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Comparative Study on the Effects of Herbal and Non herbal toothpastes on Human Dental Flora

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ABSTRACT

Toothpastes are agents produced in the form of pastes, gels, powders, or liquid usually in conjunction with a toothbrush, for cleaning dental plaque and improving of oral health. These toothpastes are now produced in the herbal and non-herbal forms to meet the demands of the teaming population in search of a healthy oral cavity, so as to improve oral health. This research was carried out to compare the effects of herbal toothpastes and their non-herbal on human dental flora. Five oral swab samples were procured from early morning mouth rinses of volunteer subjects using swab sticks to determine the effect of herbal and non-herbal toothpaste on bacterial isolates from the human dental cavity. Blood Agar, Nutrient Agar, and Sabouraud Dextrose Agar were used in culturing at 37 for 24 hours using the streak plate method. The isolates were identified using standard microbiological techniques, which included colonial examination, Gram staining technique and Biochemical tests such as citrate, catalase, sugar fermentation, motility, and haemolysis test. The results showed different bacterial isolates: *Staphylococcus spp*, *Streptococcus spp* and *Lactobacillus spp*, as well as fungal isolates: *Candida albicans* and *Aspergillus spp*. Understanding the relationship between toothpaste ingredients and the oral microbiota can aid in formulating toothpaste products that promote optimal oral health and maintain a balanced oral microbial ecosystem.

Keywords: Herbal toothpaste, Non-herbal toothpaste, Human dental flora, Oral microbial load, Dental caries.

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1. INTRODUCTION

Dentifrices are oral-care preparations available in the form of powders, pastes, gels, or liquids and are commonly used with a toothbrush to maintain oral hygiene. Among these formulations, toothpaste is the most widely used dentifrice for improving oral health in Nigeria (Regos et al., 2014). Toothpaste is a semi-solid preparation designed to facilitate mechanical cleaning of the teeth and removal of dental plaque. According to the American Dental Association (ADA), toothpastes, gels, and powders are used with a toothbrush to clean dental plaque and promote oral health (Oranusi et al., 2013). Toothpaste formulations contain various excipients, including humectants, water, abrasives, binders, buffers, surfactants, flavouring agents, colourants, preservatives, and active ingredients. Among these active ingredients, fluoride and other antimicrobial agents contribute to the prevention and control of plaque formation and associated oral diseases (Nóbrega et al., 2016). The increasing interest in natural products has also encouraged the incorporation of plant-derived ingredients into herbal dentifrices (Prasanth, 2011).

Oral and dental diseases remain major public health concerns worldwide and are associated with poor oral hygiene, periodontitis, dental caries, pain, discomfort, infection, and tooth loss (Jahangiry et al., 2020). Dental caries, in particular, continues to constitute a significant oral health burden, with a high prevalence reported in many developing countries. It has been estimated that approximately 3.5 billion people worldwide are affected by tooth decay (WHO, 2018). The development and progression of dental caries are influenced by several factors, including dental plaque formation, fermentable carbohydrates, cariogenic bacteria, salivary quantity and quality, host susceptibility, oral hygiene, and duration of exposure (Butten et al., 2019). Several microorganisms, including *Streptococcus mutans*, *Staphylococcus aureus*, *Streptococcus mitis*, and *Candida albicans*, have been implicated in dental diseases (Akhtar and Bhakuni, 2014). Consequently, effective control of dental plaque and the microorganisms associated with it remains an important component of oral disease prevention.

Mechanical plaque removal through regular tooth brushing is one of the most widely accepted approaches for controlling dental plaque and gingivitis (Barnes et al., 2010). However, the effectiveness of toothpaste in maintaining oral health also depends on its active ingredients, particularly antimicrobial and antiplaque agents that help control pathogenic oral microorganisms (Nogueria-Filho et al., 2012). Chemical agents such as triclosan and chlorhexidine have been incorporated into dentifrices and mouth rinses because of their ability to control plaque and gingivitis. However, some conventional antiplaque agents have been associated with undesirable effects, including tooth staining and altered taste (De Olivera et al., 2018). These concerns, together with increasing interest in naturally derived products, have contributed to the growing use of herbal toothpastes.

