Efficacy of Aloe vera variety Barbadensis on bacterial isolates from cultured freshwater fish in Kenya

FJ Charo, PG Muthia, LC Bebora and JM Nguta

Abstract

Aloe vera variety barbadensis is a potential alternative antimicrobial plant extract that has been widely used in humans. The objective of this study was to investigate the susceptibility of selected bacterial isolates from cultured freshwater fish to different concentrations of Aloe vera variety barbadensis crude extract. Using Minimum inhibitory concentration method by Broth dilution technique, the extract was diluted in distilled water to concentrations of 250 mg/ml, 125mg/ml, 62.5 mg/ml, 31.25mg/ml, 15.63mg/ml, 7.85 mg/ml and 3.93 mg/ml and 1.96 mg/ml. Eighteen (18) bacterial isolates namely Enterococcus faecalis NT2I3, Lysinibacillus fusiformis MT8G4, Bacillus cereus NC5K2, Bacillus cereus (MT7G3), Micrococcus luteus (MC3K2), Kurthia gibsoni (NT5G5), Bacillus thuringiensis NT4G4, Aeromonas hydrophila NT5K2, Aeromonas hydrophila MT7G2, Pseudomonas mendocina NT5G2, Pseudomonas putida MT715, Enterobacter cloaca MT6G2, Aeromonas veronii NTG41, Citrobacter braakii NC3S2, Citrobacter freundii NC3G3, Escherichia coli NC2G1(ii), Escherichia coli MT2G1 and Hafnia alvei MC3K1 were each suspended in physiological saline to a turbidity matching 0.5 Mc Farland opacity tube. Then for each extract concentration, 1 ml was mixed with 0.1 ml of respective bacterial suspension and incubated at 37 °C for 24 hours. Only 1 isolate (Kurthia gibsonii) was susceptible at a MIC of 62.5 mg/ml of Aloe vera. Seven isolates (Bacillus cereus MT7G3, Micrococcus luteus, Aeromonas hydrophila NT5K2, Aeromonas hydrophila MT7G3, Aeromonas veronii, Citrobacter braakii and Escherichia coli NC2G1) were susceptible at a MIC of 125 mg/ml of Aloe vera. Five isolates (Bacillus cereus NC3K2, Bacillus thuringiensis, Pseudomonas putida, Enterobacter cloaca and Enterococcus faecalis) were susceptible at a MIC of 250mg/ml of Aloe vera. Resistance to the highest concentration of Aloe vera was observed in five isolates namely Lysinibacillus fusiformis, Pseudomonas mendocina, Citrobacter freundii, Hafnia alvei and Escherichia coli MT2G1. Aloe vera variety barbadensis extract was shown to be efficacious against most Gram positive and Gram negative bacteria isolated from cultured fish at a concentration between 125 mg/ml to 250 mg/ml.

Keywords: Aloe vera barbadensis, minimum inhibitory concentration, efficacy and bacterial isolates

1. Introduction

Antibiotic use for treatment of diseases in cultured fish has over the years precipitated development of antimicrobial resistance and deposition of antibiotic residues in fish tissues. It has also caused suppression of the aquatic animal’s immune system (Defoirdt et al., 2007) [3]. Fish bacterial isolates have been shown to be resistant to antibiotics (Souli et al., 2008; Wanja et al., 2020) [8 12]. In order to avoid usage of antibiotics in fish, plant crude extracts like those of Viscum album, Urtica dioica and Zingiber officinale have been used in aquaculture with positive results (Christybpapita et al., 2007) [10]. The use of Aloe vera plant extract in aquaculture has however never been reported and that forms the basis of this study.

Aloe vera is a succulent plant species of the genus Aloe. It originates from the Arabian peninsula but grows wild in the tropical, semi-tropical and arid climates around the world. The botanical names are derived from Latin ‘Aloe’ meaning bitter and “vera” meaning true. It is a stemless or very short stemmed plant growing 60-100 centimetres tall. The leaves are thick and fleshy, green to grey-green with serrated margin.

