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THE ANTIMICROBIAL ACTIVITY OF THE FLOWER EXTRACT OF *Hibiscus sabdariffa* Linn. (ZOBO FLOWER) ON SOME FREQUENTLY ISOLATED BACTERIA

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ABSTRACT

Aim: this study aimed to determine the antimicrobial activity of *hibiscus sabdariffa* (zobo) flower extract on bacterial isolates. With the rise of the global prevalence of antimicrobial resistance, medicinal plants have garnered considerable attention as a promising source for discovering new antibacterial drugs.place and duration of study: *hibiscus sabdariffa*flower was obtained from the fruit garden market, d/line, port harcourt, and verified by a plant taxonomist, this study lasted between february 2024 and december 2024mehodology:blended flowers of *hibiscus sabdariffa* were prepared as ethanol and aqueous extract. Branded and unbranded commercial zobo drinks were purchased from the supermarket and mile 3 market, respectively. The preparations were tested against isolates of *staphylococcus aureus, streptococcus pyogenes, escherichia coli, klebsiella pneumoniae, proteus spp., and pseudomonas spp* obtained from alfrose diagnostic medical center, rumuigbo, port harcourt using agar disc diffusion and direct inoculation methods at 24- and 72-hours intervals. Result: results revealed a 12 mm zone of inhibition (zoi) for *s. Pyogenes* and *proteus spp*. Which were the highest, 10 mm zoi for *s. Aureus* followed by 8 mm zoi for *e. Coli*, *k. Pneumoniae,* and *pseudomonas spp*. The minimum inhibitory concentration (mic) for test organisms was generally 1:10 dilution (32 mg/ml) for all organisms. Ethanolic extract showed increased antibacterial activity compared to aqueous extracts including branded and unbranded zobo drinks. Conclusion: this present study on *hibiscus sabdariffa* extracts showed it possess antimicrobial activity at higher concentrations. I, therefore, recommend the flower of *hibiscus sabdariffa* to most pharmaceutical companies to be included in the preparation of their drugs.

1. INTRODUCTION

1.1 Background to the Study

Plants have been used for thousands of years by humans to treat various ailments, including bacterial infections. Many ancestral medicinal uses have been scientifically corroborated using modern techniques that have allowed the identification of active compounds and the characterization of their antibacterial mechanism of action. Some of the compounds of plant origin that have been most studied in recent years are complex plant extracts and essential oils as well as pure compounds such as terpenoids, polyphenols and alkaloids (Álvarez-Martínez *et al.,* 2021).

Plants produce an invaluable source of secondary metabolites in response to environmental factors such as attack by herbivores, abiotic stress, or interspecific interactions (Yang *et al*., 2018). Since ancient times, humans have used these secondary metabolites of plants in various fields, including medicine and gastronomy. The vast chemical diversity of plant secondary metabolites and their long history of traditional use make plants very attractive natural reservoirs for research into the discovery of new antimicrobial compounds. Rapid technological advancement and the application of new, increasingly efficient methodologies have allowed the identification and characterization of numerous antibacterial agents in recent years (Katz and Baltz, 2016).

Phytochemicals can act through different mechanisms and target sites compared to traditional antibiotics, hence their combination with conventional antibiotics has been proposed to provide superior efficacy in suppressing the development of resistance (Abreu *et al*., 2012).

The foundation of modern therapeutics is based on the use of plants and their extract in preparing herbal drugs, enabling man in establishing an empirical medicinal system (Gumisiriza *et al.,* 2021).

As stated by WHO, 80% of the world population uses phytochemical agents for the treatment of diseases. Bioactive compounds of plants have remarkable potential to treat diseases (Hussain *et al.,* 2018). Phytobioactive compounds have been extensively used in traditional medicines for the treatment of various diseases including type 2 diabetes. The higher antidiabetic potential of these phytobioactives has been shown in various animal models, and plant bioactive-based medicines currently are in great demand in the market due to their multiple efficacies and higher availability (Ganesan *et al.,* 2017).

Recently, with the global prevalence of antibiotic-resistant bacteria has become a cause for widespread concern. This disconcerting trend is further compounded by the absence of new antibiotic classes being developed, ultimately giving rise to what is commonly referred to as the "antibacterial crisis" (Schcolnik-Cabrera, 2023). With the rise of this global problem, medicinal plants have garnered considerable attention as a promising source for the discovery of new antibacterial drugs as these plants possess

a wide array of chemical constituents, such as alkaloids, flavonoids, terpenoids, and phenolic compounds, which exhibit diverse biological activities, including potent antibacterial properties (Sun and Shahrajabian, 2023).

Hibiscus sabdariffa L. (zobo plant) often used in traditional medicine is rich in phytochemicals like polyphenols especially anthocyanins, polysaccharides, and organic acids thus having enormous perspective in modern therapeutic uses (Riaz and Chopra, 2018).

1.2 Aim of the Study

This project research aimed to determine the antimicrobial activity of *Hibiscus sabdariffa* (zobo) extract on bacterial isolates.

1.3 Objectives of the Study

The objectives of this study are:

1. To ascertain the antibacterial activity of *Hibiscus sabdariffa* (zobo flower) extract on bacterial isolates.
2. To ascertain the MIC and different concentrations of *Hibiscus sabdariffa* (zobo flower) extract that shows antibacterial inhibition.
3. To compare the size of the inhibition zone on the bacterial isolates.
4. To ascertain if the degree of inhibition by ethanol extract of the *Hibiscus sabdariffa* (zobo flower) at different concentrations is attributed to its age.
5. To compare the degree of inhibition of ethanol, aqueous extracts of *Hibiscus sabdariffa, and* branded and unbranded commercial Zobo drinks.

