



Bacteriological Quality of Local Streams and Antibacterial Effect of *Ocimum gratissimum* and *Psidium guajava*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2021/v31i630323

Editor(s):

(1) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.

Reviewers:

(1) Rania Abozahra, , Damanhour University Egypt.

(2) Ahmed Abduljabbar Jaloob Aljanaby, University of kufa, Iraq.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/72656>

Original Research Article

Received 15 June 2021
Accepted 20 August 2021
Published 26 August 2021

ABSTRACT

Background: Streams are known sources of drinking water for most communities in the rural areas. It importance to human and other forms of life cannot be overemphasize, hence the need to evaluate it portability

Methodology: Microbiological quality of different streams in Inua Akpa of Odukpani Local Government Area of Cross River State was determined using standard bacteriological technique. The two (*Psidium guajava* and *Ocimum gratissimum*) plant materials were extracted using 70% ethanol and distilled water. Susceptibility testing was carried out using agar diffusion methods. SPSS version 20 was used for descriptive statistics, student Unpaired T-test compared the means of bacterial isolates and their distribution in different streams.

Results: *E. coli*, *Salmonella*, *Klebsiella* and *Enterococcus Spp* were isolated with percentage occurrence based on streams as: 43.0, 27.0, and 30.0%, 71.0, 27.0, 00, 83.0, 00, 18.0 and 63.0%, 38.0, 00 in Ndom Nyam, Usung Esuk and Usung Odot streams respectively. *Klebsiella* was the most frequent isolate (83.0%) followed by *Salmonella Spp* in Ndom Nyam. *Salmonella* and *Enterococcus spp* were absent in Usung Odot while *Klebsiella Spp* was undetected in Usung Esuk. *E.coli* was isolated in all the experimented streams with total occurrence of 93.0%. There was a

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significant difference at $P = 0.005$ in the occurrence of *E. coli* in all the streams compared to other isolates.

The isolated organisms were susceptible to *P. guajava* at all concentrations (50, 30, 20, 10, 5mg/mL) with highest inhibition of (24mm) observed at 50mg/mL against *Klebsiella Spp.* *Ocimum gratissimum* was effective against *E.coli* at all concentrations, *Enterococcus* was only inhibited at 50 and 30mg/mL, *Klebsiella* showed resistant at the lowest concentration (5mg/mL) while *Salmonella* resisted the extract at all concentrations. The aqueous extract of both plants showed no antimicrobial activity against any isolated organisms as well as negative controls.

Conclusion: This study suggest a regular monitoring of local streams to stop human activities that encourages the introduction of microorganisms into sources of drinking water. The expressed inhibitory zones of diameter by these two plants justify their used as alternative treatment for water related illnesses in rural communities.

Keywords: Local streams; *E. Coli*; *klebsiella*; *salmonella*; and *Enterococcus Spp.*; *Ocimum gratissimum* and *Psidium guajava*.

1. INTRODUCTION

Poor water quality continues to pose a major threat to human health while access to clean drinking water and adequate sanitation are major brake on development [1]. Globally diarrheal diseases are responsible for 1.8 million death annually, most of which are children from developing countries [2]. It is also estimated that about 88% of that burden is directly attributed to unsafe water supply, poor sanitation and personal hygiene. The economic situation in most developing countries as well as lack of effective infrastructure make the citizens to rely on untreated (highly contaminated) surface and groundwater. Stream subjected to contamination by human activity, surface run-off, farming practices etc. is the major source of water that most community in developing countries depends on for washing of clothes, food utensils, bathing and cooking [3]. Recent work has found that rural drinking water was more contaminated than urban in some parts of Mohale basin in Lesotho [4]. Other literature reports that over half drinking water sources tested in Africa were contaminated as compared to 35% in Asia and that on average, over 40% of rural drinking water sources were contaminated as compared to only 12% in urban areas. Drinking water has been established as a primary transmission pathway for diarrheal pathogens [5]. One of the preventive measure employ by developed countries against outbreak of waterborne diseases is central water treatment and distributing system [6]. Improving the microbial quality of drinking water can be achieve by employing effective and workable water treatment before distribution. This may serve as a better strategy for minimizing outbreak of water related illness. World Health Organization (WHO) guidelines recommend

Escherichia coli (EC) or thermo-tolerant faecal coliforms (FC) as indicators/index organism for the presence of faecal pathogens, it also serve as a bench mark for effective disinfection processes. Water treatment and maintenance of quality water that is safe for human consumption in the rural areas where bore-whole water is not common is a major challenge and concern.