Egyptians called the Aloe “the plant of immortality” while Greeks regarded it as a universal panacea 2000 years ago. The plant has been used for medicinal purposes in several cultures for millennia in Greece, Egypt, India, Mexico, Japan and China. Egyptian queens Nefertili and
Cleopatra used it as part of their regular beauty regimens (Surjushe et al., 2008) [11]. Alexander the Great and Christopher Columbus also used it to treat soldiers’ wounds (Surjushe et al., 2008) [11]. Aloe vera crude extracts have been reported to have antibacterial activity on Streptococcus mutans in human dentistry (Subramaniam et al., 2012) [10]. Indigenous Aloe species in Kenya include Aloe secundiflora, Aloe turkanensis, Aloe calidophia and Aloe scabrafolia. Aloe vera variety barbadensis is exotic from South Africa but grown commercially in Kenya (Newton, 2004). Aloe barbadensis is frequently used due to its high medicinal value (Grace et al., 2009) [6]. This study evaluated the susceptibility of bacterial isolates from cultured fish to Aloe vera variety barbadensis extracts.

2. Materials and Methods

Ethical clearance: Approval to carry out the study was obtained from the Faculty of Veterinary medicine, Biosafety, Animal use and Ethics committee, University of Nairobi (reference number FVM BAUEC/2020/273) and licensed by the National Council for Science, Technology and Innovation (reference number 842968), in Kenya.

Study site

The study was conducted at the University of Nairobi, department of Veterinary public health, Pharmacology and Toxicology. Aloe vera was sourced from Voi in Taita–Taveta County which lies between latitude -3° 19’ 60.00’’ S and longitude 38° 14’ 60.00’’ E. The Veterinary Public Health, Pharmacology and Toxicology department, University of Nairobi is situated at upper Kabete campus between latitude 1.2524° S, and longitude 36.7287° E.

Study design

A cross-sectional study was done. Approximately 5kg of Aloe leaves from a mature plant (20 cm long and green) were harvested and the whole plant uprooted from Voi in Taita-Taveta County. Harvested Aloe leaves were taken to the pharmacology laboratory, University of Nairobi for extraction of the crude extract while the whole plant uprooted was taken to the Herbarium at the National Museums of Kenya for taxonomic identification and authentication. It was identified as Aloe vera variety barbadensis.

Aloe vera extraction

In the laboratory, process of extraction started by sorting out the harvested leaves, washing with tap water to remove dirt and drying at room temperature. The outer skin also known as rind was removed with a sharp knife to expose the aloe fillet that was chopped into smaller pieces and put into a glass beaker. Sliced fillet pieces were then crushed with a pestle and a mortar to squeeze out the aloe juice (Noot et al., 2008) [3]. The extracted homogenized Aloe juice was then filtered to remove all fibrous materials to obtain pure aloe juice. The

![Map showing Voi town in Taita-Taveta County where Aloe vera was harvested](https://www.fisheriesjournal.com)
preparation was poured into the aqueous portion. Then, it was mixed with 1 ml of respective bacterial suspension. The final concentration was taken as the lowest antibiotic concentration (MIC) was measured. It was noted that the bacteria were susceptible to the extracts. Minimum inhibitory concentration (MIC) was determined as the lowest bacterial concentration (highest dilution) of the extract showing turbidity (no inhibition).