1.4 Significance of the Study

1. Significant determination of the validity of the use of *Hibiscus sabdariffa* in herbal and modern medicine.
2. Elucidating the therapeutic effects of *Hibiscus sabdariffa* in addition to its nutritional benefits to man.
3. To provide data on the degree of antibacterial activity of *Hibiscus sabdariffa* against frequent bacterial isolates.

1.5 Scope of the Study

This study involved the use of two gram-positive and four gram-negative frequently isolated bacteria in the Medical Microbiology Laboratory. *Hibiscus sabdariffa* (zobo flower) has secondary metabolites (active ingredients) effective for its use in herbal medicine, extracted using ethanol. This study is targeted towards determining the antibacterial activity of these active ingredients against six frequently isolated bacteria species.

2. MATERIALS AND METHODS

2.1 Study Area

This research was conducted at the Medical Microbiology Laboratory of the Faculty of Medical Laboratory Science, Rivers State University, Port Harcourt. Rivers State University is situated in the Mile 3, Diobu area of Port Harcourt, in the heart of the Niger Delta region, an urban location within Nigeria with major economic and cultural activities contributing to its status as a dynamic and vibrant community. The city is situated in the southernmost part of the country, in the delta of the Niger River, positioned at coordinates 4.75^{0}N latitude

and 7.00^{0}E longitude, situated along the Bonny River within the Niger Delta. It covers an area of 369 km² and, as of 2023, has an estimated population of 3,637,000 inhabitants, a significant increase from the 1,382,592 recorded during the 2006 census. It is famous as a center of transportation and industry, with a large number of oil refineries and petroleum-producing companies working.

Rivers State University as of 2017 had approximately 3,000 staff members and has a student body exceeding 30,000 it is equipped with state-of-the-art academic facilities, research centers, and libraries that cater to a multitude of disciplines. The campus landscape is a blend of modern infrastructure and natural surroundings, providing students and staff with a conducive environment for learning, collaboration, and personal growth. It holds the distinction of being Nigeria's first technological university and the first university established within the Niger Delta. Port Harcourt, the capital of Rivers State, Nigeria, the city of Port Harcourt features a diverse landscape of commercial and residential buildings, along with religious, educational, and healthcare institutions ranging from tertiary to primary levels.

2.2 Plant Collection and Identification

The plant material of *Hibiscus sabdariffa*flower used in this study was obtained from the Fruit Garden Market, D/Line, Port Harcourt, and was verified by a plant taxonomist in the Department of Applied and Environmental Biology, Rivers State University, Port Harcourt. Branded and unbranded commercial Zobo drinks were purchased from the supermarket and Mile 3 market, respectively.

2.3 Collection and Identification of Test Organisms

Stock cultures of *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumonia, Proteus spp., and Pseudomonas spp* were obtained from Alfrose Diagnostic Medical Center, Rumuigbo; Rivers State and identified using colony morphology, gram staining, and biochemical tests.

2.4 Gram Stain

The isolated organisms from the plates were smeared on a clean glass slide by placing a loopful of water and the inoculum on the slide and allowed to air dry. The air-dried smear was fixed and covered with a crystal violet stain for 60 seconds. After which excess stain was washed off with distilled water. All water was tipped off and covered and smeared with Lugol's iodine for 60 seconds. Iodine was washed off with distilled water and decolorized rapidly (few seconds) with acetone–alcohol. It was washed off immediately with distilled water and covered the smear with Safranin stain for 60 seconds. The stain was washed off with clean water. The back of various slides were wiped to clean and placed in a draining rack for the smear to air-dry. A drop of oil immersion was placed on the smear and examined microscopically, first with the 40X objective to check the staining and to see the distribution of material, and then with the oil immersion

objective to report the bacteria and cells (Cheesbrough, 2006).

2.5 Biochemical Test

Catalase Test

A drop of 3% hydrogen peroxide was made on a clean glass slide. With a wooden applicator, a colony of a test bacterium was brought in contact with the hydrogen peroxide, and it was observed for bubbles. The presence of bubbles indicated a positive catalase test, and no bubbles showed a negative catalase test (Davis and Pezzlo, 2023).

Coagulase Test (Free coagulase)

A clean test tube was filled with 0.5 ml of 1 in 10 diluted rabbit plasma and 0.1 ml of overnight broth culture of test bacteria were added. The test tube was incubated at 37°C and observed between 4 hours for gelling of the plasma indicating a positive test, which remains in place even after inverting the tube (Davis and Pezzlo, 2023).

Indole Test

The isolate was inoculated in 5ml peptone water and was incubated at 37°C for 24 hours. After 24 hours, 3 drops of Kovac's reagent were added to the inoculum, after which it was then shaken and observed for reaction. A positive reaction is indicated by the development of a red color in the reagent layer above the broth within a minute and a negative reaction is indicated by the reagent retaining its yellow color (Davis and Pezzlo, 2023).

Citrate Test

A suspension of the test organism was streaked and butt-stabbed on a Simmon's citrate Agar slant. It was then incubated at 37°C and observed for a bright blue color in the medium which indicates a positive result and a negative result if there is no change (Davis and Pezzlo, 2023).

