PREVALENCE OF ANTIBIOTIC RESISTANCE OF GRAM POSITIVE AND GRAM NEGATIVE ORGANISMS ISOLATED FROM ZOBO SOLD IN MAKURDI.

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Abstract: This study investigated the antibiotic susceptibility profile of thirty two (32) bacteria isolated from thirteen (13) unspiced samples of zobo drink sold in six markets including a University campus all located in Makurdi the capital city of Benue State. Bacterial isolates were morphologically, culturally and biochemically characterized using standard bacteriological techniques. Susceptibility patterns to Ten (10) antibiotics each for Gram Positive and Gram negative bacteria were determined by the Kirby-Bauer disc diffusion technique. Species belonging to nine genera from which *Staphylococcus* spp., *Escherichia coli*, and *Salmonella* spp. were the most prevalent was recorded. All Gram positive isolates were resistant to Erythromycin (E) (100%) followed by both Ciprofloxacin (CPX) and Ampiclox (APX) (85.7%) but were all sensitive to Gentamicin (CN), Rifampicin (RD), Streptomycin (S) (100%), and Chloramphenicol (CH) (85.7%). For the Gram negative bacteria isolates; all identified organisms were resistant to Ceporex (CEP), Nalidixic (NA) (100%) followed by Septrin (SXT) (92.3%) but sensitive to Gentamicin (CN) (92.3%) and Streptomycin (S) (77%). This study has provided information for public health associated with antibiotic resistance to Erythromycin among others in zobo drink. It also calls for health concern as a wide array of bacteria including pathogenic types were isolated from zobo drink sold in Makurdi. Furthermore, the antibiotic susceptibility/resistance patterns in the study would inform the choice of suitable drugs for chemotherapy by health practitioners in Makurdi and its environs.

Keywords: Antibiotic; Erythromycin; *Escherichia coli*; Gentamicin; Makurdi; *Salmonella* spp.; *Staphylococcus* spp.; Zobo.

Introduction

Zobo is the common name derived from "zoborodo" a local Hausa name for *Hibiscus sabdariffa*. The zobo drink usually is non-alcoholic, and has gained popularity over the years, known to be widely consumed in the Northern part of Nigeria mostly dominated by the Hausa's, and fairly consumed in other parts of the country (Raimi, 2013). Despite the increasing popularity of the drink as a result of its health and nutritional benefits, one of the barriers for its preparation in huge quantity is poor shelf life. The calyces used for preparation of the drink have been reported to be a major source of contamination as they house many food spoilage microbes particularly fungi and bacteria (Ogiehor and Nwafor, 2004). Zobo calyx or petals of Roselle plant has different varieties. The dried matured calyx of the flower of Hibiscus plant may be red, bright red or brown which accounts for the different colours of Zobo drinks (Shruthi and Ramachandra, 2019). The juice can be extracted either by using hot or cold water at different length of time depending on choice. Despite its medicinal and nutritional values, the drink does not have good keeping quality because of contamination. Roselle drink is reported to contain enteropathogenic microorganisms up to 2.49×10^4 cfu/mL. This is hazardous to the consumers especially when a large volume of the drink is taken (Eaton and Groopman, 2013).

According to Omemu *et al*. (2006), the drink can contain some microorganisms responsible for food spoilage. Presently, the production processes lacks modern mechanization or standardization across the country. Consequently, the shelf life of the drink is less than two days (Sarathewati and Taniwiryono, 2020). Furthermore, the method of packaging or dispensing zobo drink in nylon or plastic containers before retailing, and poor hygienic practices as well as lack of running water, toilet, proper storage and waste disposal facilities at preparation and service points has resulted in poor unsanitary conditions and exposure to potential contaminants with increased risk to public health (Raimi, 2013).

Antibiotic resistance is a growing global health challenge where many potential pathogenic bacteria show resistance to a particular or multiple antibiotics. As such, infections with antibiotic-resistance bacteria have become the main cause of tumors and heart diseases. Therefore, several susceptibility tests or assays are required to determine the ability of antibiotics to inhibit the growth of microorganisms isolated from samples of different food products (Walsh and Fanning, 2008). An increasing antimicrobial resistance has resulted in morbidity and mortality from treatment failures and increased health care costs. Appropriate use of antimicrobial drugs has unquestionable benefits, but physicians and the public frequently use these agents inappropriately; hence, it has become necessary to perform the antimicrobial susceptibility test as a routine (Uddhav and Sahthivel, 2013).

