Volume 9 Issue 1 (2021) 26-31 ISSN 2347 - 3258
International Journal of Advance Research and Innovation

Evaluation of Green Tea Effect on Oral Bacteria, Streptococcus Mutans

Riya Kukreti, Poonam Rani, Rohit Gautam
Department of Biotechnology, Khurana Block, Meerut Institute of Engineering and Technology, Meerut (UP), India

Article Info
Article history:
Received 2 January 2021
Received in revised form
28 February 2021
Accepted 5 March 2021
Available online 15 March 2021

Keywords: Green tea, Streptococcus mutans, polyphenol, medicinal properties, antibacterial.

Abstract: Tea is a gift of China to the world. It is a popular beverage consumed by almost every person globally, after water. Green tea has not gone under oxidation process and found beneficial for human consumption because of its medicinal properties and presence of some nutrients and minerals required by human beings for a healthy life. Molecular component of green tea (Polyphenols, catechins, flavonoids and minerals) makes it suitable in ailments and some microbial infections. Oral cavity has the second largest and diverse microbiota after gut, as numerous microorganisms such as bacteria, fungi and viruses exist in the mouth. Streptococcus mutans is facultative, anaerobic oral bacteria mainly responsible for tooth decay was selected for the present study because dental plaque and caries are common infections found in population. For experimental purpose 30 healthy individuals were randomly selected from the college campus (MIET, Meerut). Saliva of each individual was taken twice i.e. before and after green tea consumption. For identification and confirmation of S. mutans, biochemical tests have been done. The effect of green tea was observed by the growth and count of S. mutans on common (nutrient agar media) and special media (Mitis Salivary agar media). Plates were showing less count after green tea consumption as compared to the plates before consumption of green tea. On the basis of the results, it can be concluded that Green tea is the natural preventive and curative agents and is good to consume once in a day to reduce the count of Streptococcus mutans in mouth to maintain oral health.

1. Introduction

Green tea is considered as a nature's gift, obtained from the leaves and bud of plant Camellia sinesis plant (a small bush about 1-2m height) (Mohan et al., 2018;Kumar et al., 2018; Mahmood et al., 2010). During the period of emperor Chen Nung in China (Mohan et al., 2018; Ware et al., 2017) and now consumed in many countries such as India, Japan, Thailand, United States, Europe, Russia and Iran (Ramesh et al., 2016; Kumari et al., 2018; Mohan et al., 2018; Ware et al., 2017). In India it was firstly discovered in Assam state and its cultivation was originally started in Darjeeling district of West Bengal (Sarkar et al., 2016). Green tea serves as a good source of nutrients for human consumption as it contains various components like enzymes, amino acid, carbohydrates, lipids, sterols, vitamins, minerals, polyphenol, catechin and caffeine (Ramesh et al., 2016; Singh et al., 2016). Green tea is mainly prepared by drying and steaming the leaves of plant which inactivates the polyphenol oxidase enzyme that prevent oxidation (Mohan et al., 2018). It contains several molecular (bio active) components such as polyphenol in the form of catechin, epigallocatechin 3 gallate (EGCG), epicatechin gallate, epicatechin and flavanols, and are main components of green tea extract and make it suitable for use in ailments too as having medicinal properties. These bioactive components determine their health benefit on humans. Due to the presence of polyphenol and catechin green tea shows strong antioxidant, anti-inflammatory, anti-microbial and anti-mutagenic properties, hence prevent from several diseases such as cancer, cardiovascular, inflammatory skin disease, diabetes and oral cavities (Khamverdi et al., 2019; Mahmood et al., 2010; Ramesh et al., 2016; Singh et al., 2016). Extract of green tea is also used in cosmetics and mouthwash which is safe for every age group.