Oxidase Test

A good-sized amount of bacterial colony was picked using a wooden stick from a plate culture and placed on a piece of filter paper first. One drop of oxidase reagent was added. A positive reaction occurred within 10-20 seconds and with a bluish-purple color that progressively became more purple. The reaction was not read after 30 seconds (Davis and Pezzlo, 2023).

Urease Test

The tube of urea broth was inoculated with bacterial colony and incubated in a water bath for 3-12 hours at optimal temperature, 37°C. Urease production was indicated by a bright pink (fuchsia) color on the slant after incubation (Davis and Pezzlo, 2023).

2.6 Preparation of Plant Extracts Methodology

The *Hibiscus sabdariffa* (Zobo flower) were cleaned, sun-dried for 3 days, blended into a fine powder, and then stored in a sterile airtight bottle.

2.7 Preparation of Ethanol Extract

Thirty-two grams of the powdered *Hibiscus sabdariffa* flower was weighed using a weighing balance and dispensed into a sterile conical flask. 100ml of 95% ethanol was measured using a sterile measuring

cylinder and transferred to the conical flask, mixed thoroughly, and left standing for 24 hours. The mixture was then filtered using a sterile filter paper and sterilized by autoclaving. This was referred to as the neat or ethanol extract.

2.8 Preparation of Aqueous Extract

Thirty-two grams of the powdered *Hibiscus sabdariffa* flowerwere weighed and dissolved in 100ml of distilled water in a sterile conical flask. It was mixed thoroughly by stirring using a glass stirring rod and left to stand for 24 hours. This was then filtered using a sterile filter paper, and sterilized by autoclaving. Filtrate was referred to as the aqueous extract.

2.9 Dilution

Four separate test tubes were arranged in four rows in a test tube rack each for ethanol extract, aqueous extract, and branded and unbranded commercial Zobo drinks, respectively. 9 ml (milliliters) of distilled water was pipetted into each of the tubes properly labeled. Serial dilutions were made by transferring one milliliter (1ml) from the ethanol extract (neat tube) to tube I, it was thoroughly mixed and 1ml transferred to tube II, it was thoroughly mixed again, and 1ml to tube III, and thoroughly mixed again, and 1ml transferred to tube IV, giving a concentration of 1:10 (0.1ml), 1:100 (0.01ml), 1:1000 (0.001ml) and 1:10000 (0.0001ml) respectively.

Serial dilutions for aqueous extract, branded and unbranded commercial Zobo drinks were made by transferring one milliliter (1ml) from the respective neat tubes to 9 ml (milliliters) of distilled water pipetted into each tube in rows II, III, and IV. Serial dilutions for each row were properly carried out, with the same procedure used in the dilution of the ethanol extract giving a concentration of 1:10 (0.1ml), 1:100 (0.01ml), 1:1000 (0.001ml) and 1:10000 (0.0001ml) respectively.

2.10 Preparation of Materials

Sensitivity Discs

The sensitivity discs were prepared byperforating Whatman's filter paper into circular shapes of 6mm using a perforator. The punched circular-shaped filter paper discs were transferred into a bijou bottle, and sterilized by autoclaving at 121°C for 15 minutes.

2.11 Agar Disc Diffusion Method

The sterilized filter paper discs were impregnated with 10ul of the undiluted ethanol flower extracts (32g/100ml). Also, 10ul of the diluted ethanol flower extracts of the *Hibiscus sabdariffa* (1:10, 1:100, 1:1000, and 1:10000) were impregnated into the sterilized filter paper discs each. A positive control of the ethanol flower extract was made using 10ul of 95% ethanol, impregnated into a sterilized filter paper.

Thereafter, six well-dried and properly labeled MHA plates were inoculated with test organisms (*Staphylococcus aureus, Streptococcus pyogenes, Klebsiella spp, Proteus spp., Escherichia coli,*and *Pseudomonas spp*) by pour plate method separately on individual plates and excess was discarded. This was followed by

aseptically transferring the control (ethanol-impregnated disc), disc impregnated with the ethanol extract (32g/100ml), and discs impregnated with the serial dilutions on the surface of the inoculated plates with the aid of forceps sterilized by flaming in-between each transfer. Six Mueller Hinton Agar (MHA)plates were used for the six bacteria for each solution and each plate contained five discs representing five different concentrations i.e., the stock and four serial dilutions, except plates for ethanol extract with additional 95% ethanol impregnated disc (control) giving 6 different concentrations. 10µl of each of the undiluted concentrations and serial dilutions (1:10, 1:100, 1:1000, and 1:10000) of the aqueous extract, branded and unbranded commercial Zobo drinks were impregnated into sterilized filter paper disc each. The inoculated (MHA)plates with test discs were incubated at 37°C. After incubating for 24 hours, plates that showed clear zones of inhibition were noted, and the zone diameter was measured in millimeters (mm). Results were recorded.

2.12 Statistical Analysis

Results obtained from this study were presented in tables and comparisons were expressed in graphical representation.

3.RESULTS

The results obtained from the antibacterial activity of *Hibiscus sabdariffa* (zobo flower) flower extract on bacterial isolates are presented and explained in this chapter in tables and figures.