Materials and Methods

Sample collection and isolation

Thirteen samples of unspiced zobo were purchased using 500 mL sterile glass bottles from six locations in Makurdi; Wurukun Market (WM), Wadata Market (WDM), North Bank Market (NBM), Modern Market (MM), Kanshio Area (KA), and University of Agriculture Makurdi (UAM). Nutrient Agar (NA), Eosine Methylene Blue Agar (EMBA) and Salmonella-Shigella Agar (SSA) media were used for isolation of bacteria. Samples were inoculated by pour plate method and primary cultures streaked on appropriate media to obtain pure cultures. Discrete colonies were inoculated on slants and kept for further studies.

Biochemical characterization of bacteria isolates

Morphological characterization of isolates was done by Gram staining following microscopic examination. All bacteria were cultured on NA prior to biochemical tests (viz. oxidase, indole, catalase, coagulase, citrate, and sugar fermentation test). The resultant characteristics were compared with those of known taxa using Bergey's Manual of Determinative Bacteriology (Cheesbrough, 2005; Kigigha *et al*., 2018).

Oxidase test

A freshly prepared 0.2 g tetra-methyl-phenylene-diamine hydrochloride in 20 mL distilled water was flooded over inoculated plates after 24 hours incubation, and the excess immediately drained off. Colonies with dark purple colour were positive for coliforms (Bristone *et al*., 2018).

Indole test

An emulsified colony of the test organism was cultured in tryptophan broth and incubated at 37^{o}C for 24 hours, and 0.5 mL Kovac's reagent added to the broth culture. Positive results were characterized by development of a red alcohol layer on top of the reagent within one minute. The test was use to identify *Escherichia coli* (Cheesbrough, 2005; Ayandele, 2015).

Catalase test

The test was carried out to differentiate *Staphylococcus* spp. from *Streptococcus* spp. and other catalase-positive from catalase-negative bacteria. A pure colony of the test isolate was transferred onto a clean grease-freeglass slide. Thereafter, a drop of 3% hydrogen peroxide was added and production of gas bubbles recorded as positive result (Bristone *et al*., 2018).

Coagulase test

A pure culture was aseptically transferred to a drop of serum on a clean grease-free glass slide, emulsified and observed for clumping. Presence of clumping within 10 seconds indicated a positive test. This test was used to identify *Staphylococcus aureus* (Bristone *et al*., 2018).

Citrate utilization test

A sterile inoculating needle was use to inoculate Simon's citrate agar slants with the test organism and incubated at 37^{o}C for 24 - 72 hours. The development of a deep blue colour indicated a positive result and the test was use to identify coliforms (Cheesbrough, 2005; Izah *et al*., 2015).

Sugar fermentation test

The ability of isolates to utilize sugars was tested using glucose, lactose, sucrose, maltose and fructose. The fermentation broth comprised 0.5 g sodium chloride (NaCl) and 0.0189 mg phenol red in 100 mL of distilled water. Five milliliters (1% solution) of each sugar was separately added into each test tube containing inverted Durham tubes. The test tubes and contents were autoclaved at 121^{o}C for 10 minutes. After cooling, isolates were inoculated and incubated at 37^{o}C up to 72 hours. Change in colour from red to yellow was positive for acid production and collection of gas bubbles in Durham tubes positive for gas production. The test was conducted in replicates.

Antimicrobial Susceptibility Test

The Kirby Bauer disc diffusion method was employed (Addis and Sisay, 2015). Bacterial colonies were inoculated in sterile normal saline and incubated at 37˚C to obtain bacterial density of 3×10^{8} mL^{-1} as determined by McFarland standard scale number 1. The culture was streaked uniformly onto freshly prepared Muller Hinton's agar plates using disposable sterile swabs. Plates were allowed to dry and discs of multiple antibiotics mounted. Antibiotics used were: Ciproflox (10 mcg), Norfloxacin (10 mcg), Gentamicin (10 mcg), Amoxyl (20 mcg), Streptomycin (30 mcg), Rifampicin (20 mcg), Erythromycin (30 mcg), Chloramphenicol (30 mcg), Ampiclox (20 mcg) and Levofloxacin (20 mcg) for Gram positive bacteria. And, Travid (10 mcg), Reflacine (10 mcg), Ciproflox (10 mcg), Augmentin (30 mcg), Gentamicin (10 mcg), Streptomycin (30 mcg), Ceporex (10 mcg), Nalidixic Acid (30 mcg), Septrin (30 mcg) and Amplicin (30 mcg) for Gram negative. The plates were incubated at 37°C for 24 hours and zones of inhibition measured (Cheesbrough, 2006; Bauer *et al.*, 2006; Umar *et al.*, 2015) and interpreted using the NCCLS standard charts (2008).

