E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2023; 11(2): 110-113

www.fisheriesjournal.com
Received: 03-01-2023
Accepted: 05-02-2023

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

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DOI: https://doi.org/10.22271/fish.2023.v11.i2b.2797

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, 62.5mg/ml, 31.25mg.ml, 15.63mg/ml,7.85 mg/ml, 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* MT7I5, *Enterobacter cloaca* MT6G2, *Aeromonas veronii* NT4G1, *Citrobacter braakii* NC3S2, *Citrobacter freundii* NC3G3,*Escherichia coli* NC2G1(ii), *Escherichia coli* MT2G1 and *Hafnia alvei* MC3K1were 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 (ii)) 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 feacalis)* 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) [5]. Fish bacterial isolates have been shown to be resistant to antibiotics (Souli *et al*., 2008; Wanja *et al*., 2020) [9, 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 (Christybapita *et al*., 2007) [3]. 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 scabrifolia. 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 -3º 19' 60.00' S and longitude 38º 14'60.00' E.

The Veterinary Public Health, Pharmacology and Toxicology department, University of Nairobi is situated at upper Kabete campus between latitude 1.2524^0 S, and longitude 36.7287^0 E

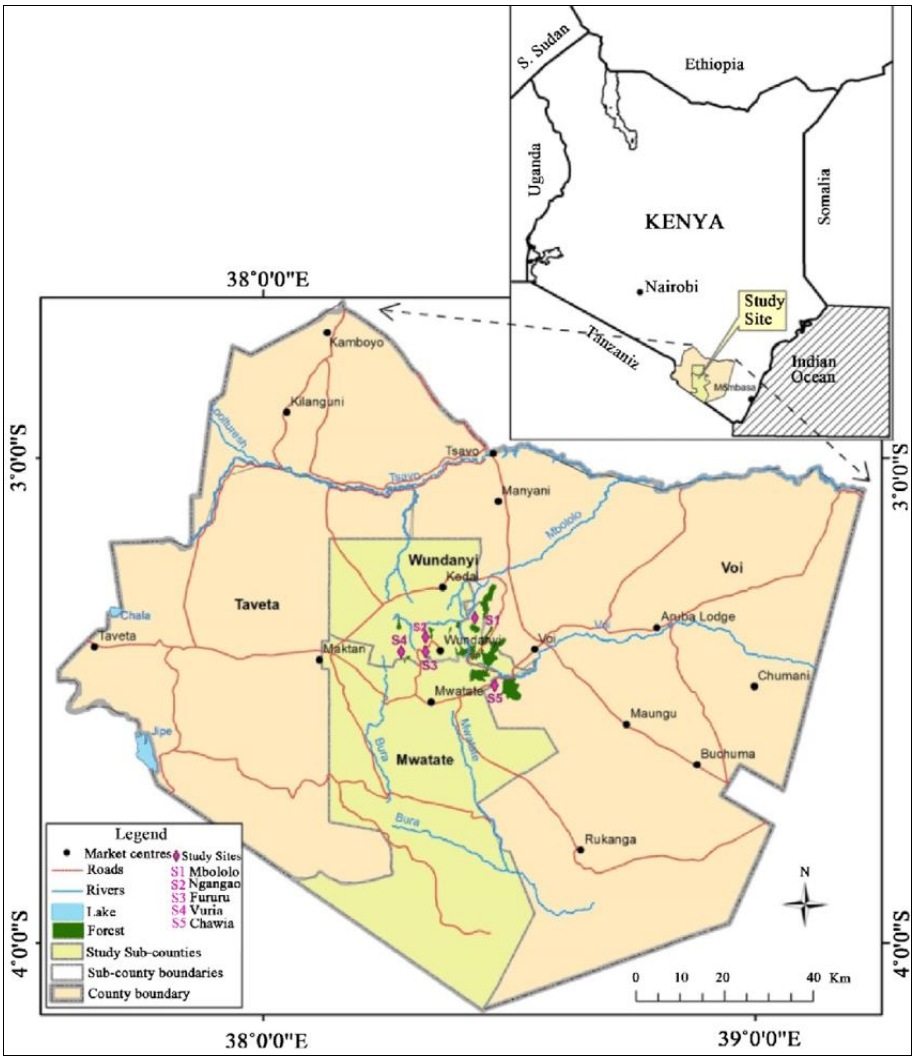

Fig 1: Map showing voi town in Taita-Taveta County where *Aloe vera* was harvested