### 3. Results

Susceptibility of the bacteria that were subjected to different concentrations of Aloe vera crude extract is shown in Table 1. The lowest concentration of Aloe vera crude extract that exerted bacterial inhibition was 62.5 mg/ml (62.5%). It inhibited growth of Kurthia gibsonii isolated from the gills of a Tilapia fish from Nyandarua County. The lowest concentration of Aloe vera crude extract that inhibited growth of Bacillus cereus (MT7G3), Micrococcus luteus (MC3K2), Aeromonas hydrophila (MT7G2), Aeromonas hydrophila (NT5K2), Aeromonas veronii (NT4G1), Citrobacter braakii (NC3S2) and Escherichia coli (NC2G1 (II)) was 125 mg/ml. Those that were inhibited by the highest concentration used for testing (250 mg/ml) were Enterobacter cloacae (MT6G2), Pseudomonas putida (MT5I5), Enterococcus faecalis (NT2I3), Bacillus thuringiensis (NT4G5) and Bacillus cereus (NC3K2). Some bacterial isolates however were resistant ( grew) to the highest concentration used for testing (250 mg/ml). These included Lysinibacillus fusiformis (MT8G4), Pseudomonas mendocina (NT5G2), Citrobacter freundii (NC3G3), Hafnia alvei (MC3K1) and Escherichia coli (MT2G1). Isolates that depicted resistance to the highest test solution of 250 mg/ml of Aloe vera represented 27.8% of the total isolates subjected to the susceptibility tests. In summary 72.2% of the bacterial isolates were susceptible to the Aloe vera crude extract at a concentration between 62.5 mg/ml to 250 mg/ml while 27.8% of the isolates showed resistance with regards to Aloe vera variety barbadensis. Aloe vera variety barbadensis had a MIC value of 125 mg/ml against E. coli from Nyandarua County (NC2G1 (II)) while E. coli from the gills of a tilapia (MT2G1) from Machakos County was resistant even at 250 mg/ml of Aloe vera. Aloe vera variety barbadensis was observed to have a MIC of 125 mg/ml against Bacillus cereus (MT7G2) from Machakos but a MIC of 250 mg/ml against Bacillus cereus (NC3K2) from a catfish kidney sample collected from Nyandarua County.

### Table 1: Minimum inhibitory concentrations of Aloe vera crude extracts against bacterial isolates

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Aloe vera Minimum Inhibitory Concentrations (MIC)</th>
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<tbody>
<tr>
<td>Enterococcus faecalis (NT2I3)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Lysinibacillus fusiformis (MT8G4)</td>
<td>&gt;250 mg/ml (Resistant)</td>
</tr>
<tr>
<td>Bacillus cereus (NC3K2)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Bacillus cereus (MT7G3)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Micrococcus luteus (MC3K2)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Kurthia gibsonii (NT5G5)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Bacillus thuringiensis (NT4G5)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Aeromonas hydrophila (NT5K2)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Aeromonas hydrophila (MT7G2)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Pseudomonas mendocina (NT5G2)</td>
<td>&gt;250 mg/ml (Resistant)</td>
</tr>
<tr>
<td>Pseudomonas putida (MT5I5)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Enterobacter cloacae (MT6G2)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Aeromonas veronii (NT4G1)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Citrobacter braakii (NC3S2)</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Citrobacter freundii (NC3G3)</td>
<td>&gt;250 mg/ml (Resistant)</td>
</tr>
<tr>
<td>Escherichia coli (NC2G1 (II))</td>
<td>&gt;250 mg/ml</td>
</tr>
<tr>
<td>Escherichia coli (MT2G1)</td>
<td>&gt;250 mg/ml (Resistant)</td>
</tr>
<tr>
<td>Hafnia alvei (MC3K1)</td>
<td>&gt;250 mg/ml (Resistant)</td>
</tr>
</tbody>
</table>

Key: M connotes Machakos; N connotes Nyandarua; T connotes Tilapia; C connotes Catfish; R connotes Rainbow trout; S connotes Skin; G is gills; K is kidney; I is intestine; mg/ml is milligrams per millilitre

