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Bacteriological Profile of Vended Local Drinks in Amakom, Kumasi, Ghana

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Microorganisms are everywhere including food. Food, although not sterile, must have the number of microbes present falling within the acceptable limit as prescribed by the World Health Organization and other regulatory bodies. Street-vended food and drinks are still a thriving industry in the majority of developing nations, providing most urban people with their daily meals. This study sought to investigate the microbial load of some locally made street vended drinks including sorrel drink ('Sobolo') made from *Hibiscus sabdariffa*, 'Brukina', (a Ghanaian drink made of ground millet and pasteurized milk), 'Emuduro' (a natural ginger drink), 'Abele' (Ghanaian ice cream) and 'Asaana' (caramelized corn drink).
Study Design: Cross-sectional study.
Place and Duration of Study: In and around Kumasi Technical University campus, Kumasi-Ghana between January 2023 to March 2023.
Methodology: Twenty-one (21) samples of local drinks were sampled randomly from various vendors aseptically and sent to the laboratory immediately for microbiological analysis. The standard plate count method and identification tests were used to determine the total aerobic count and identify microorganisms present respectively.

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Results: The study recorded a high microbial load in the samples collected. ''Emuduro'' was the most contaminated with an average viable count of 4.5 x 10^7 cfu/mL. 'Asaana' had the least contamination with an average viable count of 2.4 x 10^5 cfu/mL which fell still out of the tolerable limit of $\leq 10^5$cfu/mL. 'Sobolo', 'Brukina', and 'Abele' had counts of 4.1 x 10^7 cfu/mL, 1.4 x 10^6 cfu/mL, and 1.3 x 10^6cfu/mL respectively which are unacceptable. Pathogenic bacteria such as *Staphylococcus spp., Escherichia coli, Klebsiella spp., Pseudomonas spp.,* and *Enterobacter spp.* were identified from the samples. The high microbial load in most of the samples may result from the use of unhygienic water, insanitary surroundings and equipment, and prolonged refrigeration
Conclusion: The study revealed that street-vended locally made drinks can be harmful to people and may cause various food-borne illnesses.

Keywords: Local drinks; pathogens; microbial contamination; safety.

1. INTRODUCTION

Local drinks serve as refreshments in a lot of countries around the world including Ghana. They are common to people in a particular area and have been developed over time using different techniques and locally sourced ingredients [1]. These local drinks which are commonly patronized by Ghanaians include 'Sobolo' (Bissap), 'Brukina', 'Asaana' (also known as elewonyo), 'Emuduro' (Hausa beer), and 'Abele' walls (Ghanaian milk ice cream). 'Bissap' popularly known as 'Sobolo' is prepared from the dried calyces of the *Hibiscus sabdariifa.* It is boiled with some local spices and may be enjoyed sweetened or unsweetened [2]. 'Brukina' is a fermented millet and milk smoothie produced by mixing sweetened fermented milk with milled steamed millet [3]. 'Emuduro' is a sweetened, spiced ginger drink that may be served hot or cold. 'Asaana' is made by mixing the extract of fermented crushed corn which has been boiled with caramelized sugar [4]. 'Abele' also known as the Ghanaian ice cream is made with milk powder, condensed milk, and water.

Local drinks are gaining popularity in recent times as people are inclining towards its patronage due to their affordability [5]. They are sometimes served at special occasions such as weddings, parties, and funerals; and are also sold commercially on the streets, and in shops. The production and sale of local drinks have therefore become a lucrative business for a lot of vendors [1]. These local drinks also have health benefits. For example, bissap is known to have antimicrobial activities [6]. 'Emuduro' is also proven to have antioxidant, antimicrobial, and anti-neuroinflammation activities, and relieves common health problems such as vomiting, nausea, and pain [7,8,9]

Upsurge in the consumption of these drinks increases health concerns because their production and sale are unregulated. This is an issue of concern because street-vended foods may be associated with food-borne diseases [10]. The raw materials used, the unhygienic conditions under which these beverages are produced, and how they are handled or stored may predispose them to microbial contamination with pathogenic microorganisms (such as *Escherichia coli, Staphylococcus aureus, Salmonella, and Shigella spp.*). The presence of these organisms can be detrimental to the health of consumers [11].

