Assessment of some selected pathogenic microorganisms of selected soft drinks sold on the markets in Cape Coast Metropolis, Ghana

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ARTICLE INFO	ABSTRACT
	Soft drinks are widely consumed globally and are generally considered safe due to their carbonation and low pH. However, microbial contamination during processing and storage remains a concern. Ten different brands of soft drinks were purchased from markets in Cape Coast based on consumer preference. Bacteriological analyses were carried out on these brands of soft drinks using the standard procedures. The results indicated negative for *Escherichia coli* for all tested samples but color changes occurred on the media during some weeks. However, *Salmonella* spp. was detected in some samples, with one case exhibiting too numerous to count microbial load. Therefore, colony counts ranged from 0 cfu/mL to TNTC (too numerous to count). These findings indicate that soft drinks are safe for consumption, however, occasionally contamination may occur. Hence, regular monitoring should be carried out by regulatory bodies to ensure that soft drinks produced and sold at all times are safe for consumption.

1. Introduction

A soft drink is a globally consumed, non-alcoholic beverage, acidic beverage (pH 2.5-4.0) that distinguishes itself from alcoholic drinks (1).

They are a staple in many diets worldwide, ranking second to water as a source of hydration, energy, micronutrients, and salt replenishment (2). These drinks are commonly consumed during activities like sports, leisure, and social events such as traditional marriages, weddings, funerals, etc. (3). Soft drinks may harm dental and overall health, especially in children and adolescents due to their high sugar and acids content, which, can cause tooth decay, dental caries, obesity and increase the risk of type 2 diabetes (4).

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According to (5), the average high school student consumes 1-2 bottles per week of soft drinks.

Similarly, (6) mentions that adolescents in Ghana particularly prefer consuming carbonated soft drinks.The high level of consumption and demand for soft drinks makes quality control challenging, especially during sterilization and purification, affecting the quality of the products (3). Soft drinks have a highly acidic pH (2.5-4.0), and contain 1.5 to 4 volumes of carbon dioxide (CO_2) hence inhibiting microbial development (1). However, the acidic level may not be enough to prevent microbial growth entirely. This is due to the high water content, vitamins, and minerals, in the soft drinks which provides an attractive environment for microbes. They are a favorable medium for the growth of numerous bacteria, some of which can be dangerous to humans (7). Enteric pathogens particularly *Escherichia coli* and *Salmonella* spp. were found to survive in beer and coca cola for 48 h but not in diet cola due to differences in pH (8). Microbial contamination of soft drinks typically occurs during the production process due to the raw materials, factory environment, microbiological state of the equipment and packages, and lack of hygiene (9). Additionally, the water used for the preparation can be a major source of microbial contaminants such as total coliforms, fecal coliforms, fecal streptococci, etc (10).

The market in Ghana has experienced an expansion in both imported and domestically made non-alcoholic beverages. Although, consumer affluence determines their beverage selection (11). In Ghana, most studies on the microbiological quality of soft drinks have focused on locally produced beverages. Research on drinks such as 'Asaana', 'Sobolo', Brukina', 'Emuduro',

'Abele', and 'Asaana' (caramelized corn drink) and Lamugin have revealed significant microbial contamination (12–14). These studies focused only on locally produced drinks without consideration for industrially produced soft drinks.

In Ethiopia, industrially produced soft drinks such as energy drinks and fruit juices revealed microbial contamination (15). However there are limited studies on the microbiological safety of industrially produced soft drinks in Ghana. Hence this study seeks to assess some selected pathogenic microbes specifically *Escherichia coli* and *Salmonella* spp in selected industrially produced soft drinks sold on the markets in Cape Coast, Ghana.