Table 3.1 shows the morphological and biochemical identification of test organisms. Isolate A on gram staining showed gram-positive clustered cocci, and yielded positive results for coagulase and catalase tests to confirm*Staphylococcus aureus*. Isolate B with β hemolysis on blood agar on gram staining showed gram-positive cocci in chains, yielded negative results for coagulase and catalase tests to confirm*Streptococcus pyogenes*. Colony morphology of Isolate C with a pink color, circular, moist, smooth colonies on MacConkey agar, isolate D with pink, round, smooth, mucoid colony on MacConkey agar, isolate E with swarming characteristics on agar plates and an ammonia smell. Isolate C-F on gram staining showed negative rods, and were confirmed as *Escherichia coli*, *Klebsiella spp*, *Proteus spp*, and *Pseudomonas spp*. after carrying out indole, citrate, urease, and oxidase tests, respectively.

Table 3.1: Morphological and Biochemical Identification of Test Organisms

Unconfirmed Isolates	Gram	Coagulase	Catalase	Indole	Citrate	Urease	Oxidase	Confirmed Isolate
A	+	+	+					*S. aureus*
B	+	-	-					*S. pyogenes*
C	-			+	-			*E. coli*

D	-	-	+			*K. pneumoniae*
E	-			+	-	*Proteus spp.*
F	-			-	+	*Pseudomonas spp.*

KEY: **+ =Positive, - = Negative**

Table 3.2 below shows the antibacterial activity of *Hibiscus sabdariffa* (zobo flower) flower extract on bacterial isolates after day 1 (24 hours) using the agar diffusion method. From the table, aqueous extract was more effective than ethanolic extract against *K. pneumoniae, E. coli and Pseudomonas spp.* while ethanolic extract was more effective than aqueous extract against *Proteus spp.* The MIC was 1:10 dilution (32 mg/ml) for *S. aureus, S. pyogenes, K. pneumoniae, E. coli,*and *Proteus spp.* except *Pseudomonas spp.*

Table 3.2: Antibacterial activity of *Hibiscus sabdariffa* (zobo flower) flower extract on bacterial isolates after day 1 (24 hours) using agar diffusion method

	Diameter of Zone of Inhibition (mm)						
Solvent	**Dilutions**	***S. aureus***	***S. pyogenes***	***K. pneumoniae***	***E. coli***	***Proteus spp.***	***Pseudomonas spp.***
	Control	8mm	10mm	6mm	6mm	8mm	6mm
Ethanol	Absolute	10mm	12mm	8mm	8mm	12mm	8mm
	1:10	6mm	8mm	6mm	6mm	8mm	R
	1:100	R	R	R	R	R	R
	1:1000	R	R	R	R	R	R
	1:10000	R	R	R	R	R	R
Aqueous	Absolute	10mm	12mm	10mm	10mm	6mm	10mm
	1:10	6mm	8mm	6mm		R	6mm
	1:100	R	R	R	6mm	R	R
	1:1000	R	R	R	R	R	R

1:10000	R	R	R	R	R	R	
				R			

Table 3.3 below shows the antibacterial activity of *Hibiscus sabdariffa* (zobo flower) flower extract on bacterial isolates after day 1 (24 hours) using direct inoculation method. From the table, aqueous extract was more effective than ethanolic extract against *K. pneumoniae* and *Pseudomonas spp*. while ethanolic extract was more effective than aqueous extract against *S. aureus, S. pyogenes and Proteus spp.* The MIC was 1:10 dilution (32 mg/ml) for *S. aureus, S. pyogenes, K. pneumoniae, E. coli* except for *Pseudomonas spp* and *Proteus spp.*

Table 3.3: Antibacterial activity of *Hibiscus sabdariffa* (zobo flower) flower extract on bacterial isolates after day 1 (24 hours) using direct inoculation method

	Diameter of Zone of Inhibition (mm)						
Solvent	**Dilutions**	***S. aureus***	***S. pyogenes***	***K. pneumoniae***	***E. coli***	***Proteus spp.***	***Pseudomonas spp.***
	Control	6mm	10mm	5mm	6mm	8mm	6mm
Ethanol	Absolute	8mm	12mm	6mm	8mm	10mm	8mm
	1:10	5mm	6mm	4mm	5mm	6mm	R
	1:100	R	R	R	R	R	R
	1:1000	R	R	R	R	R	R
	1:10000	R	R	R	R	R	R
Aqueous	Absolute	8mm	10mm	8mm	8mm	5mm	10mm
	1:10	4mm	6mm	4mm	5mm	R	6mm
	1:100	R	R	R	R	R	R
	1:1000	R	R	R	R	R	R
	1:10000	R	R	R	R	R	R

Table 3.4 below shows the antibacterial activity of *Hibiscus sabdariffa* (zobo flower) flower extract on bacterial isolates after day 3 (72 hours) using agar diffusion method. From the table, there was decreasing antibacterial activity against *K. pneumoniae* after 3 days and increasing antibacterial activity against *S. pyogenes*

while there was no change in antibacterial activity against other organisms. The MIC was 1:10 dilution (32 mg/ml) for *S. aureus, S. pyogenes, K. pneumoniae, E. coli and Proteus spp.* except for *Pseudomonas spp.*

Table 3.4: Antibacterial Activity of *Hibiscus sabdariffa* (zobo flower) Flower Extract on Bacterial Isolates after day 3 (72 hours) using agar Diffusion Method