2.Oral Microbiota and Dental Caries

The human mouth is referred as one of the diverse microbiomes of human body as it consists various microorganism that include viruses, fungi, protozoa, archaea and bacteria (Wade et al., 2013). Oral cavity of human is the only major gateway to the human body and a species rich heterogenous ecological system that consist teeth, buccal mucosa, soft and hard palate and tongue that are colonized by bacteria (Forssten et al., 2010; Lu et al., 2019; Dewhirst et al., 2010). It generally consists of about 700 kinds of bacterial taxa, that constitutes the human oral microbiota (Forssten et al., 2010; Lu et al., 2019). It helps in prevention of dental caries from foreign particle (pathogen) by act as a part of host defence system against them (Forssten et al., 2010). According to the anatomical and physiological conditions, oral cavity microbiome can change and is not remain uniform (Staskova et al., 2019). Generally, the microbes present in human mouths are harmless but some of them under certain conditions can cause oral infection like periodontal disease or caries (Forssten et al., 2010). Several evidences reveal that physical state of human, such as diabetes, obesity and cancer are closely related to oral microbiota (Lu et al., 2019). Dental caries and dental plaque are caused by mixture of microorganism and food debris and are one of the most common disease worldwide (Forssten et al., 2010). Dental caries refers as a biofilm-mediated, sugar driven, multifactorial and dynamic widely spread chronic infectious disease affecting the large number of people.

Fig. 1: Fresh leaves of green tea (Moghbel et al., 2011)

2.1 Streptococcus mutans

Streptococcus mutans is a facultative, anaerobic, gram-positive cocci bacteria, include group of seven closely related species that are collectively referred as mutans streptococci and can be classified in eight serotypes (Ranganathan et al., 2019). These are mesophilic bacteria that grow at temperature about 18 – 40^{o}C and also considered as the "normal flora" of oral cavity of human and associated with dental caries (Zelnicek et al., 2016). Mouth, pharynx and intestine are primary habitat for S. mutans (Forssten et al., 2010). Among all the species S. mutans and S. sobrinus are commonly isolated from human source whereas, animal are the major source where other species are mostly found (Hung et al.,

Corresponding Author,
E-mail address: kukretiriya@gmail.com

2005). S. mutans is the primary causative agent of dental caries whose main virulence factor is associated with careiogenicity (Banas et al., 2004). Mutans streptococci can cause acidic environment (which demineralised the superficial structure of tooth) in human mouth and increase the risk of cavities by act as strong acid producer due to which it also termed as careiogenic bacteria (Forssten et al., 2010; Ranganathan et al., 2019). These are the specialized microorganism that create the slimy environment in the mouth that allow the adherence of the bacteria with the tooth surface with the help of receptors (Zelnicek et al., 2016). The main virulence factor of S. mutans associated with cariogenicity (include adhesion), acidogenicity (ability to produce large quantities of organic acids from metabolized carbohydrates) and aciduricity (ability to survive at low pH) which helps in initial attachment, colonization and accumulation of biofilm on the tooth surface (Lemon et al., 2013; Ranganathan et al., 2019).

S. mutans are the commonly found bacteria in the oral cavity of humans that play a very important role in the formation of (or causing) dental caries. And green tea consists some molecular components that show several properties which prevent humans from several diseases. Therefore, present study was conducted to "evaluate effect of green tea on oral microbiota" of human beings at, MIET, Meerut.

3.Material and Method

3.1 Nutrient agar media (NAM)

Nutrient agar is general purpose nutrient media, used for the cultivation of a wide variety of less fastidious organism. For preparation of it 28 gm of nutrient agar powder was mixed in 1000 ml of distilled water and boiled all the components get fully dissolved. After that autoclave the flak containing the media at 121degree Celsius and 15 psi pressure for about 15 to 20 minutes. Once the nutrient agar has been autoclaved allow it to cool but not to solidify. After that pour nutrient agar in to the Petri plates and leave plates on the sterile surface (laminar air flow) until the media get solidify. Once the media gets solidify put the plates in incubator for 24 hrs. After 24 hrs plates were clean and there was no contamination found so were used as media plates for practical purpose.

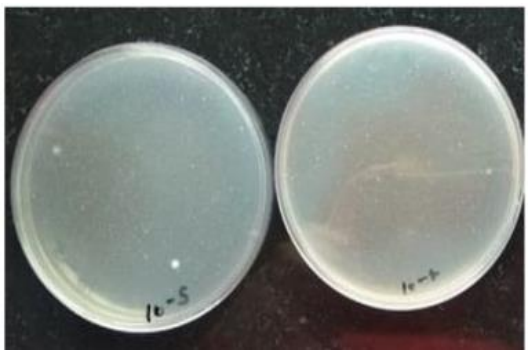

Fig.2: Nutrient agar media plates.