The use of plant materials as spices, condiments and medicinal purposes dates back to the history of mankind [7]. There are recent exploitation of medicinal values of wild plants which have gained more acceptances in many countries of the world. *C. gratissimum* is an erect perennial herb of soft shrub belonging to Lamiaceae family, genus *Ocimum* and species *gratissimum*. The report of Nakamura et al. and Lemos et al. [8,9] states that this plant has potency against some bacterial and fungal species. *Psidium guajava* in the other hand belongs to the family Myrtaceae and is popularly known as guava. Traditionally, preparations of it leaves have been used in folk medicine in several countries mainly as anti-diarrheal remedy [10]. This research set out to examine the Bacteriological quality and portability of local streams that supply drinking water to Inua Akpa community in Odukpani Local Government area and investigates the in-vitro activity of *C. gratissimum* and *P. guajava* on isolates from these streams.

2. MATERIALS AND METHODS

2.1 Water Collection and Transportation

A 10 mL of Water sample was collected at 3 different spot of each stream (Ndom Nyam, Usung Esuk, and Usung Odot) in Inua Akpa into universal bottles and stored in an ice-cold pack.

The Ice-pack was transported to Microbiology Laboratory, Cross River University of Technology Calabar within 2 hours of collection for analysis.

2.2 Enumeration of Total Bacterial Count

Bacterial count were determined by pour plating method using nutrient, MacConkey agar and *Salmonella Shigella* agar (SSA) (Liofilchem® s.r.l., Italy) respectively. A tenfold (10^{-10}) serial dilution was prepared and 1 mL of the (10^{-4}) and (10^{-9}) mL were plated in triplicate on the different medium. Plates were incubated at 37 °C for 24 hour [11].

2.3 Isolation and Identification of Faecal Coliform

After 24 hours of incubation, colonies were subjected to three successive sub-culturing and re-isolations on Nutrient and MacConkey agar to obtained pure culture. The identity of the different colonies were reaffirmed by standard bacteriological characterization.

2.4 Collection of Plant Material

Leaves of *Ocimum gratissimum* (OG) and *Psidium guajava* (PG) were collected at Inua Akpa community in Odukpani Local Government area and authenticated by Botanist and curators of Faculty of Biological Sciences and research carried out in the Department of Microbiology Cross River University of Technology where the voucher specimen is deposited. Plants were rinsed thoroughly in running tap water, air dried at room temperature for 2 weeks until it became crispy. The dried plant leaves were milled into powered using Q'Link electric blender (QBL-20<40), weighed and stored in plastic bags in the dark.

2.5 Extraction of Plant extracts

A 100g weight of each powered plant material was soaked in 100mL of solvent (Distilled water and 70% ethanol) in a 1 litre conical flask covered with cotton wool plugs. The flask was shaken vigorously at first and intermittently for 24 h leaving in water bath maintained at 40°C between the intervals of shaking. The mixture was filtered, first through three layers of clean muslin cloth, and then through a Whitman no 1 filter-paper. The filtrates were evaporated to dryness in water bath at 56°C. The percentage

yields of the crude extracts were determined using the principle of Jacqueline et al. [12].

2.6 Susceptibility Testing of Bacterial Pathogens by Pour-Plate Method

The standardization of the cultures was carried out according to the National Committee of Clinical Laboratory Standards (NCCCLS, 1997) to 0.5 McFarland standard (approximately $0.5 \times 10^6 - 1.0 \times 10^8$ CFU/mL). The turbidity of the inoculums of each test bacterial specie was adjusted at each time of the test.

Susceptibility testing was carried out according to the method reported by Okeke *et al.*, Okoli and Iroegbu, [13,14]. Pre-dried Muller Hinton agar (Fluka BioChemika) plates were prepared and 1mL of the inoculums spread evenly on the surface of the agar plate with the help of a spreader. Sterile 6mm Whitman paper disc was impregnated with the re-suspended extracts in their extraction solvents. The discs were placed on already prepared Muller Hinton agar plates (Fluka BioChemika) containing the inoculums. Discs impregnated in 70% ethanol and 100ml of distilled water were used as negative controls. The plates were incubated at 37°C for 24 hours after which the diameters zones of inhibition were measured in millimeter (mm).

2.7 Data Analysis

The statistical analysis was done using SPSS version 20 for descriptive statistics. The student Unpaired T-test compared the means of bacterial that was significant at $P= 0.05$ and their distribution in different streams.