Previous studies have investigated the potential benefits of individual herbal ingredients used in oral-care products. Vasconcelos et al. (2016), for example, reported antibacterial activity of *Punica granatum* extracts and demonstrated activity against cariogenic bacteria including *Streptococcus mutans*, *Streptococcus sanguinis*, and *Streptococcus mitis*. Similarly, Costa et al. (2010) reported antibacterial and anti-adherent activities of *Matricaria recutita* extracts against cariogenic microorganisms, including *S. mutans*, *S. sanguinis*, and *Lactobacillus casei*. (Arantes et al. 2015) also reported bacteriostatic and fungistatic activities associated with toothpaste containing *Calendula* extracts, including activity against *S. aureus*, *S. mutans*, and *Candida albicans*.

Other studies have reported potential therapeutic properties of herbal ingredients such as chamomile, sage, echinacea, myrrh, rhatany, and peppermint oil (Hebbal et al., 2013). These findings suggest that plant-derived ingredients may have potential in controlling microorganisms associated with oral diseases.

Despite these findings, much of the available research has focused on individual medicinal plants or specific herbal ingredients rather than directly comparing herbal and non-herbal toothpaste formulations in relation to the human dental flora. Furthermore, differences in toothpaste composition and active ingredients may influence their antimicrobial effects and their ability to alter the microbial environment of the oral cavity. This creates a need for comparative evaluation of commonly used herbal and non-herbal toothpastes to provide further evidence regarding their effects on oral microorganisms.

Therefore, this study was carried out to compare the effects of herbal and non-herbal toothpastes on human dental flora. Specifically, the study evaluated their effects on oral microbial load, investigated and identified microorganisms associated with the dental flora following their use, examined their influence on oral fungal populations, and compared their effectiveness in reducing the occurrence of dental caries. By evaluating these parameters comparatively, the study sought to provide additional information on the relative effects of herbal and non-herbal toothpaste formulations on microorganisms associated with human dental health.

2. MATERIALS AND METHODS

Study Area

The study was conducted using Herbal and Non-Herbal Toothpastes purchased from Eke Awka Market in Awka, Anambra State. The study was conducted at the General Microbiology Laboratory at Nnamdi Azikiwe University, Akwa, Anambra State.

Sample Collection

Five sterile swab sticks were used to collect biological samples from different individuals who use Herbal Toothpaste and also from other individuals who used Non-Herbal Toothpaste. Samples were collected in the early hours of the morning, 5 mins after brushing, from various parts of their mouths. The samples were labelled and were taken to the Microbiology Laboratory for laboratory analysis.

Isolation of Microorganisms

The swab stick samples were then inoculated unto already prepared nutrient agar and sabouraud dextrose agar (SDA) plates to check for the growth and identification of bacteria and fungi, respectively. After inoculation, the culture plates were then incubated at 37°C for 24 hours to allow for growth. After 24 hours, suspected colonies were sub-cultured on agar plates to obtain pure cultures of our isolate.

Pure Culture Maintenance

The isolated organisms were purified through repeated subculture method. Streak plate method was used for this purpose. Nutrient agar and Sabouraud Dextrose Agar (SDA) were used as media. When a plate yielded only one type of colony, the organisms were considered to be pure.

Identification and Characterization of the Isolates

The isolates were characterized by morphological and biochemical characteristics and were identified by using Bergey's Manual of Systematic Bacteriology. The microbiological identification procedures included: Gram staining method, motility test, colour / pigmentation on culture plates, smell. The biochemical tests, such as catalase test, citrate utilization test, sugar fermentation test, and hemolysis test, were equally carried out.