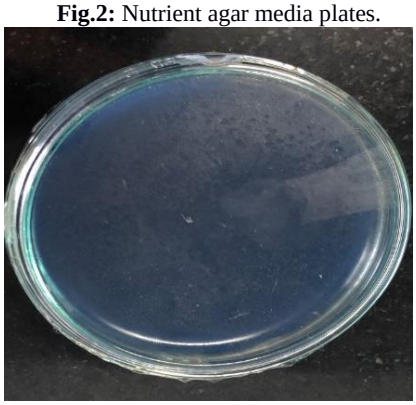

Fig. 3: Mitis salivary agar plate.

3.2 Mitis Salivarius agar media (MSA)

Mitis salivary agar is a type of selective media used for the isolation of genus streptococci especially Streptococcus mitis, Streptococcus salivarius, Streptococcus mutans etc from mixture of specimens. 18.014 gm of Mitis salivary agar powder was suspended it in 200ml of distilled water and boil till all the components get fully dissolved. After that autoclave the flak containing the media at 121º Celsius and 15 psi pressure for 15 minutes then allow it to cool .After that pour nutrient agar in to the petri plate and leave plates on the sterile surface (laminar air flow) until the agar get solidify. Once the agar gets solidify put the plates in incubator for 24 hrs. After 24 hrs plates were checked, there was no contamination found there so media plates were used for practical purpose, (Note-After the agar plates are prepared 4 plates (2 of each media) was separately placed from the other plates before the sample collection process is done. These plates were also placed in to the incubator along with the inoculating plates and named as control plates).

3.2 Volunteer's selection

For the experiment 30 healthy individual who are studying in our institution were randomly selected for testing purpose. All the volunteers' (students) have good oral hygiene and aged between 18-30 years. From each volunteer two samples were taken one before consumption and after consumption of green tea.

Sample collection before green tea consumption and inoculation of the bacteria on the already prepared media plates: For sample collection initially all volunteers were called out and make them comfortable. Now saliva sample from each of the volunteer was collected under aseptic condition in sterile containers and put them separately in laminar hood so that the sample was not come in contact with any other kind of contamination. As the samples were collected in sterile containers now the sample is inoculated (by streaking method) into the media plates (NAM and MSA) with the help of inoculating loop. Now all the inoculated plates were placed separately with in the laminar hood.

3.3 Green tea and its preparation

Green tea is considered as one of the most beneficial tea for human consumption. For the experiment Lipton green tea having honey lemon flavour is used. Fresh Lipton green tea (honey lemon flavour) was procured from local market which is available in the form of green tea dip bags was used for the experimental purpose. For preparation of green tea firstly boil the water. Pour the boiled water in disposable paper cup and dipped green tea powder sachet (2gm) for 2 minutes in cup. After 2 minutes take out the green tea powder sachet and put it in dustbin. After that freshly prepared green tea was consumed by the volunteers one by one.

3.4 Sample collection after tea consumption

As the green tea was consumed by the volunteers now again process of sample collection is repeated. Now all the volunteers that consumed green tea were called out after 30 minutes of tea consumption. Again, make sure that the media plates are prepared before sample collection step and do not contain any kind of contamination. Now again saliva sample from each of the volunteer was collected under aseptic condition in sterile containers and placed them separately. After sample collection now again, the sample was inoculated in to already prepared media plates with the help of inoculating loop. Again, all the inoculated plates were placed separately in laminar hood.

3.5 Incubation of plates

Now as the sample was inoculated in to the petri plates now all the plates were placed (incubated) in to the incubator in different chamber (before tea and after tea) very carefully. Along with these inoculating plates, control plates were also placed in to the incubator in different chamber. After placed the plated in incubator record the observation after 24hrs, 48hrs and 72hrs of incubation.

3.6 Identification of microorganism

For identification of the culture firstly microscopic examination of the culture should be done. Microscopically it was done by gram staining method.

3.7 Gram Stain Reagents

First of all microscopic slide was prepared by fixing the specimen on microscopic slide by forming a smear on it. Cover the smear with crystal violet (drop wise), the primary stain for 1 min. Gently, rinse off the stain with water. Now cover the smear with gram's iodine (drop wise), the mordant, for 1 min. Again, rinse off the iodine solution with water. Run the acid-alcohol decolourizer over the smear (30 sec) until the solution appears clear. Gently rinse the slide with water. At last cover the smear with safranin (drop wise), the secondary or counterstain for 1 min. Gently rinse the stain with water. Allow the slide to get air dry and our slide is ready to observe under the microscope.