Results and Discussion

Tables 1 and 2 presents the antimicrobial sensitivity pattern of Gram positive and Gram negative bacteria with reference to the percentage of resistance in both Gram Positive and Gram negative bacteria isolated from zobo. The antibiotic susceptibility pattern of the isolates from zobo revealed alarming results that calls for prompt attention against unsanitary production and consumption of product as well as indiscriminate use of antibiotics by the public. All the isolates of Gram positive bacteria were resistant to erythromycin (100%), followed by a high resistance to ciprofloxacin and ampiclox both at 85.7%. Gram positive isolates were also highly resistant to norfloxacin and amoxyl both at 71.4%. However, none of the Gram positive isolates from zobo were resistant to gentamicin, streptomycin and rifampicin. Etang *et al*. (2017) also reported that all the gram positive isolates from kunuzaki in Calabar were 100% resistant to erythromycin but showed increased sensitivity to gentamicin which corroborates our study.

Table 1: Antimicrobial sensitivity pattern for Gram positive bacteria isolated from zobo drink

Isolate	Identified Organism	CPX	NB	CN	AML	S	RD	E	CH	APX	LEV
MM6	*Staphylococcus* spp.	S	S	S	R	S	S	R	S	R	R
NB3	*Staphylococcus* spp.	R	S	S	R	S	S	R	S	S	R
KA1	*Staphylococcus* spp.	R	R	S	R	S	S	R	S	R	S
KA2	*Bacillus* spp.	R	R	S	R	S	S	R	S	R	S
WU3	*Bacillus* spp.	R	R	S	R	S	S	R	S	R	S
UA4	*Staphylococcus* spp.	R	R	S	S	S	S	R	S	R	S
WD5	*Staphylococcus* spp.	R	R	S	S	S	S	R	R	R	S
Percentage resistance (%)		**85.7**	**71.4**	**0**	**71.4**	**0**	**0**	**100**	**85.7**	**85.7**	**71.4**

R = Resistance, S = Sensitive, CPX = Ciproflox, NB = Norfloxacin, CN = Gentamicin, AML = Amoxyl, S = Streptomycin, RD = Rifampicin, E = Erythromycin, CH = Chloramphenicol, APX = Ampiclox, LEV =Levofloxac

Table 2: Antimicrobial sensitivity pattern for Gram negative bacteria isolated from zobo drink

Isolate	Identified Organism	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
MM1	*E. coli*	R	R	R	R	S	S	R	R	R	R
MM2	*Proteus* spp.	R	R	R	R	S	S	R	R	R	R
MM3	*Klebsiella* spp.	R	R	R	R	S	R	R	R	R	R
MM4	*Pseudomonas* spp.	R	R	R	R	S	S	R	R	R	R
NB2	*Klebsiella* spp.	R	R	R	R	S	R	R	R	R	R
NB5	*E. coli*	R	R	S	S	S	R	R	R	R	R
KA3	*Salmonella* spp.	R	R	R	R	S	S	R	R	R	R
WU1	*Pseudomonas* spp.	S	R	R	R	S	S	R	R	R	R
UA2	*Pseudomonas* spp.	R	R	S	S	S	S	R	R	R	R
UA5	*Salmonella* spp.	S	S	S	R	S	S	R	R	S	S
UA6	*E. coli*	R	R	R	R	S	S	R	R	R	R
WD1	*E. coli*	R	S	R	R	R	S	R	R	R	R
WD3	*Pseudomonas* spp.	S	S	S	S	S	S	R	R	R	S
	Percentage resistance (%)	**76.9**	**76.9**	**69.2**	**76.9**	**92.3**	**76.9**	**100**	**100**	**92.3**	**84.6**

R = Resistance, S = Sensitive, OFX = Travid, PEF = Reflacine, CPX = Ciproflox, AU = Augmentin,
CN = Gentamicin, S = Streptomycin, CEP = Ceporex, NA = Nalidixic Acid, SXT = Septrin, PN = Ampicillin

Table 3: Zone of inhibitions (mm) produced by different antimicrobials on Gram positive bacteria from zobo