Gram staining

The diluted suspensions of the bacteria were smeared on clean slides, air dried, heat fixed by passing over a flame for 2 to 3 times. The slides were flooded with crystal violet solution for one minute, washed with water, and flooded with Grams iodine for one minute. The slides were washed with water and decolorized with 95% ethyl alcohol dropped from a dropping bottle until no violet color was visible. The slides were washed with water and counter stained with safranin stain for about 30 second and washed with water. The slides were air dried and examined under a microscope using 100x objectives using a daylight filter under oil immersion. Cells were then identified by color, observed purple for Gram positive and pink or red for Gram negative cells (Varghese and Joy, 2014).

Biochemical Tests

Biochemical tests are used for microbial identification based on difference in their biochemical activities exhibited by different types of bacteria. Different biochemical tests are listed below that are used for identification of gram positive and negative organism.

Citrate test

This was done as described by Cheesebrough (2010). This detects the ability of an organisms to use citrate as the sole source of carbon. Simon citrate was prepared by weighing 2.5g of Sodium citrate, 1.5g of Ammonium phosphate, 0.2g of Magnesium sulphate, 1g of potassium dehydrogenate phosphate and 0.1g of Bromothymol blue and dissolved in 1 litre of distilled water, homogenized and dispensed in test tubes then corked with cotton wool. A speck of each isolate was inoculated into Simon citrate medium and incubated at 37 degrees for 72 hours. A positive result is confirmed by formation of blue color, while the initial green color denotes a negative test result. Control test was also read and recorded.

Catalase Test

Catalase test is performed to check the ability of bacterial isolates to produce the enzymes catalase that breaks Hydrogen Peroxide (H_2O_2) into water (H_2O) and Oxygen (O_2). A suspension of the organism was made on a clean grease free slide by emulsifying the organism with a loop full of normal saline. Three drops of 3% hydrogen peroxide was added to the suspension and checked for the production of active bubbles which is a positive result (Varghese and Joy, 2014).

Sugar Fermentation Test

Seven sugars (1% each) were used for the test and include: Glucose, Fructose, Lactose, Maltose, Sucrose, Dextrose, Sorbitol and Mannitol. Each of the sugars (1.5g) were dissolved in 150ml of water in a conical flask. Peptone water (2.25g) and methyl red indicator were added to each of the flasks, after which the final solution were transferred into the test tubes containing inverted Durham tubes.

The test tubes are plugged with cotton wool and labelled accordingly. The test tubes and their contents were autoclaved at 115°C and allowed to cool. Using a syringe, 1ml of 24 hours old nutrient broth culture (for bacterial isolates) and 72 hours old sabouraud dextrose broth culture (for fungal isolates) was inoculated into the different sugars and incubated for 24 hours at 37°C. Acid production is indicated by a yellow color change of the indicator, while gas production indicated by gas accumulation in the submerged Durham tubes; thus, evidence of carbohydrate fermentation (Varghese and Joy, 2014).

Motility Test

This test is used to determine whether an organism is motile or non-motile. Motile organisms contain flagella, which helps them to travel beyond point of inoculation. Semi-solid agar was prepared for this and then autoclaved. After this it was poured into test tubes and allowed to cool. The test organisms were inoculated into the media using stab inoculation method and then incubated at 37°C for 24 hours before observations were made. Diffused growth indicates motile organism, while growth at stab line indicates no-motile organism. (Varghese and Joy, 2014).

Hemolysis Test

This test is performed to identify and characterize various bacterial species, particularly those belonging to the *Streptococcus* genus. This test determines the ability of these organisms to cause the breakdown of red blood cells.

During the test, a blood agar plate was inoculated with the test organism by streaking. A greenish coloration around the bacterial colonies indicates alpha (partial) haemolysis, a clear zone of complete haemolysis indicates beta (complete) haemolysis and no observable haemolysis around the bacterial colonies indicates gamma haemolysis (no haemolysis). (Versalovic et al., 2011).

3. RESULTS AND DISCUSSION

Table 4.1. Morphological Characterization of Bacterial Isolates from Herbal Toothpaste.

Isolate	Elevation	Margin	Colour	Opacity	Shape	Probable Isolate
1	Flat	Entire	Milky	Opaque	Circular	<i>Streptococcus spp</i>
2	Raised	Undulate	White	Opaque	Irregular	<i>Lactobacillus spp</i>

Table 4.2. Morphological Characterization of Bacterial Isolates from Non- Herbal Toothpaste.