3.7 Sub-culturing

After the staining procedure sub-culturing of the culture should be done. This step will help us in identification and confirmation of the bacterial colony. For this step again media plates (NAM & MSA) were prepared before the further procedure by using the same method as mention earlier. All the steps involved in the media preparation process remain same except that when the autoclaved media poured in the petri plates and allow it to solidify then as the media plates get solidify now the plates are placed in incubator for 24 hrs at 37^0C.On the very next day plates were checked, whether the plates contain any kind of contamination or not if no contamination is found then the plates are ready for sub-culturing. Now, again the culture inoculated in freshly prepared media plates. After, inoculated plates were placed separately in incubator.

After that observe the inoculated plates and record the observation at a time interval of about 24hrs. After 24 hrs when growth is shown inoculated petri plates now biochemical test will be done which is the next step in bacterial identification and confirmation.

4.Biochemical tests and results

4.1 Catalase test

The test was performed under aseptic condition with the help of laminar hood. With the help of an inoculating loop or a needle, transfer growth from the centre of a colony to the surface of a glass slide. Add one drop of 3% hydrogen peroxide and observe for bubble formation.

4.2 Antibiotic sensitivity test

For this technique firstly nutrient agar media plates were prepared. Now as the Petri plates are prepared then the plates are over layered or streak with the help of inoculating loop with the culture on which test will be done. After that, incubated these plates for 24-48 hours at 37°C. After 48 hours when fully growth is shown on the Petri plates then the antibiotic disc with the help of flamed forceps were placed on the surface of the Petri plate at a particular distance so that the difference between two discs is sufficient so that they do not overlap with each other. As the disc is placed on the Petri plates then allow it to settle on the agar media and then incubate the plates for 24 to 48 hours at 37°c. After 48 hours of incubation, a zone of inhibition is created on the plates with a diameter proportional to amount of antimicrobial drug added on the plate.

4.3 Numeriacal Equations

To calculate the statistical analysis of the data firstly arithmetic mean and then standard deviation of the data was calculated.

Arithmetic mean (A) = **(**Sum of all observations) / (Total number of observation)

Standard deviation –

$$\sigma = \sqrt{\frac{\sum(x_i - \mu)^2}{N}} \quad (1)$$

Where σ = population standard deviation

N = the size of the population

xi = each value from the population

μ = the population mean

4.4 Observation Observations was recorded in both the cases before and after the consumption of green tea given by tables 1 and figures 4 to 9.

Table 1: Showing colonies count before and after consumption of green tea

Number of Colonies			
S. No	**Students**	**Before Tea Consumption**	**After Tea Consumption**
1.	A	200	60
2.	B	85	15
3.	C	110	35
4.	D	90	20
5.	E	100	25
6.	F	150	40
7.	G	175	55
8.	H	145	33
9.	I	75	10
10.	J	125	28

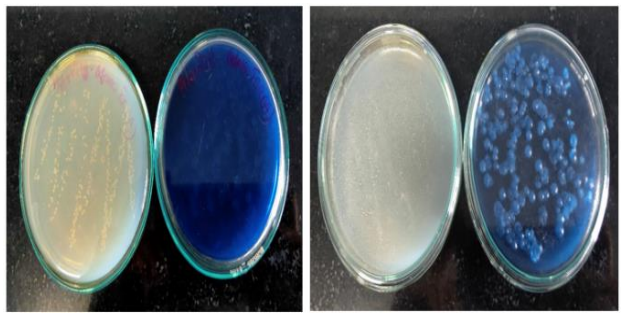

Fig. 4: Growth after 72 hours of incubation (Before consumption of tea)

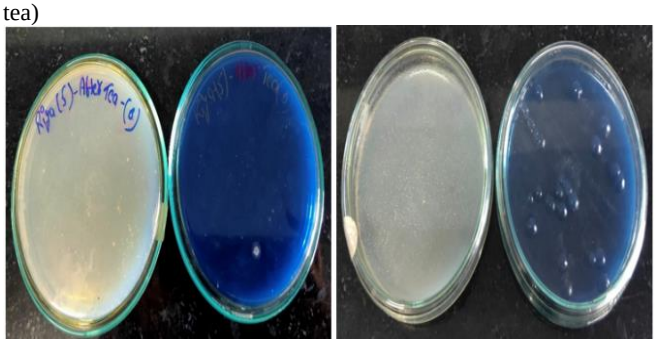

Fig. 5: Growth after 72 hours of incubation (After consumption of green tea)