Isolate	Identified Organism	CPX	NB	CN	AML	S	RD	E	CH	APX	LEV
MM6	*Staphylococcus* spp.	12	12	20	-	18	18	-	12	-	-
NB3	*Staphylococcus* spp.	-	14	16	-	20	18	-	12	16	-
KA1	*Staphylococcus* spp.	-	-	16	-	18	20	-	12	-	16
KA2	*Bacillus* spp.	-	-	18	-	18	18	-	12	-	14
WU3	*Bacillus* spp.	-	-	22	-	18	22	-	12	-	16
UA4	*Staphylococcus* spp.	-	-	14	14	16	16	-	14	-	14
WD5	*Staphylococcus* spp.	-	-	18	12	18	18	-	-	-	12

CPX = Ciproflox, NB = Norfloxacin, CN = Gentamicin, AML = Amoxyl, S = Streptomycin, RD = Rifampicin,
E = Erythromycin, CH = Chloramphenicol, APX = Ampiclox, LEV = Levofloxacin

Table 4: Zone of inhibitions (mm) produced by different antimicrobials on Gram negative bacteria from zobo

Isolate	Identified Organism	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
MM1	*E. coli*	-	-	-	-	14	12	-	-	-	-
MM2	*Proteus* spp.	-	-	-	-	14	12	-	-	-	-
MM3	*Klebsiella* spp.	-	-	-	-	22	-	-	-	-	-
MM4	*Pseudomonas* spp.	-	-	-	-	22	14	-	-	-	-
NB2	*Klebsiella* spp.	-	-	-	-	22	-	-	-	-	-
NB5	*E. coli*	-	-	18	12	18	-	-	-	-	-
KA3	*Salmonella* spp.	-	-	-	-	22	12	-	-	-	-
WU1	*Pseudomonas* spp.	28	-	-	-	20	16	-	-	-	-
UA2	*Pseudomonas* spp.	-	-	24	20	16	14	-	-	-	-
UA5	*Salmonella* spp.	24	24	18	-	14	22	-	-	20	18
UA6	*E. coli*	-	-	-	-	20	20	-	-	-	-
WD1	*E. coli*	-	26	-	-	-	16	-	-	-	-
WD3	*Pseudomonas* spp.	30	26	30	24	16	12	-	-	-	18

OFX = Travid, PEF = Reflacine, CPX = Ciproflox, AU = Augmentin, CN = Gentamicin, S = Streptomycin, CEP = Ceporex, NA = Nalidixic Acid, SXT = Septrin, PN = Ampicillin

Most of the Gram negative bacterial isolates in the zobo drinks showed a higher percentage of resistance to ceporex and nalidixic (100%) closely followed by high resistance to septrin (92.3%), ampicillin (84.6%), travid, reflacine and augmentin all at 76.9%. The highest sensitivity shown by bacteria isolated from zobo was both to gentamicin and streptomycin at 92.3% and 77% respectively. Studies have earlier reported antibiotic resistant bacteria in different drinking water supplies (McKeon *et al*., 1995; Nwachukwu and Emeruem, 2007). Oyagade and Fasuan (2004) also isolated antibiotic resistant *E. coli* strains from well water. In another study, bacteria isolated from kunuzaki exhibited multiple drug resistance (MDR), of which *E. coli* recorded 90% resistance to ampicillin similar to our findings (Kelechi *et al*., 2019). The *E. coli* strains obtained in the study showed 100% MDR to at least seven antibiotics which is very alarming. The high rate of resistance may be due to indiscriminate administration of antibiotics or the acquisition of plasmids with resistant genes (Kelechi *et al*., 2019). Similarly, another work reported multiple antibiotic resistance indexes greater than 0.2 by all the Enterobacteriaceae isolates obtain from kunuzaki in Kaduna metropolis and stated that all isolates were resistant to more than two antibiotics irrespective of the antibiotic category (Parom *et al*., 2020). The antibiotics, namely: ampicillin, erythromycin, gentamicin, travid, septrin were employed in this study because most of them are readily available and affordable and are used in many health centers in sub-Saharan Africa (Okeke, 2003; Okereke, *et al*., 2015). Therefore the unregulated and continuous use of these drugs by people for treatment of many bacterial infections may be inadequate or inappropriate and therefore responsible for this seemingly high resistance. Thus, the potential public health hazard associated with antibiotic resistance commonly found in drinks for human consumption should not be overlooked. Among other problems, these resistant organisms in drinks including zobo may promote multiple antibiotic resistant organisms in humans (Walter and Vennes, 1985).