Several studies have been carried out in Africa to assess the microbiological quality of local drinks. A recent study by Aboagye and colleagues [1] in Ghana revealed high microbial counts in all 'Sobolo' samples collected from two different campuses of a University in Ghana. Furthermore, unacceptable microbial counts from some samples of local drinks including 'Sobolo', 'Emuduro', Zoomkoom, 'Asaana', Fura da no-no, and Burkina were reported in a similar study conducted in Kumasi [12].

Sometimes the microbial content in a specific local drink may fall within the acceptable limit, but may still not be appropriate to use as it may contain pathogenic microorganisms and food safety indicators such as coliforms, *Escherichia coli, Salmonella spp., Staphylococcus aureus, and Bacillus cereus.* [13]. For instance, Minamor and colleagues [14] carried out microbiological analysis of Pito, an alcoholic local drink in Ghana, and their findings showed that the total viable counts of bacteria were within the acceptable limits. However, they identified the presence of pathogenic microorganisms such as *E. coli, K. pneumoniae, Shigella spp., Enterobacter spp., Staphylococcus aureus, and P. aeruginosa.*

This study was therefore conducted to assess the bacteriological quality of selected local drinks sold in Amakom, Kumasi as these products are continually patronized by the masses.

2. MATERIALS AND METHODS

2.1 Sampling

Five (5) samples each of 'Sobolo', 'Emuduro' and 'Brukina'; three (3) samples each of 'Asaana' and 'Abele' were randomly procured from vendors in Amakom,Kumasi around Kumasi Technical University campus. The samples were appropriately labelled, kept in ice and transported to the microbiology laboratory for analysis

2.2 Microbiological Analysis

2.2.1 Enumeration of bacteria

Microbiological analysis was done to enumerate the total number of viable microorganisms present in the samples and identify some pathogenic microorganisms. The standard plate count method by Jahan and colleagues (2022) [15] with slight modifications was used in the determination of the total viable count.

One milliliter (1mL) of each sample was diluted in 9 mL of sterile water to make 1: 10 dilutions from which further dilutions were made. A volume of 0.1mL of the final dilution was introduced onto Mueller Hinton agar plate and spread uniformly with a sterile glass rod. The colonies on each plate were counted after incubating at 37^{o}C for 24 hours. The total viable count was calculated in colony-forming units per milliliter (cfu/mL) by using the formula:

$$\text{Total viable count (cfu/mL)} = \frac{\text{number of colonies counted}}{\text{total dilution factor x volume inoculated}}$$

2.2.2 Identification of bacteria

Identification of the microbes was carried out by using the method employed by Khan et al (2015) [16]. Selective and differential media were employed for the identification of bacterial colonies using the streak plate method. The manufacturing company's guidelines regarding how to use the various culture media were adhered to. MacConkey agar was used to identify Gram-negative enteric bacteria. Mannitol salt agar was used to identify *Staphylococcus aureus*. Salmonella Shigella (SS) agar was used to identify and differentiate between *Salmonella spp.* and *Shigella spp.* Agar plates of the various selective and differential media were streaked with each sample using a sterile inoculating loop. The plates were incubated at 37^{o}C for 24 - 48h. After incubation, microbes on the plates were identified based on a careful study of the morphological and biochemical features of their colonies on each plate.

3. RESULTS AND DISCUSSION

Consuming food contaminated with food-borne pathogens can be harmful to health. Consumption of unhygienic food can give rise to about 600 million cases of foodborne diseases ranging from diarrhoea to cancer, and approximately 420,000 deaths globally every year [17]. Controlling foodborne pathogens is therefore a crucial step in halting the emergence and spread of diseases in a population [18].