3. RESULTS

3.1 Percentage Distribution of Isolates According to Streams

Prominent bacteria *Spp* were isolated from the three streams (Ndom Nyam (NN), Usung Esuk (UE), and Usung Odot (UO)) analyzed in Inua Akpa. Variation in occurrence of isolated organisms according to stream was observed as follows; In NN, *E.coli* occurred 40 times (43%) out of 93, *Salmonella Spp* occurred 25 times (71%) out of 35, *Klebsiella Spp* occurred 15 times (83%) out of 18 and *Enterococcus Spp* occurred 5 times (63%) out of 8 respectively. This was followed by UE that had three (*E.coli*, *Salmonella Spp*, and *Enterococcus Spp*) coliform bacteria with prevalence of 25(27%), 10(29%),

and 3 (38%) respectively. *Klebsiella Spp* was absent in this stream. The third stream was UO stream that had just two organisms (*E.coli* and *Klebsiella Spp*) isolates, the first occurring 28 times out of 93 making a prevalence of 30% and the later occurred 3(17%) times out of 18, *Salmonella* and *Enterococcus Spp* were absent in this stream (Fig. 1).

3.2 Total Number of Isolates in All Streams

Among all the isolated organisms, *E. coli* was the most abundant occurring 93 times in all the streams, this was followed by *Salmonella Spp* 35, *Klebsiella Spp* and *Enterococcus Spp* 8 respectively (Fig. 2).