		Diameter of Zone of Inhibition (mm)					
Solvent	**Dilutions**	***S. aureus***	***S. pyogenes***	***K. pneumoniae***	***E. coli***	***Proteus spp.***	***Pseudomonas spp.***
	Control	8mm	10mm	6mm	7mm	10mm	6mm
Ethanol	Absolute	10mm	13mm	10mm	8mm	12mm	8mm
	1:10	6mm	8mm	8mm	6mm	8mm	R
	1:100	R	R	R	R	R	R
	1:1000	R	R	R	R	R	R
	1:10000	R	R	R	R	R	R

Table 3.5 below shows the antibacterial activity of Ethanolic *Hibiscus sabdariffa* (zobo flower) flower extract on bacterial isolates after day 3 (72 hours) using the direct inoculation method. From the table, increasing antibacterial activity was observed against *S. aureus, K. pneumonia,*and *Proteus spp.* while there was no change in antibacterial activity against other organisms. The MIC was 1:10 dilution (32 mg/ml) for all test organisms except for *Pseudomonas spp.*

Figure 3.1 below shows the comparative antibacterial activity of branded and unbranded commercially sold *Hibiscus sabdariffa* drink. From the figure, branded commercially sold *Hibiscus sabdariffa* drink showed antibacterial activity against *K. pneumoniae* and *E. coli* while unbranded commercially sold *Hibiscus sabdariffa* drink showed antibacterial activity against *S. aureus* and *E. coli*. Also, antibacterial resistance was observed against *S. pyogenes,Proteus spp,* and *Pseudomonas spp*. Overall, there was lesser antibacterial activity of branded and unbranded commercially sold *Hibiscus sabdariffa* drink when compared with ethanolic and aqueous extracts.

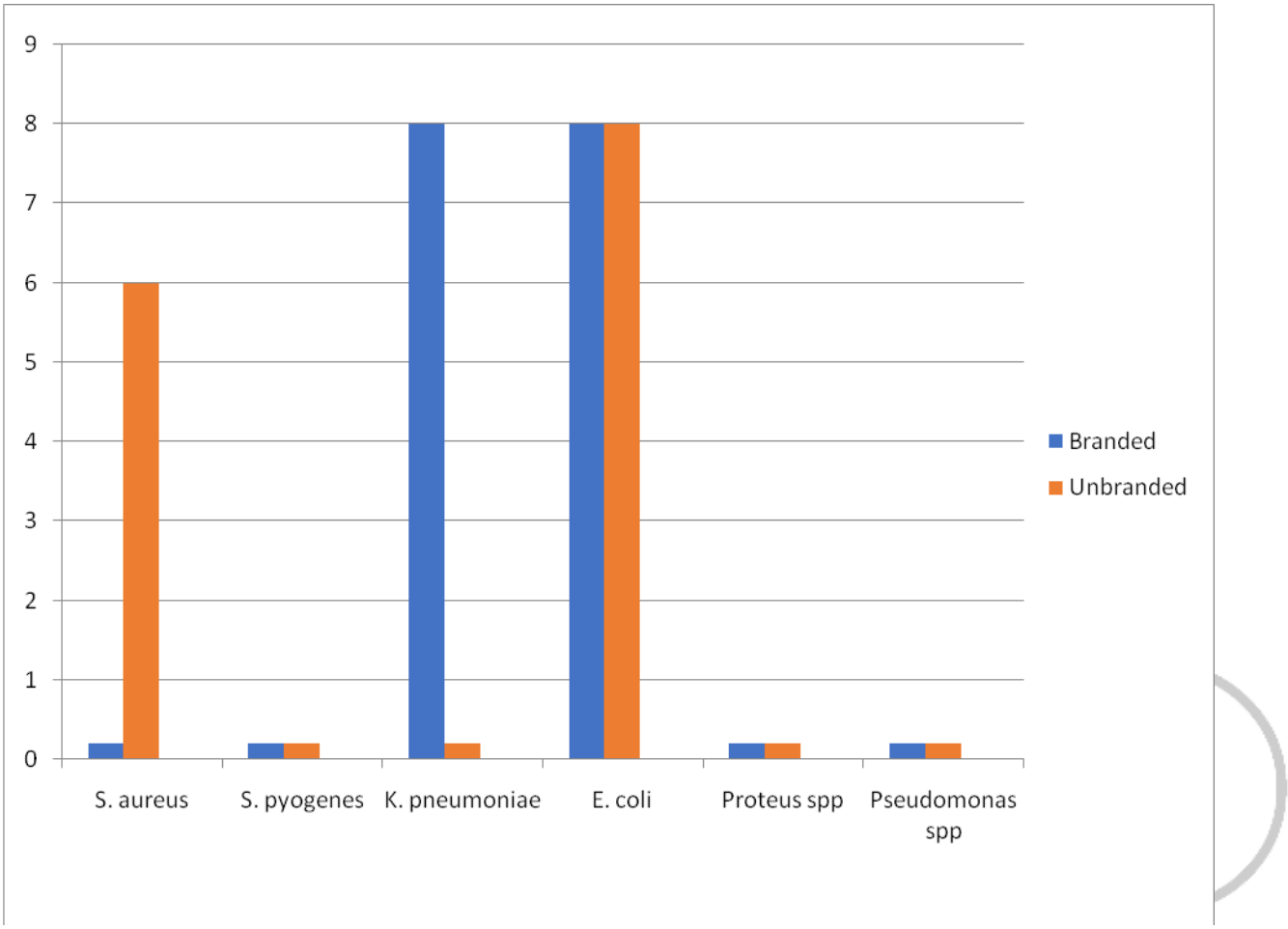

Figure 3.1: Comparative Antibacterial Activity of Branded and Unbranded Commercially Sold *Hibiscus sabdariffa* Drink

Figure 3.2 below shows the comparative antibacterial activity between agar disc diffusion and direct inoculation methods of day 1 ethanolic *Hibiscus sabdariffa* flower extract. Higher antibacterial activity was observed for *S. aureus*, *Klebsiella spp.* and *Proteus spp.* in agar disc diffusion than direct inoculation method.