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 authentification. 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 (Noor *et al*., 2008) [8]. The extracted homogenized *Aloe* juice was then filtered to remove all fibrous materials to obtain pure aloe juice. The

extraction was ethanolic where 50% ethanol was used for extraction of the crude extract. 4 liters of 50% ethanol were mixed with 1 liter of aloe juice (4:1) and mixture continuously stirred using a magnetic stirrer for 72 hours (Noor *et al*., 2008) [8].

Rota – evaporation of the mixture was done to remove the ethanol whereas the aqueous portion was taken to the Kenya Medical Research Institute's Centre for Traditional Medicine and Drug Research for freeze-drying. The final crude extract was then collected and used for bacterial susceptibility tests.

Preparation of test solutions and evaluation of activity of Aloe crude extract

Susceptibility of the selected bacterial isolates to the *Aloe vera* variety *barbadensis* extract was tested using Minimum inhibitory concentration method given by Broth dilution technique. The extract was diluted in distilled water to concentrations of 250 mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg.ml, 15.63mg/ml,7.85 mg/ml, 3.93 mg/ml and 1.96 mg/ml respectively. Then 18 bacterial isolates from fish namely *Aeromonas hydrophila* MT7G2, *Aeromonas hydrophila* NT5K2, *Aeromonas veronii* NT4G1, *Bacillus cereus* MT7G3, *Bacillus cereus* NC5K2, *Bacillus thuringiensis* NT4G4, *Citrobacter braakii* NC3S2, *Citrobacter freundii* NC3G3, *Enterobacter cloaca* MT6G2, *Enterococcus faecalis* NT2I3, *Escherichia coli* MT2G1, *Escherichia coli* NC2G1 (ii), *Hafnia alvei* MC3K1, *Kurthia gibsonii* NT5G5, *Lysinibacillus fusiformis* MT8G4, *Micrococcus luteus* MC3K2, *Pseudomonas mendocina* NT5G2 and *Pseudomonas putida* MT7I5 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 ºC for 24 hours. Observation of no bacterial growth (no turbidity) denoted that the bacteria were susceptible to the extracts. Minimum inhibitory concentration (MIC) was taken as the lowest antibacterial concentration (highest dilution) of the extract showing turbidity (no susceptibility).

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 effected bacterial growth 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 braaakii* (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 cloaca* (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.*

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

Bacterial Isolates	*Aloe vera* Minimum Inhibitory Concentrations (MIC)
Enterococcus feacalis (NT2I3)	250 mg/ml
Lysinibacillus fusiformis (MT8G4)	>250 mg/ml (resistant)
Bacillus cereus (NC3K2)	250 mg/ml
Bacillus cereus (MT7G3)	125 mg/ml
Micrococcus luteus (MC3K2)	125 mg/ml
Kurthia gibsonii (NT5G5)	62.5 mg/ml
Bacillus thuringiensis (NT4G5)	250 mg/ml
Aeromonas hydrophila (NT5K2)	125 mg/ml
Aeromonas hydrophila (MT7G2)	125 mg/ml
Pseudomonas mendocina (NT5G2)	>250 mg/ml (Resistant)
Pseudomonas putida (MT7I5)	250 mg/ml
Enterobacter cloaca (MT6G2)	250 mg/ml
Aeromonas veronii (NT4G1)	125 mg/ml
Citrobacter braakii (NC3S2)	125 mg/ml
Citrobacter freundii (NC3G3)	>250 mg/ml (Resistant)
Escherischia coli (NC2G1(II))	125 mg/ml
Escherichia coli (MT2G1)	>250 mg/ml (Resistant)
Hafnia alvei (MC3K1)	>250 mg/ml (Resistant)

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* (MT715) while *Pseudomonas mendocina* (NT5G2) was resistant at same concentration of 250 mg/ml of *Aloe vera*. Similarly, *Aloe vera* variety *barbadensis* had a 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, Kurthia 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 lipopolysaccharide 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 braakii* 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 cultured 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.

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