In the disk diffusion antibiotic sensitivity test (The Kirby-Bauer), the thin bacterial film applied to a plate was subjected to various antibiotics and the results presented in Table 3 and 4. The zones of inhibition of specific antimicrobial-impregnated discs revealed the extent of antibiotic resistance in the zobo micro flora. Hence, the most resistant isolates showed the smallest zones of inhibition. Consequently, the zone of inhibition produced by gentamicin and rifampicin on *Bacillus* spp. were the widest zone; whereas, those produced by chloramphenicol among others on *Staphylococcus* spp. were the smallest. However, there was no zone of inhibition of any isolate to erythromycin at all the recordings which represent the highest rate of resistance (Table 3). In a previous study, Makut *et al*. (2014) stated that the antibiotic resistance pattern exhibited by *Staphylococcus aureus*, *E. coli*, *Enterobacter aerogenes* and *Streptococcus* spp. isolated from the zobo sold in Keffi indicated possible abuse of the use of antibiotics and that the zobo contained potentially pathogenic species that could lead to failures in antibiotic chemotherapy among consumers.

For the Gram negative bacteria isolated from zobo, the zone of inhibition produced by ciprofloxacin on *Pseudomonas* spp. showed the widest zone while there was a high multiple drug resistance recorded across eight different antimicrobial used. They include: septrin, ampicillin, ceporex, tarivid, nalidixic etc (Table 4). The high level of resistance calls for an awakening to a war against abuse and misuse of antibiotics in our society. This widespread antimicrobial resistance is of serious concern because some of these resistant bacteria may be transmitted to humans through foods and drinks. Bacterial strains that are resistant to an antibiotic can produce enzymes that inactivate the drug or alter their own genes through mutation or by acquiring resistant genes from other organisms (Trevors *et al*., 1987; Spratt, 1994). In Vietnam, antibiotic resistance has been reported to occur in human bacterial isolates, including *Salmonella typhi* and other diarrhea-causing pathogens (Doublet *et al*., 2003).

Conclusion

This study has provided awareness about the possible presence of antibiotic resistant bacteria in zobo drinks and a focus for monitoring. The isolates were screened for antibiotic resistance against 20 antimicrobials both for Gram positive and Gram negative bacteria, *Pseudomonas* spp. and *Staphylococcus* spp. were resistant to at least three or more antibiotics. The study confirmed that zobo drinks can serve as a reservoir of antibiotic resistance bacteria that may contain a pool of mobile genetic element, which are ready to disseminate antibiotic resistance genes to other human pathogens and so constitute a public health problem. In order to reduce or eliminate the microbial load or antibiotic resistant bacteria, the production, processing environment and handlers should adopt good hygienic practices. Again, ingredients used for processing of zobo, drinks should be properly washed and water sterilized to prevent contamination. Thus, proper hygiene of processors and retailers of zobo and the prudent use of antibiotics are important keys to prevent contamination of the product and spread of antibiotic resistant bacteria.

Declaration of conflicting interest

The authors declared no potential conflicts of interest.

References

Addis M & Sisay D 2015. A review on major food borne bacterial illnesses. *Journal of Tropical Diseases*,3(4): 176.doi:10.4176/2329891X.1000176.

Ayandele AA 2015. Microbiological analyses of hawked kunun and zobo drinks within LAUTECH Campus, Ogbomoso, Oyo State, Nigeria. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 9(10): 52 - 56.

Bristone C, Mariyam K, Ogori AF, Badau MH & Joeguluba O 2018. Microbial quality evaluation of *zobo* drink sold in university of Maiduguri. *Food Science Nutrition and Technology*, 3(1): 000137.

Cheesbrough M 2005. Biochemical test to identify bacteria: District laboratory practices in tropical countries, 2nd edn, Cambridge University Press, UK, pp. 1 - 454.

Doublet B, Lailler R, Meunier D, Brisabois A, Boyd D, Mulvey M, Chaslus-Dancla RE & Cloeckaert A 2003. Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster in *Salmonella enterica* serovar Albany. *Emerg. Infect. Dis.*, 9: 585 - 591.

Eaton DL & Groopman JD 2013. The toxicology of aflatoxins In: *Human Health, Veterinary, and Agricultural Significance*, 1st edn, Academic Press, New York, USA, pp. 383 - 426.