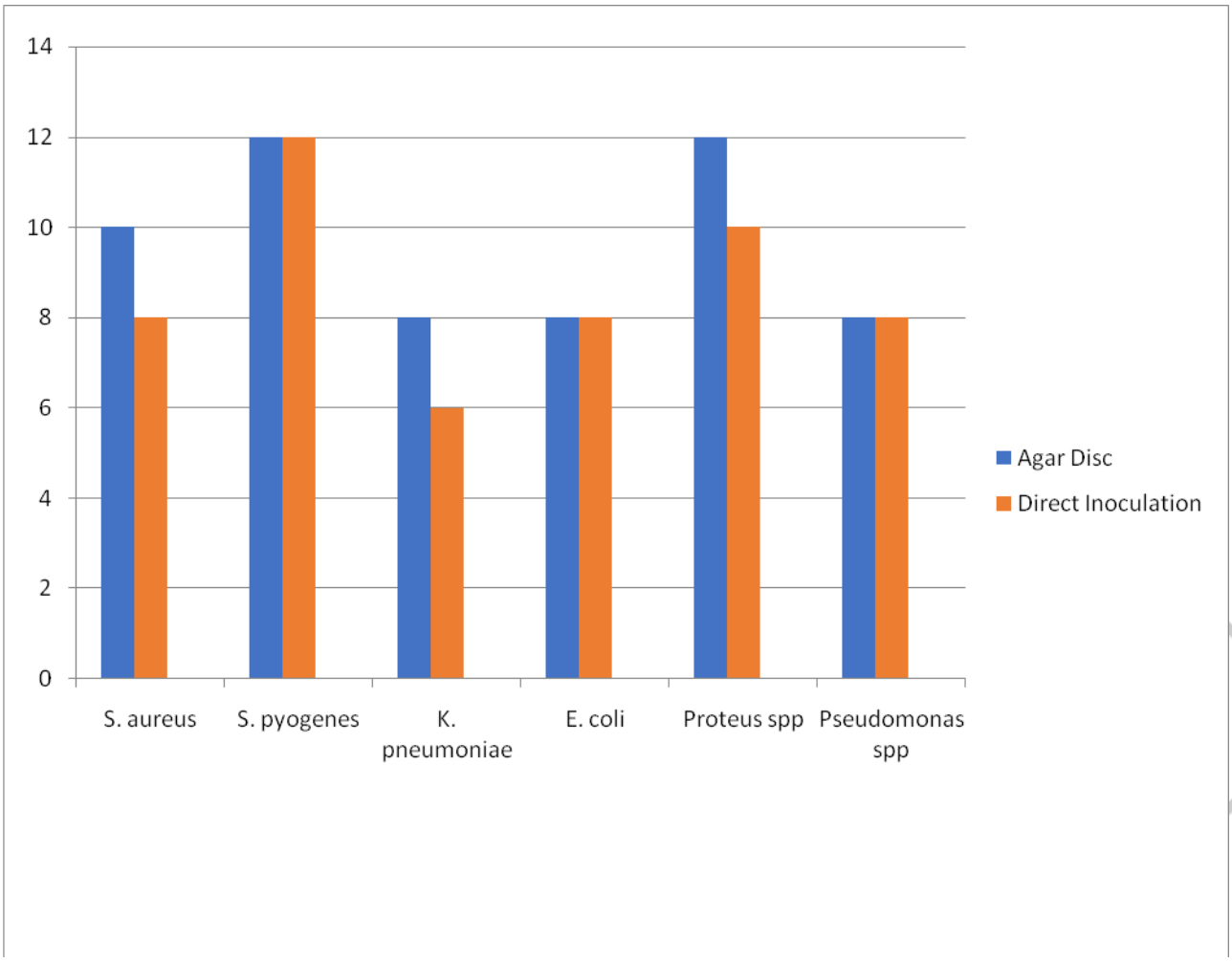

Figure 3.2:Comparative Antibacterial Activity between Agar Disc Diffusion and direct Inoculation Methods of Day 1 Ethanolic *Hibiscus sabdariffa* Flower Extract

Figure 3.3 below shows the comparative antibacterial activity between agar disc diffusion and direct inoculation methods of day 1 ethanolic *Hibiscus sabdariffa* flower extract. Higher antibacterial activity was observed for *S. pyogenes* in agar disc diffusion than direct inoculation method.