Isolate	Elevation	Margin	Colour	Opacity	Shape	Probable Isolate
1	Raised	Entire	White	Opaque	Irregular	<i>Staphylococcus spp</i>
2	Raised	Entire	White	Opaque	Irregular	<i>Staphylococcus spp</i>

Table 4.3. Morphological Characterization of Fungal Isolates from Herbal and Non- Herbal Toothpaste.

Isolate	Elevation	Margin	Colour	Opacity	Shape	Probable Isolate
1	Raised	Entire	White	Opaque	Circular	<i>Candida spp.</i>
2	Flat	Entire	White	Undulate	Irregular	<i>Aspergillus spp.</i>

Table 4.4. Biochemical Characteristics of Bacterial Isolates from Herbal and Non-Herbal Toothpaste.

Isolate s	Gra m Stain rxn	CA T	CU T	H T	M T	Gl u	Fr u	La c	Su c	Ma l	So r	De x	Ma n	Probable Organism
Sample 1	+ve	+ve	+ve		+ve	A	AG	A	AG	AG	A	AG	AG	<i>Lactobacillus</i> <i>Spp</i>
Sample 2	+ve	+ve	+ve		+ve	A	AG	AG	A	A	AG	AG	AG	<i>Streptococcus</i> <i>spp</i>
Sample 3	+ve	+ve	+ve		-ve	A	AG	A	AG	AG	AG	AG	AG	<i>Staphylococcus</i> <i>spp</i>
Sample 4	+ve	+ve	-ve		+ve	A	AG	A	A	A	AG	AG	AG	<i>Staphylococcus</i> <i>Spp</i>

Key: + = Positive & - = Negative

CAT = Catalase test

MT = Motility test

CUT = Citrate utilization test

HT = Haemolysis Test

Ve = Positive

Glu= Glucose

Suc =Sucrose

Dex= Dextrose

A =Acid

Fru= Fructose

Mal = Maltose

Man =Mannitol

Lac = Lactose

Sor=Sorbitol

AG =Acid and Gas

Table 4.5. Sugar Fermentation of Fungi Isolates from Herbal and Non-Herbal Toothpaste.

Sugars	Sample 1	Sample 2	Sample 3	Sample 4
Glucose	A	A	A	A
Fructose	A	AG	A	AG
Lactose	Nil	AG	A	A
Sucrose	AG	AG	AG	A
Maltose	AG	AG	AG	A
Sorbitol	AG	AG	AG	AG
Dextrose	AG	AG	A	AG
Mannitol	AG	AG	AG	AG

AG= Acid and Gas

A= Acid

The study highlighted comparative studies on the effects of herbal dentifrice toothpaste to non-herbal toothpaste on human dental flora. Toothpastes are agents produced in the form of powder, paste, gel or liquid used to improve oral health, usually in conjunction with a toothbrush (Regos et al., 2014). Toothpastes are now produced in the herbal and non-herbal forms to meet the demands of the teeming population in search of a healthy oral cavity. Oral and dental diseases are considered one of the most common health problems in the world. Oral diseases caused by various microorganisms are common around the world. Maintaining a good oral hygiene prevents common oral diseases. Oral hygiene plays an important role in the prevention of oral diseases, including periodontitis, cavity and oral candidiasis. The oral microbial flora contributes to most of the oral diseases. The benefits of oral health, however, do not lie squarely on brushing with toothpaste, but on a number of factors (Nogueria-Filho et al., 2012). The primary purpose of this study is to compare the effectiveness of herbal dentifrice toothpaste to non-herbal toothpaste on human dental flora.