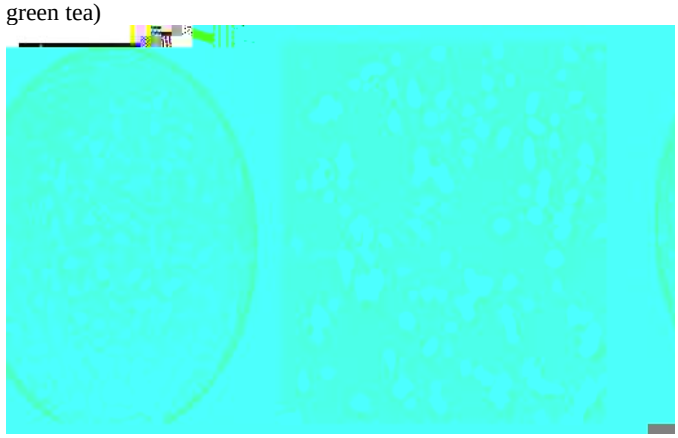

Fig 6: Gram stain of gram- positive cocci (Microscopic Examination)

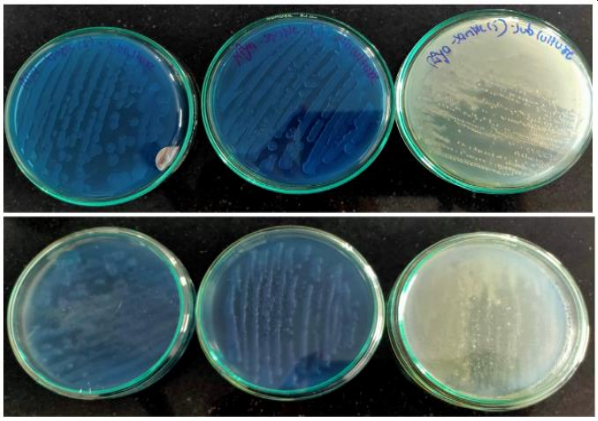

Fig. 7: Observation after 48 hours of subculturing (Subculturing).

Fig. 8: Catalase test showing no bubble formation **(**Biochemical tests)

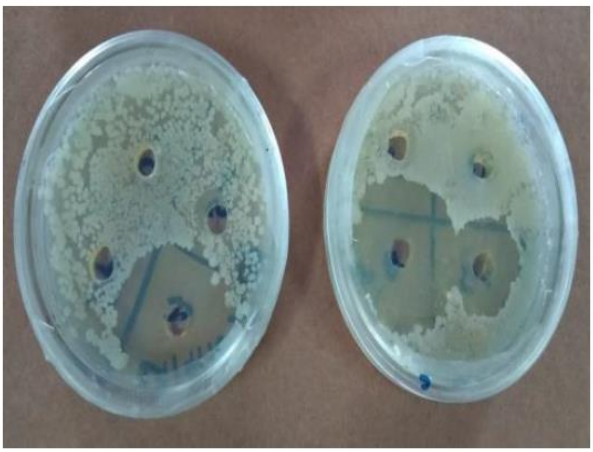

Fig 9. Zone of inhibition (ABST), **(**Biochemical tests)

4.5.Results

All of the observation observed were –

a) Good growth on mitis salivary agar media as it is a selective type of media for Streptococcus mutans.
b) Microscopic examination by Gram staining method indicate that the culture contains gram positive bacteria.
c) Culture bacteria consisting of cocci shape.
d) Catalase test is negative as no bubble formation is seen.
e) A zone of inhibition is also observed on petri plate indicated that the culture is affected by the antibiotic ampicillin and cefotaxime.

All the above observation states that the culture media consist Streptococcus mutans only.

- **Mean of the following data is** – Before consumption of tea – 1255 colony- After consumption of tea – 321 colony.
- **Standard deviation of the data** – Before tea consumption – 41.2613621- After tea consumption – 16.2237823.

