrared machine. The samples were introduced to treatment beakers at room temperature at a material to liquor ratio of 1:10. The concentration of berberine chloride was 10%, 20% and 30%, on weight of fabric (owf). The temperature was gradually raised to 130 °C, and the treatment continued for 90 minutes. The high pressure-high temperature method was used to ensure the exhaustion of berberine chloride onto the synthetic and blend fabric substrates. The samples treated were then rinsed in deionised water and air dried. Untreated samples of the substrates were used as control.

Determination of the antibacterial activity of the treated fabrics

Two methods were used to test for antibacterial activity. In the first method, evaluation of the treated fabrics was made by AATCC Test Method 147: Antibacterial Activity of Textile Materials: Parallel Streak Method. In this method, five streaks of the microorganisms were inoculated on nutrient agar plates. Fabric samples were placed in intimate contact with the bacteria inoculated agar. The plates were incubated and then observed for the presence of a clear area of interrupted growth underneath and adjacent to the test fabric, which gives an indication of the antibacterial activity of the fabric. The zone of inhibition of the samples was calculated using ***Equation 1***:

$$W = \frac{(T - D)}{2} \tag{1}$$

where:

W - width of the clear zone of inhibition in mm,

T - total diameter of the test specimen and clear zone in mm,

D - diameter of the test specimen in mm.

The second method involved counting the colony forming units (CFU), as described by Gupta et al. [15]. The CFU's were enumerated using a Reichert Darkfield Quebec Colony Counter and calculating the percent reduction in bacteria using ***Equation 2***:

$$R\% = \frac{(B - A)}{B} \times 100 \tag{2}$$

where:

R - percent reduction in bacteria,

A - CFU of treated fabric,

B - CFU of untreated (control) fabric.

Durability of the treated fabrics and antibacterial activity against laundering

The fabrics treated were laundered in an Atlas Launder-Ometer according to AATCC Test Method 61, Test 1A. The test is intended to simulate five home launderings. The antibacterial properties of the laundered fabrics were evaluated once more.

Durability of the treated fabrics and antibacterial activity against light

Independent of laundering, the fabrics treated were subjected to lightfastness by exposing the samples in an Atlas Sun Test XLS+ Weatherometer chamber with the following parameters: a Black Standard Temperature (BST) of 63 °C, a phase time of 300 minutes, an irradiance (E) of 500 W/m^2, and a final dosage of 9000 kJ/m^2. The samples were exposed front and back in the chamber over successive days. The antibacterial properties of the light-exposed samples were then evaluated.

All the experiments were done in triplicate, and the values reported are the means of the three replications.

Results and discussion

Antibacterial activity of berberine chloride in solution

At the outset, the Berberine chloride was screened for its antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis* in solution. The results showed that berberine chloride was effective against both microbes since a clear zone of inhibition was seen at treatment concentrations of 0.2%, 0.3%, and 0.4%. The minimum concentration of the agent at which the zone of inhibition was greater than 2 mm was considered as the Minimum Inhibitory Concentration (MIC), and therefore against both the microbes the MIC was 0.2%. At this concentration, the growth of bacteria adjacent to and beneath the filter paper was completely inhibited. At a concentration of 0.1%, berberine chloride inhibited bacterial growth beneath the filter paper, but the zone of inhibition was less than 2 mm.

Determination of the antibacterial activity of the treated fabrics

Subsequent to the activity of berberine chloride in the solution being established, the next step was to evaluate its effectiveness on the three textile substrates. An agent bound to a textile fibre may be expected to show lower activity than in

solution since some functional groups are modified by interaction with the fibre during the treatment process [15]. Therefore, to offset the expected decrease, fabric samples were treated at higher berberine chloride concentrations of 10%, 20% and 30% (on weight of fabric).

Evaluation of the samples treated was first done according to AATCC Test Method 147. A sample was considered to possess good antibacterial activity if its zone of inhibition was more than 2 mm. The mean zones of inhibition of a polyester, nylon and polyester-cotton blend treated with berberine chloride at various concentrations against *Staphylococcus aureus* and *Enterococcus faecalis* are shown in ***Tables 2***, respectively. As is clearly seen from the data in the tables, berberine chloride is an effective antibacterial agent when applied to all three test substrates. The zone of inhibition of the treated substrates at all concentrations was greater than 2 mm. Since a 10% concentration (on weight of fabric) was sufficient to exhibit good antibacterial activity throughout all the substrates, a statistical analysis was made to further gauge whether differences between the substrates were statistically significant at this level of treatment. The data were analysed using the least square means (LSD) procedure at a 95% confidence interval (SAS, Version 9.2). It was found that the zones of inhibition were significantly different among the three substrates. However, it is pertinent to note that the difference in the size of the zone of inhibition, even when statistically significant, cannot be interpreted as a measure of quantitative distinction in antibacterial activity [16]. To test the durability of the antibacterial activity of berberine chloride, the samples treated were laundered and exposed to light under conditions described previously, the results of which are given in ***Table 2***. Values of the zone of inhibition for the samples laundered clearly show that in post-laundering, berberine chloride retains its antibacterial activity against *S. aureus* and *E. faecalis*. However, laundering does diminish the effectiveness of berberine chloride, but the zones of inhibition were still greater than 2 mm. Likewise, exposure to light also diminishes the effectiveness of berberine chloride as an antibacterial agent. However, once again the zones of inhibition of all three substrates are greater than 2 mm, which indicates that they have sufficient durability against light exposure.