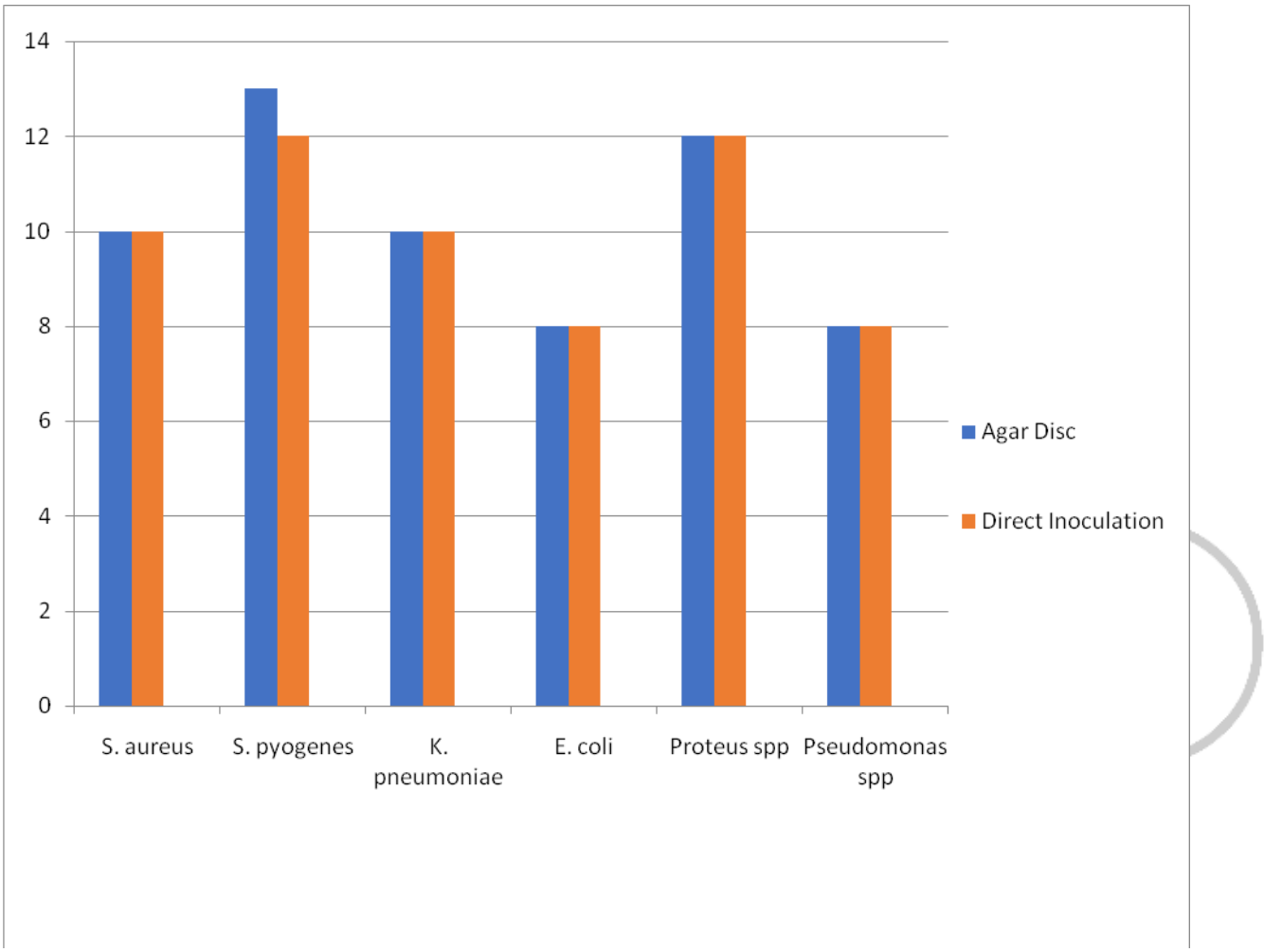

Figure 3.3:Comparative Antibacterial Activity between Agar Disc Diffusion and Direct Inoculation Methods of Day 3 Ethanolic *Hibiscus sabdariffa* Flower Extract

4.

5. **DISCUSSION**

The antimicrobial properties of plants have been investigated by several studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties. In this present study, crude *H. sabdariffa* flower extracts showed antimicrobial activity of 12 mm zone of inhibition for *S. pyogenes* and *Proteus spp.* which were the highest, 10 mm zone of inhibition for *S. aureus* followed by 8 mm zone of inhibition for *E. coli*, *K. pneumoniae* and *Pseudomonas spp*. at 320 mg/mL concentration. The minimum inhibitory concentration (MIC) for test organisms was generally 1:10 dilution (32 mg/ml) for *S. aureus, S. pyogenes, K. pneumoniae, E. coli,*and *Proteus spp.* except for *Pseudomonas spp.* The antibacterial activity of *H. sabdariffa* may be attributed to the presence of various bioactive compounds such as hibiscus acid. These compounds possess antimicrobial properties and have been found to disrupt membrane integrity and increase the permeability of bacterial cells. In addition to hibiscus acid, other polyphenols present in *H. sabdariffa* may contribute to its antibacterial activity. Polyphenols are known to possess antioxidant and anti-carcinogenic activities and exhibit antibacterial properties (Hafiz *et al*., 2021).