Local drinks are non-sterile products, but they must contain microorganisms within specified limits. High microbial load was observed in the samples with average viable counts ranging from 2.4 x 10^5 to 4.5 x 10^7cfu/mL (Table 1). The average microbial counts for all the drinks exceeded the specified microbial limits of $\leq 10^5$ cfu/mL for ready-to-eat foods in which all components are fully cooked for immediate sale or consumption [19]. The high bacterial counts can be attributed to the unstandardized conditions under which these products were prepared. Unsanitary environments and highly contaminated raw materials are sources of microbial contamination. Additional sources of contamination may include inadequate cleansing of utensils used before, during, and after production; dispensing of the beverages into their final containers, and incorporation of additives such as sweeteners, spices, and flavors [16,1].

Although not all the microorganisms present in the samples are disease-causing, the following pathogens were identified: *Staphylococcus spp.* (33% of samples), *Klebsiella spp.* (29%), *Enterobacter spp.* (19%), *Escherichia coli* (14%), *and Pseudomonas spp.* (14%) (Table 1 and Fig. 1). The pathogenic microbes identified in individual drinks are presented in the Appendix. Ideally, these pathogens are not supposed to be in food as they can result in food-borne illnesses. Foodborne illnesses are mostly diagnosed as gastroenteritis with associated symptoms including nausea, diarrhoea, abdominal pain, severe fever, and headaches [20]. Risiquat [21]

also identified *E. coli, Bacillus spp., S. aureus, S. faecalis, Proteus spp., Enterobacter spp., Klebsiella spp., E coli, Bacillus spp.* in Zobo ('Sobolo'), in a similar study.

Enterobacteriaceae is responsible for many serious human illnesses, including urinary tract infections, septicemia, and gastroenteritis, and poses a health risk to consumers when present in food [22]. Enterobacteriaceae therefore serve as indicator bacteria for the microbiological quality of food and the degree of hygiene in manufacturing processes [23]. This study indicated the presence of members of Enterobacteriaceae including *Klebsiella spp., Enterobacter spp., Escherichia coli, and Pseudomonas spp.* in the local drinks studied.

Pathogenic *E. coli* produces toxins that cause gastrointestinal symptoms including diarrhoea, abdominal cramps, nausea, and infantile diarrhoea. Contaminated food spreads some of these pathogenic strains, posing a significant public health threat [24,25]. There are increasing reports implicating resistant and virulent *Pseudomonas spp.* in food-borne infections. The pathogen is known to cause urinary tract infections, respiratory tract infections, bacteraemia, and gastrointestinal and systemic infections [26,27]. *Klebsiella spp.,* predominantly *K. pneumoniae* is associated not only with nosocomial illness but also with food-borne illnesses. *K. pneumoniae* isolated from food products are reported to show multi-drug resistance which is a cause for concern [28,29]. Numerous investigations have found *Enterobacter spp.* in food, which is a causative agent of hospital sepsis and urinary tract infections [16,22,]. *Salmonella spp.* infections, including Salmonellosis and enteric fever, characterized by stomach flu and potentially fatal outcomes, have a high occurrence globally [30]. Fortunately, *Salmonella* was not present in any of the local drinks sampled in this study. The periodic mandatory health screening testing conducted among food vendors in Ghana could account for this finding.

Staphylococcus spp. form part of the normal flora of the skin and mucosal surfaces of humans. However, they can produce heat-stable toxins that can cause gastrointestinal illness characterized by nausea, vomiting, and stomach cramps [31]. *Staphylococcus spp.,* contained in local drinks can transmitted into the human body not only through the gastrointestinal system but also through breeches such as wounds on the skin to cause infection.

Reports from both developed and developing countries indicate that most food-borne gastrointestinal illnesses are caused by unsanitary handling practices and/or unhygienic environments both during and after food preparation [32]. The main ways through which humans are exposed to gastrointestinal pathogens are through contaminated food or direct or indirect contact with animal and human feces [33]. These microorganisms could have been introduced into the beverages especially 'Sobolo', 'Asaana', and 'Emuduro' during packaging and handling and not during preparation because a lot of heat is employed during the preparation of these beverages [34].