Etang UE, Ikon GM, Udofia SM, Umo AN, Udo EE, Uyanga FZ & Ohagim PI 2017. Microbiological analyses of kunu drinks locally produced and sold in Calabar, Southern Nigeria. *Journal of Advances in Microbiology*, 5(2): 1 - 8.

Izah SC, Orutugu LA & Kigigha LT 2015. A review of the Quality assessment of zobo drink consumed in Nigeria. *ASIO Journal of Microbiology, Food Science and Biotechnological Innovations*, 1(1): 34 - 43.

Kelechi CE, Alozie CE, Owuamalam PO & Onyekachi OV 2019. Screening of kunun-zaki for the presence of extended spectrum beta lactamase (esbl) and carbapenemase producing *Escherichia coli*. *SAR Journal*, 2(4): 158 - 166.

Kigigha LT, Samson GAS, Izah C & Aseibai ER 2018. Microbial assessment of zobo drink sold in some locations in Yenagoa metropolis, Nigeria. *EC Nutrition* I, 13(7): 470 - 476.

Makwin DM, Abigail IO, Ameh-Eleyi JO, Aisha EA 2014. Antibiotic susceptibility pattern of bacteria isolated from zobo drinks sold in Keffi, Nigeria. *Malaysian Journal of Microbiology*, 10(3): 169 - 173.

McKeon DM, Calabrese JP & Bisonnette GK 1995. Antibiotic resistant gram-negative bacteria in rural ground water supplies. *Science Direct*, 29 (8): 1902 - 1908.

Nwachukwu E & Emeruem CM 2007. Presence of antibiotic resistant bacteria in sachet water produced and sold in the Eastern Nigeria. *Research Journal of Microbiology*, 2(10): 782 -786.

Ogiehor IS & Nwafor OE 2004. Associated microbiological, biochemical and chemical quality changes in zobo beverage produced from *Hibiscus sabdariffa Linn*. *Nigerian Annals of Natural Sciences*, 5: 1 - 10.

Okeke IN 2003. Antibiotic resistance in Africa-discerning the enemy and plotting a defence. *Africa Health*, 3: 10 - 15.

Okereke CN, Iroka FC & Chukwuma MO 2015. Phytochemical analysis and medicinal uses of *Hibiscus sabdariffa*. *International Journal of Herbal Medicine*, 2(6): 16 - 19.

Omemu A M, Edema MO, Atayese AO & Obadina AO 2006. A survey of the microflora of H*ibiscus sabdariffa* (Roselle) and the resulting Zobo Juice. *Afr. J. Biotechnol.*, 5 (3): 254 - 259.

Oyagade JO Fasuan OO 2004. Antibiotic resistant *E. coli* strains isolated from well water. *Nigerian Journal of Microbiology*, 18(1-2): 256 - 261.

Parom SK, Gamaleon GD, Ishaku SG, Igwe JC, Obajuluwa AF 2020. Antibiotic susceptibility profile of enterobacteriaceae isolates from a locally preferred drink – kunun-zaki in Kaduna State. *Nig. J. Pharm. Res.*, S(1): 149 - 157.

Raimi, OR 2013. Bacteriology Quality of zobo drinks consumed in some parts Osun State, Nigeria. *Journal of Applied Science Environmental Management*, 17 (1): 113 - 117.

Saraswati AR, Mardiah D & Taniwiryono D2020. Formulation of ready to drink (rtd) made from roselle (*Hibiscus sabdariffa. L*) tea and stevia (stevia rebaudiana) leaf safe for diabetics. *Indonesian Journal of Applied Research (IJAR)*, 1(1): 1 - 9.

Shruthi VH & Ramachandra CT 2019. Roselle (*Hibiscus sabdariffa L.*) calyces: a potential source of natural color and its health benefits. *Food Bioactives: Functionality and Applications in Human Health* Apple Academic Press pp. 169 - 190.

Spratt BG 1994. Resistance to antibioticsmediated by target Alterations. *Science*, 264(5157): 388 - 93. doi: 10.1126/science.8153626. PMID: 8153626.

Trevors JT, Barkley T & Bourquin W 1987.Gene transfer Among bacteria in soil and aquatic `environment: A review. *Canadian Journal of Microbiology*, 33: 191 - 198.

Uddhav SB & Sivagurunathan MS 2016.Antibiotic Susceptibility testing: A review on current practices. *International Journal of Pharmacy*, 6(3): 11 – 17.

Walsh C, Fanning S 2008. Antimicrobial resistance in Food-borne pathogens. A cause for concern. *Curr. Drug Targets*, 9(9): 808 - 815.