2. Materials and Methods

2.1. Study area

The study was conducted in Cape Coast Metropolis from June 2022 to August 2022. Cape Coast Metropolis is the only metropolis out of twenty-three (23) districts in the Central Region. It lies within latitude 5°20' and longitudes 1°11' to 1°41' west of the Greenwich Meridian and bounded to the south by the Gulf of Guinea. According to (16), the population of the metropolis stands at one hundred and eighty-nine thousand, nine hundred and twenty-five (189,925).The metropolis occupies an area of approximately 122 square kilometers. The capital town of the Cape Coast Metropolis is Cape Coast. It's known for its role in the transatlantic slave trade. The land area of Cape Coast is mostly sloppy with many hills and valleys and the

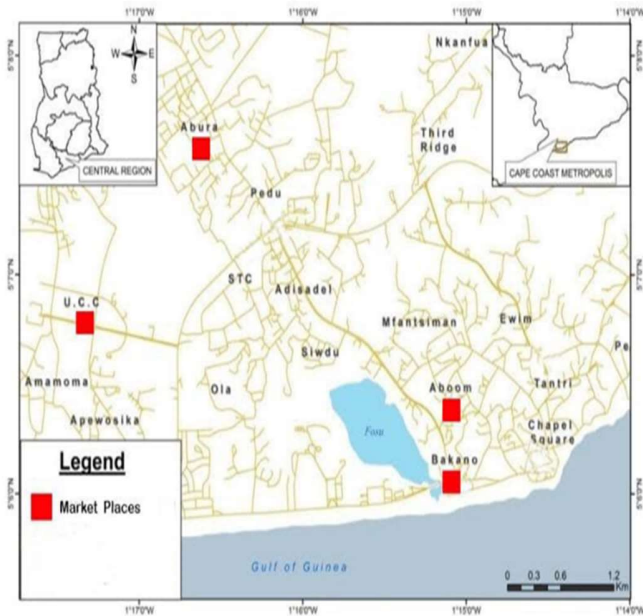locals are chiefly involved in fishing and related activities.

Figure 1. A map showing the marketplaces in Cape Coast Metropolis of Ghana (17).

2.2. Data collection

A stratified random sampling technique was used to divide Cape Coast Metropolis into five strata namely Kotokuraba, Abura, UCC Science, Bakaano, and Kingsway. A market survey was conducted at different retail shops within each town in the metropolis to determine the common brands of soft drinks that were mostly purchased. At the end of the market survey, eighteen (18) different brands of soft drinks were the most common brands of soft drinks.

The three (3) most purchased soft drink brands across all retail stores were Bel-squeeze, Tropical Splash, and Kaesar apple. On the other hand, planet cocktail, Bigoo

cola, Puma cocktail, Veraldo pear malt drink, Special Ice red grape, Malta Guinness, and Coca-Cola were not purchased across all the retail shops but appeared to be purchased in most of the retail shops.

2.3. Microbial loads analyses

The soft drink samples were purchased from retail shops that were aware of how frequently and in what order the soft drinks were purchased.

The soft drink samples were purchased on the same day as the microbiological analysis was scheduled.

2.4. Preparation of brilliant green agar

The Brilliant Green Agar (Sigma Aldrich) was prepared following the manufacturer's instructions. A measured 26.3g of brilliant green powder was dissolved in 500 mL of distilled water and swirled until a uniform mixture was obtained. The prepared media was allowed to cool and distributed into the various Petri dishes under sterile conditions in a laminar flow hood (Cruma EN ISO 9001:2015 Barcelona).

2.5. Test for microbial loads

A 100 µL aliquot of each sample of soft drink was pipetted onto both the Endo Agar and Brilliant Green Agar media and allowed to solidify. After incubation, the colonies of Escherichia coli and Salmonella spp were counted.

2.6. Preparation for endo agar

The Endo Agar (VWR BDH Chemicals, Geldenaaksebaan 464B3001 Leuven Belgium) was prepared following the manufacturer's instructions. A measured 20.75g of Endo Agar dissolved in 500 mL of distilled water. The media was dispensed into various Petri dishes in a laminar flow hood (Cruma EN ISO 9001:2015 Barcelona).

2.7 Test for microbial loads

A 100 µL aliquot of each sample of soft drink was pipetted onto both the Endo Agar and Brilliant Green Agar media and allowed to solidify. After incubation, the colonies of *Escherichia coli* and *Salmonella* spp were counted.

2.8. Statistical analysis

The data was analyzed using Microsoft Excel version 2013. The software was used to determine the most commonly purchased brands of soft drinks by consumers across all the retail shops.

3. Results

A market survey was conducted to determine the most purchased brands of soft drinks sold on the markets in Cape Coast Metropolis to assess the microbiological safety of the soft drink for human consumption. In addition, all the sampled soft drinks were not physically damaged or near it expiration.Microbial analyses were carried out on ten (10) of the most purchased brands of soft drinks in the Cape Coast Metropolis.