Table 1. Microbiological composition of local beverages

Sample	Microorganism identified
'Asaana'	*Staphylococcus spp.* *Pseudomonas spp.* *Enterobacter spp.*
'Emuduro'	*Staphylococcus spp.* *Esherichia coli* *Klebsiella spp.* *Pseudomonas spp.*
'Brukina'	*Staphylococcus spp.* *Enterobacter spp.* *Escherichia coli*
'Abele'	*Klebsiella spp.* *Pseudomonas spp.*
'Sobolo'	*Staphylococcus spp.* *Klebsiella spp.* *Enterobacter spp.*

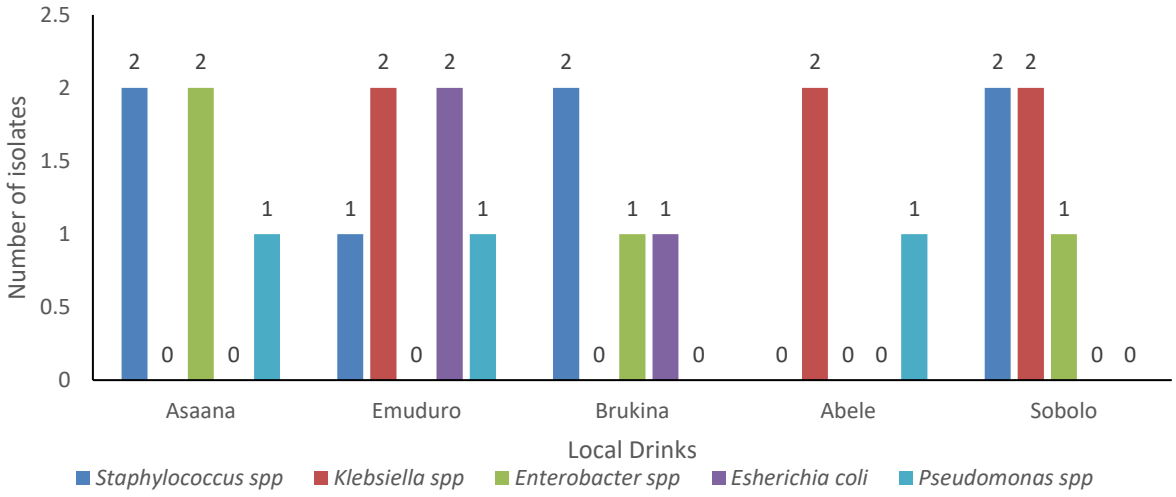

Fig. 1. Frequencies of occurrence of the microorganisms in the samples

Table 2. Total viable count of local beverages

Type of local drink	Number of samples	Average total viable count (cfu/mL) ± SEM
'Sobolo'	5	$4.1 \times 10^7 \pm 3.8 \times 10^7$
'Emuduro'	5	$4.5 \times 10^7 \pm 1.8 \times 10^7$
'Brukina'	5	$1.4 \times 10^6 \pm 4.8 \times 10^5$
'Asaana'	3	$2.4 \times 10^5 \pm 4.1 \times 10^4$
'Abele'	3	$1.3 \times 10^6 \pm 5.0 \times 10^5$

'Emuduro' had the highest contamination with a total average viable count of 4.5×10^7cfu/mL. A study conducted by Kouassi and others [35] revealed a similar high microbial count ranging from 6.5×10^9 to 3.9×10^{10} cfu/mL in Gnamakoudji, a non-alcoholic beverage made from ginger in Cote d'Ivoire. In the manufacture of 'Emuduro', chopped ginger, peppercorns, and water are ground together. The mixture is strained through a cloth strainer and the extract is boiled for a few minutes with water. Sugar is normally added after boiling. The ginger is often ground with the skin intact. Microorganisms may still be present if the washing of the ginger is insufficient. The cleanliness of the commercial machine used to grind the peppercorn and ginger may be in question since it may have been used for more than one client and may not have been thoroughly cleaned. The mixture is often strained through a strainer cloth with the aid of bare hands. Without proper hygiene, many microorganisms, including faecal ones, may be carried on the hands [35]. These unhygienic practices often result in microbial contamination.