This study agrees with that of Alaga *et al*. (2014) who demonstrated that *Hibiscus sabdariffa* extracts exhibit significant antibacterial activity against various pathogens, including *Staphylococcus aureus* (44.7mm on aqueous extract and 47.9mm on ethanol extract) and *Escherichia coli* (43.5mm on aqueous extract and 45.8mm on ethanol extract). However, there were higher zones of inhibitions in comparison to this present study. One study showed that methanolic extracts had the highest antibacterial effect, suggesting that the solvent used for extraction plays a crucial role in antimicrobial efficacy (Hafiz *et al*., 2021). These studies also suggested that the antimicrobial activity of *Hibiscus sabdariffa* is attributed to its rich phytochemical composition, including flavonoids, anthocyanins, and organic acids. These compounds may disrupt microbial cell membranes and interfere with metabolic processes (Okwu *et al*., 2023).This study is also in agreement with the study by Venkatesan *et al*. (2024) which investigated the antimicrobial activity of *H. sabdariffa* (Roselle) crude extract against a range of pathogens and found that *H. sabdariffa* (Roselle) extract had a significant antimicrobial effect, against *Staphylococcus aureus* (13mm zone of inhibition) and *Escherichia coli* (11mm zone of inhibition). However, in contrast to this present study,*Pseudomonas aeruginosa* was resistant. Also, in contrast to this present study, the minimum inhibitory concentration (MIC) by Venkatesan *et al*. (2024) was 128 µg/mL for *S. aureus* concentration followed by *E. coli* 256 µg/mL. These studies collectively affirm the potential of *Hibiscus sabdariffa* as a source of natural antimicrobial agents, with promising applications in food preservation and medicine.

Furthermore, results from day 1 and 3 experiments as well as branded and unbranded drinks showed reduced antimicrobial activity and antimicrobial resistance to some test organisms. This indicates the stability and shelf-life of the extracts influence their antibacterial efficacy over time. Also observed in this study were the variations of ingredients used in the preparation of branded and unbranded *Hibiscus sabdariffa* drinks. While most unbranded *Hibiscus sabdariffa* drinks contained garlic and honey, branded *Hibiscus sabdariffa* drinks contained additional ingredients such as clove, dates, and beetroots including garlic. The presence of garlic, honey, and additional ingredients like clove, dates, and beetroots in *Hibiscus sabdariffa* drinks can have significant implications for antimicrobial activity.

Known for its antimicrobial properties, garlic contains allicin, which can inhibit the growth of various bacteria and fungi. Its inclusion in both unbranded and branded drinks enhance their overall antimicrobial potential. Honey is recognized for its antibacterial qualities, largely due to its low water activity and the presence of hydrogen peroxide. It can help preserve the drink and provide a natural sweetness without compromising microbial activity. Clove oil has potent antimicrobial properties due to compounds like eugenol, which can act against bacteria and fungi. Its addition in branded drinks may enhance their effectiveness against a broader range of pathogens. While primarily a source of natural sugars and nutrients, dates may also have some antimicrobial properties, although they are less pronounced compared to other ingredients. Their role is likely more about nutritional benefits rather than direct antimicrobial effects. Beetroots have been studied for their health benefits, including potential antimicrobial activity, though this is less established compared to garlic or clove.

They may contribute to the overall health profile of the drink. In summary, the combination of these ingredients in branded hibiscus drinks may lead to enhanced antimicrobial activity, offering not only flavor and nutrition but also potential health benefits. This could make these drinks more appealing to health-conscious consumers looking for natural ways to support their immune systems. However, the specific antimicrobial efficacy would depend on the concentrations of these ingredients and the types of microorganisms targeted.

6. Conclusion

To conclude, this present study on hibiscus sabdariffa extracts showed it possesses antimicrobial activity at higher concentration. These findings highlight the potential of *H. sabdariffa* as a natural source of antibacterial agents that could be utilized in the development of new therapeutic agents against bacterial infections. However, it is important to note that most of the studies conducted on the antibacterial activity of *H. sabdariffa* were

performed in vitro. To confirm the efficacy and safety of *H. sabdariffa* as an antibacterial agent, further studies are needed, particularly, purification, pharmacokinetics, in vivo animal models, and clinical trials.

Recommendations

1. Standardization of Extracts: It should be ensured that both ethanolic, aqueous, methanolic and other solvent extracts are standardized for concentration and active compounds. This will facilitate comparison between the extraction methods.
2. Selection of Bacterial Strains: A diverse range of bacterial strains, including both Gram-positive and Gram-negative bacteria, should be used to comprehensively assess antibacterial activity.
3. Control Experiments: This study used 95% ethanol as positive control. Further studies of control samples with known antibiotics to benchmark the effectiveness of Hibiscus extracts against bacterial growth.
4. Phytochemical Analysis: A thorough phytochemical screening of various Hibiscus sabdariffa extracts should be carried out to identify the active compounds responsible for their antibacterial activity.
5. Mechanism of Action Studies: Further studies should explore the potential mechanisms through which the extracts exert their antibacterial effects, such as disrupting cell membranes or inhibiting enzymatic activity.
6. In Vivo Studies: Further studies should consider conducting in vivo studies to assess the therapeutic potential, toxicity, and safety of Hibiscus extracts in a biological system.

Contribution to Knowledge

This study revealed that:

1. *Hibiscus sabdariffa* flower extracts possess antimicrobial activity at higher concentration.
2. *S. pyogenes and Proteus spp.* are much susceptible to *Hibiscus sabdariffa* flower extracts compared to *S. aureus, E. coli, K. pneumoniae and Pseudomonas spp.*
3. Branded and unbranded Zobo drinks are good antimicrobial agents depending on the concentration of *Hibiscus sabdariffa* flower extracts and another ingredient with antimicrobial properties used in its preparation.
4. The minimum inhibitory concentration (MIC) for these test organisms is generally 1:10 dilution (32 mg/ml) as further dilutions showed no significant antimicrobial activity.

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