The data obtained from the market survey across the five markets in the Metropolis is presented as follows: Where √ indicates that the soft drink is present× indicates the absence of the soft drink.

The table and graph below represent specifically the number of soft drinks present across the shops in the five markets of the Metropolis. The market survey was conducted across three shops in each of the markets. Across all the shops in the Cape Coast Metropolis, Three brands of soft drinks were the most purchased across all the shops in the Cape Coast metropolis; Kaesar Apple, Bel Squeeze, and Tropical Splash
Other brands of soft drinks which include Bigoo Cola, Puma Cocktail, Bel Cola, and Pukka Herbal appeared to be sold in most of the shops across the markets. The rest of the soft drinks appeared in one or two shops across some of the markets in the Metropolis.

Table 1. The most purchased brands of soft drinks across all the markets in Cape Coast Metropolis

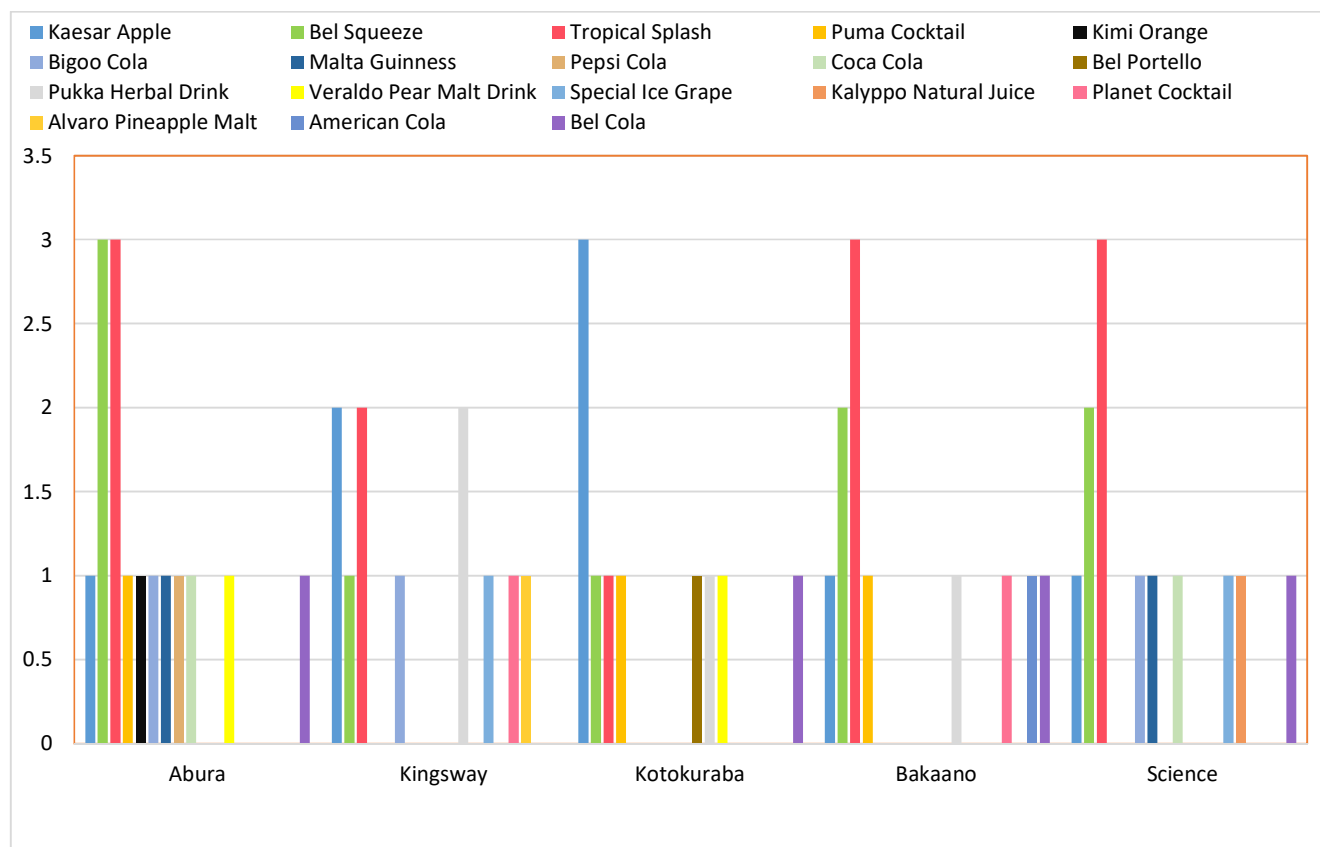| Drinks/ Towns	Abura	Kingsway	Kotokuraba	Bakaano	Science
Kaesar Apple	√	√	√	√	√
Bel Squeeze	√	√	√	√	√
Tropical Splash	√	√	√	√	√
Bel Cola	√	×	√	√	√
Puma Cocktail	√	×	√	√	×
Kimi Orange	√	×	×	×	×
Bigoo Cola	√	√	×	×	√
Malta Guinness	√	×	×	×	√
Pepsi Cola	√	×	×	×	×
Coca Cola	√	×	×	×	√
Bel Portello	×	×	√	×	×
Pukka Herbal	×	√	√	√	×
Veraldo Pear Malt	√	×	√	×	×
Special Ice Grape	×	√	×	×	√
Kalyppo Natural Juice	×	×	×	×	√
Planet Cocktail	×	√	×	√	×
Alvaro Pineapple Malt	×	√	×	×	×
AmericanCola	×	×	×	√	×