'Asaana' was the least contaminated with a total viable count of 2.4×10^5 cfu/mL. Addo-Glover and others [36] however observed lower microbial counts between 1.82 -2.71 log10 cfu/mL in 'Asaana' collected in Kumasi, which increased after a storage period of 72 hours. Aboagye et al., [1] also reported high counts of bacteria in 'Asaana', which ranged between 1.0×10^6 and 5.94×10^8 cfu/ml for two of their samples. The disparity in microbial loads may result from the variations in the method used in the preparation of 'Asaana' and the environment within which they were prepared [36]. In the preparation of 'Asaana', the maize is steeped, malted, ground, mashed, boiled, and left overnight to allow the settling of particles. The supernatant is caramelized before drinking [4]. Microbial contaminants can be introduced during all the phases of production owing to the use of unclean utensils, unsanitary environments and the non-observance of personal hygiene by producers. 'Asaana' is mostly sold in large containers with ice cubes to cool the beverage. The ice cubes could be a source of contamination in cases where the water used in the making of the ice cubes is not properly treated or the ice cubes are not made under hygienic conditions. The ice cubes are usually made by packaging water in transparent

polyethene bags and freezing them. Some sellers introduce the ice cubes into the drink without taking off the packaging which may be contaminated from handling. Some sellers of 'Asaana' have the habit of blowing air into transparent polyethene bags into which 'Asaana' is dispensed during the sale. This could eventually lead to the transfer of microbes from the breath of the seller into the local drinks.

From this study, 'Sobolo' had a high viable count of 4.1 x 10^7 cfu/mL, which is comparable to that obtained by Aboagye et al. [1], who recorded a viable count ranging from 7.60 x 10^6 – 1.58 x 10^8cfu/mL in 'Sobolo' sampled in Accra, Ghana. In contrast, a lower total bacterial plate count ranging from 6.9 x 10^3 to 9.6 x 10^3cfu/mL was found in 'Sobolo' drinks (Zobo) by Bristone et al. [37] in Nigeria. Again, this variation in microbial loads may result from differences in the methods used in the preparation of 'Sobolo' in each location. 'Sobolo' is a refreshing drink made from the *Hibiscus sabdariffa* calyxes which has many nutritional and medicinal benefits including the management of hypertension, liver diseases, and pyrexia [6]. It is made by extracting the calyx of the flower, which is typically done with hot water for some minutes. The flowers are removed by sieving through a colander after which flavors and sweeteners are added. Contaminants could have been introduced when the hot extract is cooled down, when flavors and sweeteners are added, or when the extract is poured into sieve meshes, bottles, or polyethene bags.

Extracts of *Hibiscus sabdariffa* calyxes are known to have antimicrobial activity against Gram-positive and Gram-negative bacteria as well as *Candida albicans* [6]. Nonetheless, the concentration of active ingredients responsible for the antimicrobial activity of *Hibiscus sabdariffa* calyxes may not be enough to inhibit the growth of microorganisms when it is formulated as a drink [38].

This study reported a viable count of 1.4 x 10^6 cfu/mL in 'Brukina'. Similarly, Baidoo and Kunadu [28] recorded a high total viable count of 7.48 log10 cfu/mL in 'Brukina' in Accra, Ghana. 'Brukina' is made from millet and fermented milk, which can serve as a rich source of nutrients for microorganisms. Given that milk left at ambient temperatures to ferment creates a good environment for the growth of microorganisms because of its high nutrient content and a near-neutral pH, it is believed that such an uncontrolled fermentation procedure could support the growth of some pathogenic microorganisms as in the case of this study [39]. In some cases, fresh milk directly sourced from cows is used in the production of 'Brukina'. The fresh milk may not be expressed under hygienic conditions, and may not be pasteurized. These practices could lead to microbial contamination.