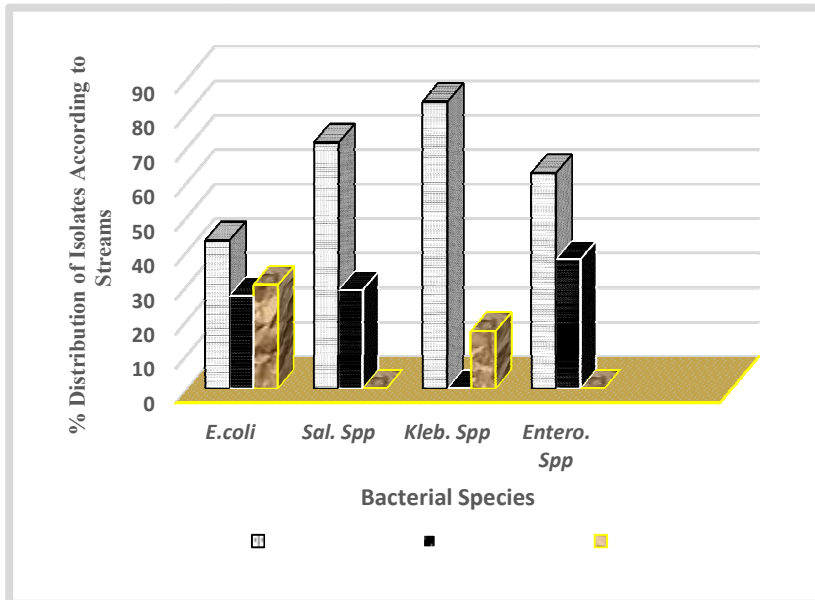


Fig. 1. The Distribution of different bacteria species in different stream

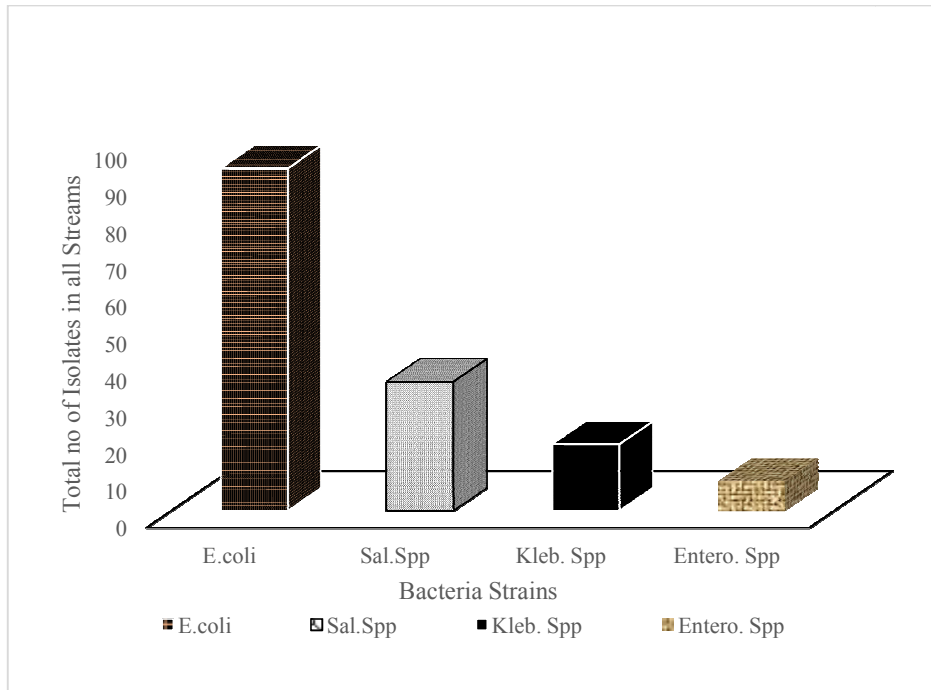


Fig. 2. Total number of each isolate in all streams

3.3 Total Number of Isolates according to Stream

In all the three streams analyzed, NN was the most contaminated with 85 (55%) isolates out of 154 followed by UE with 38 (25%) and 31 (20%) recorded for UO respectively (Fig. 3).

3.4 Antimicrobial Activity of PG

Inhibition zone of diameters (IZDs) achieved with extracts of PG ranged from 9 -24 mm for ethanolic extracts and 0 – 5 mm for aqueous extracts. The 50mg/mL ethanolic extract expressed the highest inhibition of 24mm on *Klebsiella Spp* followed by *Enterococcus Spp* 23mm and *E.coli* and *Salmonella Spp* 17mm respectively. Decrease in efficacy was observed

according to decrease in concentration of the extracts irrespective of the organisms. All isolates were resistant to the aqueous extract of PG (Table 1).

3.5 Antimicrobial Activity of OG

The diameters of inhibition achieved by ethanolic extract of OG ranged from 8 - 25 mm with highest inhibition of 25 mm observed at 30mg/mL concentration against *Klebsiella Spp*. This was followed by 20 mm at 20 and 10 mg/mL against *E.coli* and *Klebsiella Spp* respectively. Variation in antimicrobial activity based on different concentration and isolates involved was observed. *Salmonella Spp* was resistant to OG in all concentrations, 5mg/mL was only sensitive to *E.coli*. All isolates were resistant to the aqueous extract of plant materials (Table 2).

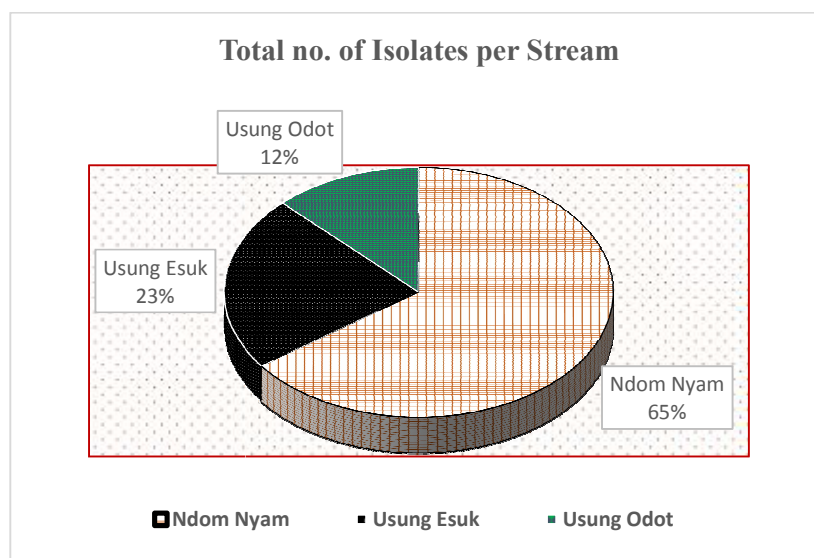


Fig. 3. The level of contamination of each stream

Table 1. Inhibition Zone of Diameter (IZD) (mm) of *Psidium guajava* Extracts on Bacterial

Ethanolic Concentration of Extracts (mg/mL)	<i>E.coli</i>	<i>Klebsiella Spp</i>	<i>Salmonella Spp</i>	<i>Enterobacter Spp</i>
50	17 (S)	24 (S)	17 (S)	23(S)
30	15(S)	22(S)	16(S)	20(S)
20	14(S)	20(S)	13(S)	15(S)
10	12(S)	17(S)	10(S)	12(S)
5	10(S)	10(S)	10(S)	9(S)
Aqueous Extract				
50	0 (R)	0(R)	0(R)	0(R)
30	0(R)	3(R)	0(R)	0(R)
20	0(R)	0(R)	5(R)	5(R)
10	0(R)	0(R)	5(R)	0(R)
5	0(R)	0(R)	0(R)	0(R)