Figure 2. The most purchased brands of soft drinks sold on the markets in Cape Coast Metropolis.

Microbial analysis of the soft drinks

Samples from ten brands of soft drinks were analyzed for their microbial loads. Throughout the six-week experiment, a negative control was compared with the samples to confirm the growth of the media originating from the soft drink samples. The following codes were used to represent the sampled soft drinks.

BiC- Bigoo Cola

KA- Kaesar Apple

PC- Puma Cocktail

PlC- Planet Cocktail

BS- Bel Squeeze

BC- Bel Cola

TS- Tropical Splash

SG- Special Grape

PH- Pukka Herbal

V- Veraldo Pear Malt

The microbial analyses for *Escherichia coli* over the six weeks experiments showed no growth on both media but few color changes were observed during the first week in some of the samples on the Brilliant Green Media. Additionally, the microbial analyses for *Salmonella typhi* over six weeks showed minimal growth, with only one instance recorded in sample PC during the fourth week with too numerous to count (TNTC). While slight color changes were observed in some media during the second and fourth weeks, these did not correspond to microbial growth. Overall, the majority of samples throughout the experiment

showed no growth, indicating the general absence of

Salmonella spp.

Table 2. Results for the microbial analyses of *Escherichia coli* on endo agar (cfu/mL) for six weeks.

Weeks/Samples	KA	BiC	PC	PlC	BS	TS	SG	PH	V	BC
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0

As shown in Table 2, for the six-week experiments, no growth or color change was recorded to indicate the presence of *Escherichia coli* in the sampled soft drinks on the Endo media.

Table 3. Results for the microbial analyses of *Salmonella* spp. on endo agar (cfu/mL) for six weeks.

Weeks/Samples	KA	BiC	PC	PlC	BS	TS	SG	PH	V	BC
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	TNTC	0	0	0	0	2	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0

As shown in Table 3, *Salmonella typhi* was generally absent in the sampled drinks throughout the six-week study. However, notable exceptions were observed in week 4, where PC exhibited significant growth, and PH a low count of 2.

Table 4. Results for microbial analyses of *Escherichia coli* on brilliant green agar for six weeks (cfu/mL).

Weeks/Samples	KA	BiC	PC	PlC	BS	TS	SG	PH	V	BC
1	0	0	Color Change	0	0	0	0	0	Color Change	Half changed color
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0

From Table 4, the microbial analyses for six weeks did not detect the presence of *Escherichia coli* in any of the samples. However, slight color changes were observed in some media during the first week.

Table 5. Results for the microbial analyses of *Salmonella* spp. on brilliant green agar (cfu/mL) for six weeks.