'Abele', also known as Ghanaian ice cream, is a frozen dairy product. Although ice cream is highly nutritious, it is also a great environment for bacterial growth [3]. This can account for the high bacterial count observed for 'Abele' (1.3 x 10^6 cfu/mL). In the preparation of 'Abele', powdered milk, sugar, and water are mixed, dispensed into reusable plastic cups or small transparent polyethene bags, and then frozen. The reusable plastic cups and utensils could be sources of contamination if they are not cleaned properly. The water used in the preparation could be a prime source of contamination especially if the water is not boiled or well-treated before use. To reduce microbial counts in products involving milk, producers must be trained to pasteurize milk and dispense it into sterile containers before use.

These local drinks are normally sold in plastic bottles which may be new or used. The bottles are usually rinsed in a large bowl containing water which does not facilitate proper cleansing of the bottles. Already used bottles used in the packaging of local drinks may also contain microbial contaminants, especially on the mouth of the bottle. A study by Obeng et al. [40] revealed a relatively higher occurrence of microbial contaminants in used plastic bottles than unused plastic bottles used in the packaging of 'Sobolo'.

Water, a main ingredient in producing these local drinks, can be a prime source of microbial contamination. The primary cause of the contamination of drinks made locally is water used in processing [21]. Microbial contaminants can be introduced in water through the containers in which it is stored or transported and how it is handled. Pathogenic microorganisms can be introduced into water through burst PVC pipes delivering water from the mains to consumers. Practices involving the distribution of domestic wastewater in soil can potentially cause microbial contamination of groundwater [41].

To curb the incidence of high microbial counts in local drinks which are affordable and readily available, producers and vendors of local drinks

must be educated and trained about food safety and hygienic practices. Standard Operating Procedures (SOPs) must be developed to guide producers in the preparation of these local drinks. Governmental health agencies must develop a regular monitoring system to ensure that measures regarding food safety are implemented. Producers of local drinks adhering to the necessary policies can also be approved as outlets for the wholesaling or retailing of local drinks.

4. CONCLUSION

This study assessed the bacteriological quality of local drinks sold in Amakom, Kumasi. The average viable counts of all local drinks studied were unsatisfactory as they all exceeded the acceptable limits by Food Standards Australia New Zealand [19]. Pathogenic microorganisms such as *Staphylococcus spp., Klebsiella spp., Enterobacter spp., Escherichia coli, and Pseudomonas spp.* were identified in the samples, and their consumption can be harmful to the health of consumers. Standard guidelines and good hygienic practices by producers must be enforced to ensure that wholesome local drinks are produced.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

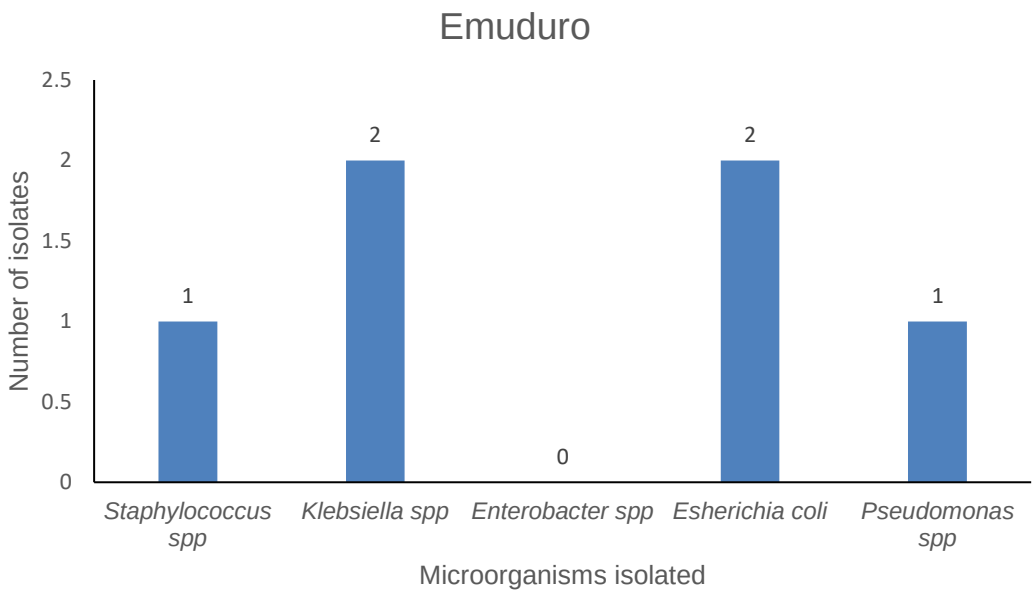

Fig. A1. Microorganisms isolated from 'Emuduro'