Aloe vera variety barbadensis had a MIC value of 125 mg/ml against E. coli from Nyandarua County (NC2G1 (II)) while E. coli from the gills of a tilapia (MT2G1) from Machakos County was resistant even at 250 mg/ml of Aloe vera. Aloe vera variety barbadensis was observed to have a MIC of 125 mg/ml against Bacillus cereus (MT7G2) from Machakos but a MIC of 250 mg/ml against Bacillus cereus (NC3K2) from a catfish kidney sample collected from Nyandarua County. Aloe vera variety, Barbadensis also depicted different MIC values against different Citrobacter and Pseudomonas species. It had a MIC value of 250 mg/ml against Pseudomonas putida (MT7I5) while Pseudomonas mendocina (NT5G2) was resistant at same concentration of 250 mg/ml of Aloe vera. Similarly, Aloe vera variety barbadensis had the same MIC value of 125 mg/ml against Citrobacter braakii (NC3S2) while Citrobacter freundii (NC3G3) was resistant even at 250 mg/ml of it. Aloe vera variety barbadensis had the same MIC value of 125 mg/ml against two different species of Aeromonas namely Aeromonas hydrophila and Aeromonas veronii. Similarly, Aloe vera variety barbadensis had the same MIC value of 125 mg/ml against two similar species of Aeromonas namely Aeromonas hydrophila from Machakos (MT7G2) and Aeromonas hydrophila from Nyandarua (NT5K2).

### 4. Discussion

Aloe vera variety barbadensis has been used to treat skin problems such as wounds, acne, burns and dermatitis in humans (Zhang et al., 2006) [13]. Its use in treatment of gastrointestinal ailments, sexual vitality, immune modulation and various skin diseases in humans has also been reported by Chatterjee et al., (2015) [2]. Numerous studies have reported effectiveness of Aloe vera variety barbadensis when applied.
topically (Dal’Belo et al., 2006) [4]. In this study Aloe vera variety barbadensis was found to have antimicrobial activity against Gram negative and Gram positive bacteria isolated from fish such as members of the genera Aeromonas, Pseudomonas, Citrobacter, Enterococcus, Micrococcus, Bacillus, Escherichia coli, Karthia and Enterobacter among others. Similar efficacy of Aloe vera against bacteria isolated from non-human hosts was reported by Arbab et al., (2020) [1] who demonstrated its efficacy on common donkey skin infection bacteria pathogens such as Escherichia, Shigella, Salmonella and Staphylococcus. In contrast to Arbab et al., (2020) [1] who reported susceptibility of all Gram positive and Gram negative bacteria isolates to the ethanol extract of Aloe vera, this study showed resistance to Aloe vera by some bacterial isolates from fish such as Citrobacter freundii, Hafnia alvei, E. coli, Lysinibacillus fisiformis and Pseudomonas mendocina. Most of the bacteria that exhibited resistance to Aloe vera ethanol extracts were Gram negative. The presence of more additional lipopoly saccharide layer in the Gram positive layer makes them more susceptible to ethanol extracts of Aloe vera (Matu et al., 2003) [7].

Manifestation of different susceptibilities to Aloe vera variety barbadensis by different bacterial species (same genera) such as Pseudomonas mendocina and Pseudomonas putida; Citrobacter freundii and Citrobacter brakii could be due to presence of different susceptibility genes in the different bacterial species. Similar species with different MIC values from different counties may be due to previous exposure of the resistant species as is the case of E. coli (MT2G1) from Machakos County and E. coli (NC2G1 (II)) from Nyandarua; Bacillus cereus (NC3K2) from Nyandarua County and Bacillus cereus (MT7G3) from Machakos County. About twenty eight percent (28%) of the tested bacterial isolates showed resistance to the highest test dilution of Aloe vera (250 mg/ml).

5. Conclusion
Aloe vera is in vitro efficacious against most Gram positive and Gram negative bacteria isolated from cultivated fish at a concentration between 125 mg/ml to 250 mg/ml. It can therefore be recommended as a potential alternative plant extract to treat bacterial infection in aquaculture and mitigate the global surge in antimicrobial resistance.

6. Conflicts of interest
The authors declare that there are no conflicts of interest.

7. Acknowledgements
Kenya Climate Smart Agricultural project (KCSAP) for funding the study, Kenya Medical Research institute, Veterinary Pathology, Microbiology and Parasitology and the Veterinary Public Health, Pharmacology and Toxicology departments, University of Nairobi their for technical support.

8. References