Weeks/Samples	KA	BiC	PC	PlC	BS	TS	SG	PH	V	BC
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	Quarter changed color	Quarter changed color	0	0	0	0
3	1	0	0	0	0	0	0	0	0	0
4	Quarter changed color	0	Quarter changed color	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0

From Table 5, color changes were observed on some of the samples during the second and fourth week and KA had a minimal growth of 1 during the third week.

4. Discussion

Recent economic policies in Ghana have been flexible, and as a result, several companies in the country are involved in the production of soft drinks (18).

Although it is commonly believed that soft drinks are safe for consumption and will not result in food poisoning, the likelihood cannot be completely ignored. Due to the influx of soft drinks into the country, this study analyzed the most purchased brands of soft drinks that are sold on the markets in Cape Coast. Based on this study, the most purchased brands of soft drinks were carbonated soft drinks packaged in plastic bottles.

This confirms the findings of (5), the average high school student consumes 1-2 bottles per week of soft drinks and (6), these students particularly preferred carbonated soft drinks. The carbonated soft drinks being produced in the country are affordable for purchase as stated by (11) that the preference for these drinks is significantly influenced by income. This study also assessed the microbial quality of the most purchased soft drinks sold on the market in the Cape Coast Metropolis. These soft drinks have been accepted and approved by the regulatory standards provided by the Food and Drugs Authority which result in their commercialization. In this study, the average microbial load of the carbonated soft drinks was 0-2 cfu/µL for Salmonella spp. on endo agar and 0 cfu/µL for Escherichia coli. There was an exception where there was a growth that was too numerous to count for Salmonella spp. Additionally, the average microbial load for Salmonella spp. on BGA was 0-1 cfu/µL and color changes for Escherichia coli.

The acidity of the carbonated soft drinks inhibits the growth of pathogenic microbes but other spoilage organisms survive in a low pH environment. Hence *Escherichia coli* and *Salmonella* spp are not able to grow during the storage period which may have accounted for their low detection in the sampled soft drinks. However, carbonated soft drinks have been found to contain some traces of *Escherichia coli* and *Salmonella spp.* as reported by Sheth et al. (8). (15) reported higher microbial loads in industrially produced drinks, including juices, energy drink, and bottled water with mean count of 3.56 ± 0.32 log cfu/mL for aerobic count plate, 3.44 ± 0.16 log cfu/mL for Enterobacteriaceae count, 3.40 ± 0.06 log cfu/mL for total coliform count, and 3.42± 0.12 log cfu/mL for total mold and yeasts count.

There was an exception where there was a growth that was too numerous to count for *Salmonella* spp. Additionally, the average microbial load for *Salmonella* spp. on BGA was 0-1 cfu/µL and color changes for *Escherichia coli.*

(15) reported significantly higher microbial loads and a diverse range of microbial organisms in industrially produced drinks, including juices, energy drink, and bottled water. The mean count was 3.56 ± 0.32 log cfu/mL for the aerobic count plate, 3.44 ± 0.16 log cfu/mL Enterobacteriaceae count, 3.40 ± 0.06 log cfu/mL total coliform count, and 3.42± 0.12 log cfu/mL total mold and yeasts count were observed in the products. The acidity of the carbonated soft drinks inhibits the growth of pathogenic microbes but other spoilage organisms survive in a low pH environment. However, carbonated soft drinks have been found to

contain some traces of *Escherichia coli* and *Salmonella* spp. as reported by Sheth et al. (8). Hence *Escherichia coli* and *Salmonella* spp. are not able to grow during the storage period which may have accounted for their low detection in the sampled soft drinks.

According to the Ghana Standards Authority, the acceptable limit for coliform counts in soft drinks is <1*10² cfu/mL which shows that the growth observed in the sampled soft drinks was within the acceptable limits. Whereas the industrially produced carbonated soft drinks are safe for consumption, the locally produced beverages as reported by (12–14) contained an array of microbes including *E.coli* in significantly higher quantities. Although most of the sampled soft drinks recorded no growth, most of the drinks recorded color changes for the Brilliant green agar.

Limitations

Since most businesses did not engage in the sale of all the carbonated soft drinks manufactured in the nation, the complete amount of carbonated soft drinks sold on the market could not be ascertained. Additionally, it was impossible to ascertain the length of time that drinks had been available on the market because most of them are provided by wholesalers rather than manufacturers.