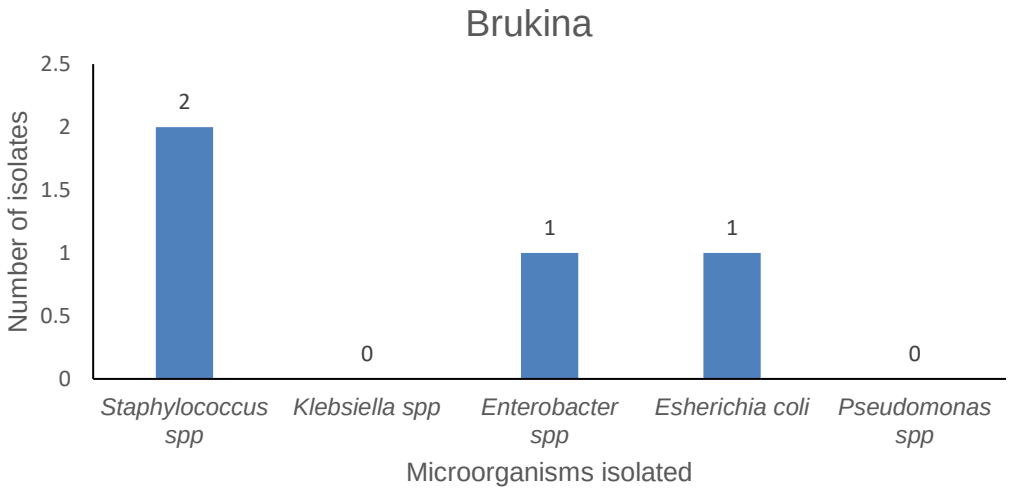

Fig. A2. Microorganisms Isolated from 'Brukina'

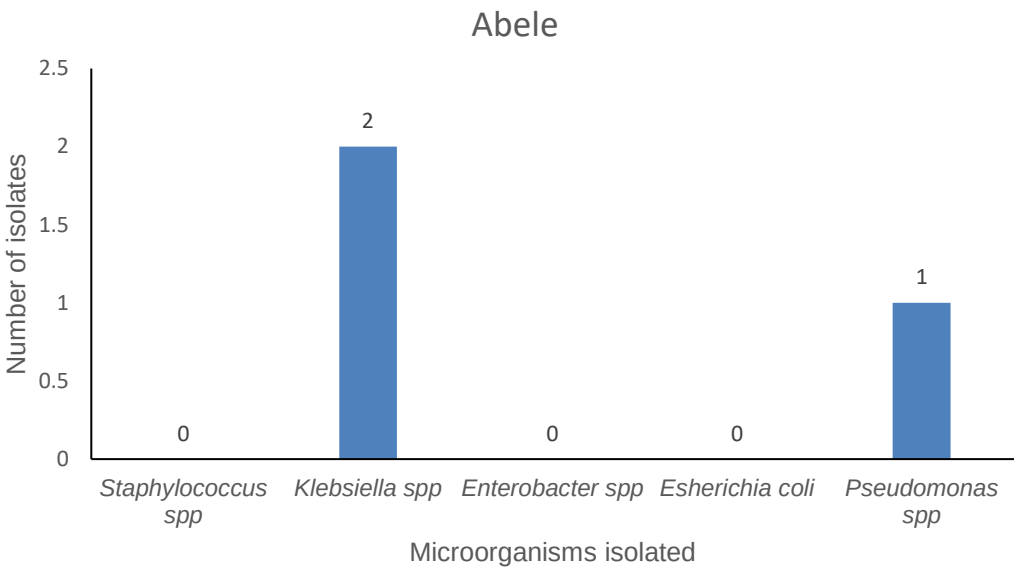

Fig. A3. Microorganisms isolated from 'Abele'