In this study, five (5) organisms were isolated. They are: *Staphylococcus spp.*, *Streptococcus spp.*, *Lactobacillus spp.*, *Candida spp.*, and *Aspergillus spp.*, with *Staphylococcus spp.* showing more occurrence from the isolated samples. The above organisms can be opportunistic pathogenic organisms. Standard microbiological techniques were used for the identification and characterization of the isolates by observing clearly their colonial morphology. The results of the biochemical test carried on the isolates are shown in table 1, 2, 3, 4 and 5.

Streptococcus spp and *Lactobacillus spp.* were able to utilize all the sugars as indicated in the table 4.4, which suggest good carbon utilization ability and showed the highest growth, which was observed during morphological studies. This suggests that these two bacteria isolates could be isolated from the environment. These results agrees with the research of (Marsh et al., 2016) who indicated that *Streptococcus spp* produces extracellular polysaccharides early in the process of biofilm formation, in effect forming a “glue” for the biofilm.

The study shows the morphological characteristics of bacterial isolates on Herbal and Non-Herbal Toothpaste as shown in Table 4.1. & 4.2 and also the morphology/cultural characteristics of fungal isolates from Herbal and Non-Herbal Toothpaste as shown in Table 4.3. The Morphological Characteristics include; shape, margin, elevation, opacity, colour, and size of the Isolates.

Table 4.4 shows the biochemical test of the isolates which include: Gram stain reaction, Citrate utilization test, Catalase test, Motility, Haemolysis test and Sugar fermentation test. Gram staining was done as a presumptive form of identification of bacterial isolates. The probable bacterial organisms isolated and identified include; *Streptococcus spp*, *Staphylococcus spp*, *Lactobacillus spp*, as shown in Table 4.4. The probable fungal organisms isolated and identified includes; *Candida spp* and *Aspergillus spp*. The *Candida spp* isolated from this research work disagrees with the research of (Lorenzo et al., 2017). The use of herbal products in oral cavity has shown an increased popularity and potential benefits due to their additional anti-inflammatory and antioxidant properties, as well as the lack of side effects related to their use. The results obtained from this study suggest that, the use of Herbal toothpaste and Non-Herbal toothpaste products should be minimized for non-medical reasons because, over-utilization may reduce the resident micro flora thereby giving way to transient micro flora, which may grow opportunistically above the normal threshold level, creating several attendance skin and detrimental health effects (Ehrenkranz et al., 2012).

Despite the fact that most individuals claim to brush their teeth frequently, the prevalence of certain dental complications remain high in most populations. Maintaining a good oral hygiene prevents common oral diseases. The oral microbial flora contributes to most of the oral diseases and malodour, thereby incorporating antimicrobial agents in the toothpastes can help the oral environment maintain a consistent phase. The toothpastes tested in this study contains several different constituents with anti-fungal and antibacterial properties, which could be useful in controlling oral microbial population. Fluorides are abundantly used in many non-herbal oral care products, as they help in preventing caries, reducing caries and preventing tooth decay compared with no fluoride therapy. Excess of fluoride concentrations can lead to dental fluorosis. Non-herbal toothpaste formulations typically contain fluoride, triclosan, and sodium lauryl sulfate, which have been extensively studied for their antimicrobial and plaque-inhibiting properties (Marsh, 2020).

Several studies stated that toothbrushing habit has significantly improved the hygiene of both young and old. Mullally et al. (2015) found a significantly reduced plaque and gingival index score, but no significant difference was found between the herbal and non-herbal toothpastes.

The impact of toothpastes, regardless of herbal and non-herbal composition on the oral microbiota is a complex and dynamic process. The oral microbiota is composed of a diverse range of microorganisms that interact with each other and the host playing both beneficial and pathogenic roles. The regular use of toothpastes, regardless of its composition, contributes to the mechanical removal of plaque and biofilm, which is crucial for maintaining oral hygiene and preventing dental diseases. Fluoride, in particular, has been proven effective in preventing dental caries by promoting remineralization of enamel and inhibiting acid-producing bacteria (Marinho et al., 2013).