5. Conclusion

The overall analysis revealed that, among all the soft drinks sold in the Cape Coast Metropolis, carbonated soft drinks are the most frequently purchased, while traditional soft drinks are no longer in demand compared to newer options on the market. Furthermore, microbial analysis indicated that most

carbonated soft drinks produced in the country are safe for consumption.

References

1. Azeredo DR, Alvarenga V, Sant'Ana AS, Srur AU. An overview of microorganisms and factors contributing for the microbial stability of carbonated soft drinks. Food. Res. Int, 2016; 82:136-44.

2. Fazeenah, A. A. A literary review on potential health risks of "soft drinks". World J Pharm Med Res. 2021; 7(1):62-70.

3. Godwill EA, Jane IC, Scholastica IU, Marcellus U, Eugene AL, Gloria OA. Determination of some soft drink constituents and contamination by some heavy metals in Nigeria. Toxicol Rep. 2015; 2:384-90.

4. Tahmassebi JF, Banihani AJ. Impact of soft drinks to health and economy: A critical review. Eur. Arch. Paediatr. Dent. 2020; 21(1):109-17.

5. Frederick V, Obed H. Knowledge, practice and perception of taking soft drinks with food and the metabolic effect on high school students in Ghana. Endocrinol Metab. 2017;1(1):103-19.

6. Atorkey P, Akwei M, Asare-Doku W. Consumption of carbonated soft drinks among Ghanaian adolescents: Associations with socio-demographic factors, health risk factors and psychological distress. Nutr. Health. 2021; 27(3):329-36.

7. Khan MM, Islam MT, Chowdhury MM, Alim SR. Assessment of microbiological quality of some drinks sold in the streets of Dhaka University Campus in Bangladesh. Int. J. Food Contam. 2015; 2:1-5.

8. Sheth NK, Wisniewski TR, Franson TR. Survival of Enteric Pathogens in Common Beverages: An *in vitro* study. Am. J. Gastroenterol (Springer Nature). 1988; 83(6):658.

9. Park YJ, Chen J. Microbial quality of soft drinks served by the dispensing machines in fast food restaurants and

convenience stores in Griffin, Georgia, and surrounding areas. J. Food Prot. 2009; 72(12):2607-10.

10. Rashed N, Aftab UM, Azizul HM, Saurab KM, Mrityunjoy A, Majibur RM. Microbiological study of vendor and packed fruit juices locally available in Dhaka city, Bangladesh. Int. Food Res. J. 2013; 20(2):1011.

12. Aboagye G, Gbolonyo-Cass S, Kortei NK, Annan T. Microbial evaluation and some proposed good manufacturing practices of locally prepared malted corn drink ("asaana") and Hibiscus sabdarifa calyxes extract ("sobolo") beverages sold at a university cafeteria in Ghana. Sci. Afr. 2020; 8:e00330.

13. Baidoo EA, Osei-Djarbeng SN, Amonoo-Neizer J. Bacteriological Profile of Vended Local Drinks in Amakom, Kumasi, Ghana. J. Adv. Microbiol. 2023; 23(12):65-76.

14. Asante-Poku A, Sakyi S, Darko IN, Asare P, Osei-Wusu S, Bonsu C, Yeboah-Manu D, Otchere ID. Microbial Quality Assessment of Locally Produced Millet Drink "Lamugin" and Hibiscus sabdariffa Calyxes Extract Beverage "Sobolo" in Accra, Ghana. Adv. Microbiol. 2024; 14(10):544-54.

15. Hiko A, Muktar Y. Levels of microbial contamination in non-alcoholic beverages from selected eastern Ethiopian towns markets. Sci. Afr. 2020; 7:e00223.

16. Ghana Statistical Service. Ghana 2021 population and housing census. SERVICE. 2021;

17. Sam EF, Adu-Boahen K, Kissah-Korsah K. Assessing the factors that influence public transport mode preference and patronage: Perspectives of students of University of Cape Coast (UCC), Ghana. Int J Dev Sustain. 2014;3(2):323–36.

18. Akande BR. Nutritional Value and Trace Metal Contamination of Soft Drinks Sold on the Ghanaian Market [PhD Thesis]. University of Ghana; 2019.