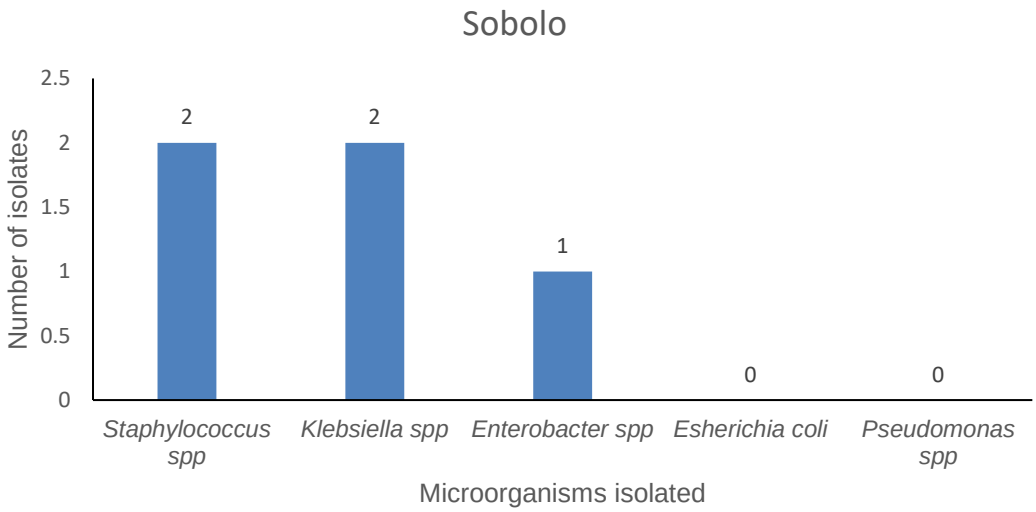

Fig. A4. Microorganisms isolated from 'Sobolo'

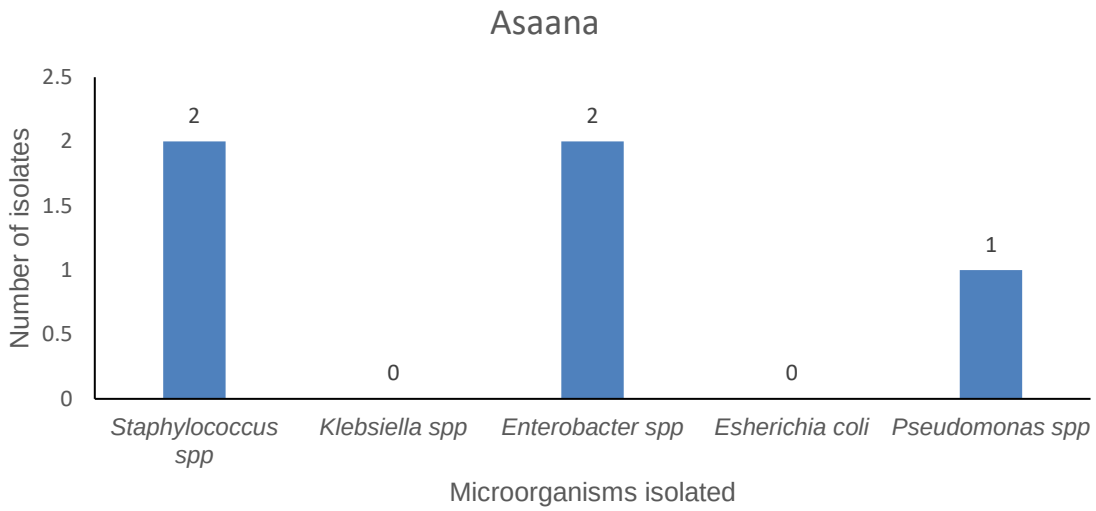

Fig A5. Microorganisms isolated from 'Asaana'

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