4. CONCLUSION

The conclusion of this study is that there is an increasing interest in the use of herbal products, including herbal toothpastes, among people. Based on the findings of this study, herbal toothpaste showed less microbial growth compared to non-herbal toothpaste, suggesting that the herbal toothpaste tested may have better antimicrobial activity. This may be attributed to the antimicrobial properties of some natural ingredients used in herbal toothpaste. However, non-herbal toothpastes have also been widely studied and have proven benefits, particularly in cavity prevention and plaque control. The choice between herbal and non-herbal toothpaste may therefore depend on individual preference, oral health condition, and other personal considerations. It is also important for individuals to seek advice from dental professionals when choosing oral hygiene products. Based on the findings of this study, herbal toothpaste is recommended as a suitable alternative to conventional non-herbal toothpaste, particularly because less microbial growth was observed in the herbal toothpaste tested. Further studies are recommended to evaluate more brands and formulations of herbal and non-herbal toothpastes using a larger number of samples and different microorganisms.

References

- [1] Akhtar, M. S. and Bhakuni, V. (2014). Streptococcus pneumoniae Hyaluronate Lyase; An overview. *Current Science*, **86**(2):285–295.
- [2] Arantes, A. B., Luz, M. M. S., Santos, C.A.M. and Sato, M. E. O. (2015). Desenvolvimento de dentifrícios com extratos fluídos de Calendula officinalis L. (Asteraceae) e Casearia sylvestris Sw. (Flacourtiaceae) destinado ao combate à placa bacteriana. *Revista Brasileira de Farmacognosia*; **86**(2): 61-64.
- [3] Barnes, V. M., Richter, R. and De Vizio, W. (2010). Comparison of Short-Term Antiplaque/Antibacterial Efficacy of Two Commercial Dentifrices. *Journal Clinical Dentistry*, **21**(4):101-104.
- [4] Butten, K., Johnson, N.W., Hall, K. K., Anderson, J. and Toombs, Y. and King, N. (2019) Risk Factors for Oral Health in Young, Urban, Aboriginal and Torres Strait Islander Children. *Aust Dentistry Journal*, **64**(1):72–81.
- [5] Cheesebrough, M. (2010). Laboratory Investigation of Microbial Biochemical Method. *District Laboratory Practice in Tropical Countries*, **2**:62-70.
- [6] Chung, J.Y., Choo, J. H., Lee, M. H and Hwang, J. K. (2016). Anticariogenic Activity of Macelignan Isolated from Myristica fragrans (nutmeg) against Streptococcus mutans. *Phytomedicine*, **13**(4):261-266.