Table 2. Inhibition Zone of Diameter (IZD) (mm) of *Ocimum gratissimum* Extracts on Bacterial Isolates

Ethanollic Concentration of Extracts (mg/mL)	<i>E.coli</i>	<i>Klebsiella Spp</i>	<i>Salmonella Spp</i>	<i>Enterobacter Spp</i>
50	19 (S)	16 (S)	9 (R)	13(S)
30	17(S)	25(S)	9(R)	12(S)
20	20(S)	13(S)	9(R)	8(R)
10	18(S)	20(S)	0(R)	8(R)
5	18(S)	0(R)	8(R)	9(R)
Aqueous Extract				
50	0 (R)	0(R)	0(R)	0(R)
30	0(R)	9(R)	9(R)	0(R)
20	0(R)	9(R)	9(R)	0(R)
10	0(R)	0(R)	0(R)	0(R)
5	0(R)	0(R)	0(R)	0(R)

4. DISCUSSION

The value of water to human life and other living things on earth is indispensable, however, the challenge in having access to portable water in most rural communities is on the increase. Stream contamination and the efficacy of PG and OG plant extracts on isolated pathogens was the aim of the study. The presence of *Klebsiella*, *Salmonella*, *Enterococcus Spp* and *E.coli* in drinking water as indicated by the result of the index study is unhealthy to the to human life. Among all isolated organisms, *E.coli* was the most frequently isolated in all experimented streams. *Isolated organisms were within the common coliforms bacteria found in the digestive track of humans and animals, forming part of the normal flora of the human gut. It usually not calls for concern except for few strains that cause serious disease in humans. For example E. coli O157:H7 is the common cause of Traveler's diarrhea, serious hemorrhagic diarrhea, cholera, hemorrhagic colitis etc. with clinical presentation ranging from nausea, abdominal cramps, fever, vomiting including long term if not fatal complications.*

The occurrence of similar pathogens from water source that supplied water to the Pakistanian public was reported by Ehsan et al. [15]. Frederick et al. [16] also reported high occurrence of pathogenic organisms in water source that supply drinking water to humans and animals in Nyankpala Community in Ghana.

The ethanolic extract of *Psidium guajava* expressed reasonable inhibitory activity against all isolated organisms at all concentrations (50, 30, 20, 10, 5mg/mL). The antimicrobial potentials of *Ocimum gratissimum* also expressed efficacy

against *E. coli* at all concentration with variations in efficacy among other isolates based on concentrations. The growth of *Salmonella Spp* was not inhibited by extract of OG at all concentrations, *Enterococcus* resisted extract activity at 20, 10, and 5mg/mL. The least concentration (5mg/mL) was observed to be ineffective against the growth of *Klebsiella*, *salmonella* and *Enterococcus* respectively. The aqueous extract of both plant (PG and OG) were unable to inhibit the growth of any of the isolated organism.

The report is supported by work done by Geidam et al. [17] who observed antimicrobial potentials in the phytochemical properties of PG leaves against gram positive and negative bacteria including *E.coli* and *Klebsiella Spp* isolated from day-old chicken.

Jasmin et al. [18] also reported on susceptibility of pathogens especially those directly involved in gastroenteritis and diarrhea with variation in efficacy of same plant materials base on different concentration (10, 50, 75, and 100%).

The possible sources of stream contamination may be linked to human activity such as direct defecation into the streams, agricultural runoff, wildlife that uses the stream environment as their natural habitat, runoff from areas contaminated with pet manure, wastewater treatment plants, and on-site septic systems. Heavy precipitation may also cause these organisms to be washed into creeks, rivers, streams, lakes, or ground water. High contamination of NN stream compared to other streams may be as a result of high human activity due to close proximity of this stream to the community.

The results of the index study apparently justify the use of these two plants in the treatment of water related illnesses.

5. CONCLUSION

The presence of bacterial pathogens in water bodies that supply drinking water to the public are on the increase in rural areas due to unhealthy human activities. The bacterial strains (*E.coli*, *Klebsiella*, *Salmonella* and *Enterococcus Spp*) isolated and identified in three reputable streams in the area mentioned above is unsafe for continuous use. The inhibitory potentials of the two plant extracts to all isolated organisms have provided a novel information about the antimicrobial activities of these plants against pathogens implicated in diarrheal infections and can be useful against drug-resistant pathogens. Further investigation on the purified state of the Bio-active components of each plant extract is strongly suggested for consideration as alternative remedy to waterborne diseases including diarrhea cases in rural communities.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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