Table 2. *Antibacterial activity of berberine chloride treated polyester, nylon and polyester-cotton against S. aureus and E. faecalis (Zone of inhibition).*

Fabric samples		Mean zone of inhibition, mm					
		Treated		Laundered		Light exposed	
		S. aureus	*E. faecalis*	*S. aureus*	*E. faecalis*	*S. aureus*	*E. faecalis*
Polyester	10% owf	6.3	7.3	4.1	4.6	4.0	4.1
	20% owf	6.8	8.1	6.0	6.8	5.6	5.6
	30% owf	7.1	8.6	6.8	7.1	6.3	6.6
Nylon	10% owf	7.5	8.1	2.3	5.1	2.0	4.6
	20% owf	8.1	9.1	3.5	7.6	2.6	6.8
	30% owf	9.8	9.6	4.8	8.1	3.6	7.1
Polyester-cotton blend	10% owf	3.8	5.1	2.0	3.6	2.3	3.5
	20% owf	5.1	5.8	3.3	4.6	4.6	4.1
	30% owf	6.8	6.6	5.0	5.1	6.8	4.8

Table 3. *Antibacterial activity of berberine chloride treated polyester, nylon and polyester-cotton against S. aureus and E. faecalis (Percentage reduction in bacteria).*

Fabric samples		Reduction in bacteria, %					
		Treated		Laundered		Light exposed	
		S. aureus	*E. faecalis*	*S. aureus*	*E. faecalis*	*S. aureus*	*E. faecalis*
Polyester	10% owf	44.6	49.6	37.2	40.9	37.4	39.6
	20% owf	54.3	57.2	46.7	52.2	48.5	49.4
	30% owf	61.8	66.9	63.0	62.9	62.5	58.2
Nylon	10% owf	41.4	49.1	36.9	39.6	34.4	40.0
	20% owf	57.7	63.3	49.8	57.3	47.1	53.5
	30% owf	70.9	73.3	65.6	64.2	61.0	62.6
Polyester-cotton blend	10% owf	48.2	51.5	38.9	42.1	33.2	39.7
	20% owf	60.0	65.4	55.2	56.1	54.4	56.5
	30% owf	73.3	76.6	74.5	67.8	70.2	69.1

The second method of evaluation was calculating the percentage reduction in bacteria by counting the Colony Forming Units, data of which are presented in ***Table 3*** for polyester, nylon and polyester-cotton, respectively. In the case of polyester (***Table 3***), the data indicate that an increase in agent concentration from 10% on weight of fabric to 30% on weight of fabric results in the higher effectiveness of berberine chloride as an antibacterial agent. For *S. aureus*, there is a minor but statistically insignificant increase in the percentage reduction after laundering and after exposure to light at 30% concentration, whereas there is a decrease in the case of *E. faecalis* at all three concentrations. In the case of nylon (***Table 3***), the data show that berberine chloride treated nylon has good antibacterial effectiveness against both *S. aureus* and *E. faecalis*. In line with the zone inhibition results, the percentage reduction data confirm that the effectiveness decreases after laundering and on exposure to light. For example, in the case of *E. faecalis*, there is a reduction of about 9 - 12% after laundering and on exposure to light. Statistical analysis at a 95% confidence interval revealed that at a 10% concentration (on weight of fabric) the results of the substrates are not significantly different. Moreover, there is no statistically significant difference between nylon and polyester-cotton at concentration levels of 20% (on weight of fabric) and 30% (on weight of fabric). Between polyester and polyester-cotton, there is moderate difference at a 20% concentration (p-value 0.077) and a higher significant difference at a 30% concentration (p-value 0.0191) ***Table 3*** bears out this analysis as it shows that the berberine chloride treated polyester-cotton blend gave the highest bacterial population reduction of 73.3% against *S. aureus* and 76.6% against *E. faecalis*

Conclusions

Berberine chloride was successfully applied as an antibacterial agent to three textile substrates - polyester, nylon and a polyester-cotton blend. It was found that berberine chloride is an effective antibacterial agent against *Staphylococcus aureus* and *Enterococcus faecalis*. The results showed that a concentration of 10% on weight of fabric was sufficient to impart antibacterial properties

to the substrates. The antibacterial efficacy of berberine chloride on all three substrates was also found to be durable against laundering and light exposure. A suggested future work could focus on comparing the efficacy of berberine chloride with other natural and commercially available antibacterial agents.

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