- [7] Costa, M.R., Albuquerque, A.C., Pereira, L., Vieira, A., Diniz, D.N., Pereira, M.S.V; Pereira, J.V and Trevisan, L.F.A. (2010). Efeito antimicrobiano do extrato da *Myrciaria cauliflora* Berg e *Matricaria recutita* Linn. sobre microrganismos do biofilme dental. *Revista de Biologia e Farmácia*, **4**(1):19-25.
- [8] De Olivera, S. M., Torres, T.C., Pereira, S.L., Mota, O. M. and Carlos, M. X. (2018). Effect of Dentifrice Containing Aloe Vera on Plaque and Gingivitis Control: A Double Blind Clinical Study in Humans. *Journal Applied Oral Science*, **16**(4):293-296.
- [9] Ehrenkranz, N.J., Taplin, D. and Butt, P. (2012). Antibiotic Resistant Bacteria on the Nose and Skin: Colonization and Cross-Infection. *Antimicrobial Agents and Chemotherapy*, **30**(4):255-264.
- [10] Hebbal, M., Ankola, A.V., Sharma, R. and Johri, S. (2012). Effectiveness of Herbal and Fluoridated Toothpaste on Plaque and Gingival Scores Among Residents of a Working Women's Hostel-A Randomised Controlled Trial. *Oral Health Prevention Dental*, **10**(4):389–395.
- [11] Jahangiry, L., Bagheri, R., Darabi, F., Sarbakhsh, P., Sistani, M. M. and Ponnet, K. (2020). Oral Health Status and Associated Lifestyle Behaviors in a Sample of Iranian Adults: An Exploratory Household Survey. *BMC Oral Health*, **20**(1):82-89.
- [12] Joiner, A. (2010): Whitening Toothpastes: A Review of the Literature. *Journal Dentistry*, **38**:17–24.s
- [13] Jose, M., Deepa, K. C. and Prabhu, V. (2011). Ethnomedicinal Practices for Oral Health and Hygiene of Tribal Population of Wayanad Kerala. *In International Journal of Research in Ayurveda & Pharmacy (IJRAP)*, **2**(4):1246-1250.
- [14] Kleber, C. J., Moore, M. H. and Nelson, B. J. (2018). Laboratory Assessments of Tooth Whitening by Sodium Bicarbonate Dentifrices. *Journal Clinical Dentistry*, **9**(1):72-75.
- [15] Kumar, G. N. (2017). Preparation, Evaluation and Comparison of Herbal Toothpaste with Markedly Available Tooth Pastes. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, **12**(6):68-70.
- [16] Lorenzo, M. R.O., Madrigal, R.G. and Pineda, P.J. (2017). Efectos de la tintura de calendula al 10 por ciento en adolescentes afectados por gingivitis crónica. *Mediciego*, **3**(2):33-36.
- [17] Marinho, V.C., Higgins, J. P., Logan, S. and Sheiham, A. (2013). Fluoride Toothpastes for Preventing Dental Caries in Children and Adolescents. *Cochrane Database of Systematic Reviews*, **7**:1-3.
- [18] Marsh, P. D and Percival, R.S. (2016). The Oral Microflora—Friend or Foe? Can We Decide? *International Dentistry Journal*, **56**(4):233–239.
- [19] Marsh, P. D. (2020). Contemporary Perspective on the Use of Fluoride Products in caries Prevention. *British Dental Journal*, **228**(11):803-809.
- [20] Mullally, B. H., James, J.A., Coulter, W.A. and Linden, G.J. (2015). The Efficacy of Herbal-Based Toothpaste on the Control of Plaque and Gingivitis. *Journal Clinical Periodontology*, **22**(9):686-689.

- [21] Nóbrega, D. F., Fernández, C. E., Del Bel, O., Cury, A. A., Tenuta, L.M. and Cury, J. A. (2013). Frequency of Fluoride Dentifrice Use and Caries Lesions Inhibition and Repair. *Caries Resistance*, **50**(2):133–140.
- [22] Nogueira-Filho, G. R., Duarte, P.M., Toledo, S., Tabchoury, C.P. and Cury, J.A. (2012). Effect of Triclosan Dentifrices on Mouth Volatile Sulphur Compounds and Dental Plaque Trypsin-Like Activity during Experimental Gingivitis Development. *Journal Clinical Periodontology*, **29**(12):1059-1064.
- [23] Oranusi, S.U. and Akhigbe E. (2013). Antimicrobial Activity and Micro-Flora Quality Evaluation of Commonly Used Toothpastes. *International Journal Biology Pharmacy Allied Science*, **2**(7):1388–1399.
- [24] Prasanth, M. (2011). Antimicrobial Efficacy of Different Toothpastes and Mouth Rinses: An In vitro study. *Dentistry Resistance Journal*, **8**(2):85-94.
- [25] Regos, J. and Hitz, H. R. (2014). Investigations on the Mode of Action of Triclosan, a Broad-Spectrum Antimicrobial Agent. *Zentbl. Bakteriöl. Parasitenkd. Infektkrankh. Hygiene Abt 1 Originale*, **226**(3):390-401.
- [26] Vasconcelos, L.C.S., Sampaio, F.C., Sampaio, M.C.C., Pereira, M.S.V., Higino, J.S. and Peixoto, M. H. P. (2016). Minimum Inhibitory Concentration of Adherence of *Punica granatum* Linn (pomegranate) gel Against *S. mutans*, *S. mitis* and *C. albicans*. *Brazilian Dental Journal*, **17**(3):223-227.
- [27] World Health Organization. (2018). Oral health. Geneva, Switzerland: *World Health Organization*