4.6 Effect of green tea on streptococcus mutans

a) Before consumption of green tea, fully growth on the petri plates is observed after 72 hours of incubation period.
b) After consumption green tea, not much growth or some growth is observed on the plates even after 72 hours of incubation period.

Both the observation itself conclude that green tea affects Streptococcus mutans by decreasing its count. As we studied that streptococcus mutans is responsible or is the main cause of dental plaque or caries (in human mouth) therefore it also decreases the risk/chances, for development of dental caries and plaque in the oral cavity of humans. We concluded that green tea act as natural preventive and curative agents that help in maintain the oral health of humans.

5. Conclusions

Tea is one of the most common refreshing beverages. Among all, green tea consists various beneficial or medicinal effect on the human health. Regular consumption of green tea can surely decrease the count of Streptococcus mutans (main cause for dental caries and plaque) in the oral cavity of humans which ultimately results in decreasing the risk of dental plaque and caries formation in human mouth. Therefore, green tea consumption on daily basis, in limited amount is economical and beneficial for health.

Acknowledgment –
Authors would like to thanks to Department of Biotechnology (CCS), MIET, Meerut for laboratory, Library and other support and assistance.

REFERENCES -

[1]. JA Aas, BJ Paster, LN Stokes, I Olsen, FE Dewhirst. Defining the Normal Bacterial Flora of the Oral Cavity, Journal of clinical Microbiology, 43(11), 2005, 5721-5732.
[2]. S Aryal. Nutrient agar – composition, preparation and uses, Microbiology Info.com, 2018.
[3]. S Aryal. Gram staining, principle, reagent, procedure steps and result, Microbe notes, 2020. http://microbenotes.com/gram-stain-principle-reagent-procedure-and-result-interpretation/.
[4]. JA Banas. Virulence properties of Streptococcus mutans, Frontiers in bioscience, a journal and virtual library, 9, 2004, 1267-1277.
[5]. C Cabera, R Artacho, R Gimenez. Beneficial effects of green tea-A review, J Am Coll Nutr, 25, 2006, 79-99.
[6]. A Choudhary. Working principle of pH meter, Pharmaceutical guidelines, 2015.
[7]. A Connelly. The laboratory balances a practical guide, andyconnelly.wordpress.com, 2017.
[8]. ME Davey, GA O'toole. Microbial biofilms: from ecology to molecular genetics, Microbiology and Molecular Biology Reviews**,** 64, 2000, 847-867.
[9]. FE Dewhirst, T Chen, J Izard, BJ Paster, ACR Tanner, WH Yu, A Lakshmanan, WG Wade. The human oral microbiome, Journal of Bacteriology, American Society for Microbiology, 2010.
[10]. TM Elattar, AS Virji. Effect of tea polyphenols on growth of oral squamous carcinoma cells in vitro, Anticancer Res. 20, 2000, 3459–3465.
[11]. M Elvin-Lewis, R Steelman. The anticariogenic effects of tea drinking among Dallas school children, J. Dent. Res, 65, 1986, 198.
[12]. GF Ferrazzano, I Amato, A Ingenito, ADe Natale. Anticariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea), Fitoterapia, 80, 2009, 255-262.

[13]. SD Forssten, M Bjorklund, C Ovwehand. Streptococcus mutans, caries and stimulation models, Nutrients, 2010, 1-10.
[14]. M Friedman. Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas, Molecular Nutrition and Food Research, 51, 2007, 16-34.
[15]. Garg M, Top 4 tests for antimicrobial drug susceptibility, Biology Discussion, http://www.biologydiscussion.com/media-microbiology/top-4-tests-for-antimicribial-drug-susceptibility.
[16]. JMT Hamilton-Miller. Anti-cariogenic properties of tea (Camellia sinensis), Journal of Medical Microbiology, 50(4), 2001, 299–302.
[17]. Y Hilal. Morphology, Manufacturing, Types, Composition and Medicinal Properties of Tea (Camellia sinensis), Journal of Basic and Applied Plant Sciences, 1, 2017.
[18]. K Hirao, H Yumoto, T Nakanishi, K Mukai, K Takahashi, D Takegawa. Tea catechins reduce inflammatory reactions via mitogen-activated protein kinase pathways in toll-like receptor 2 ligand-stimulated dental pulp cells, Life Sci, 86, 2010, 654-60.
[19]. M Hirasawa, K Takada. Multiple effects of green tea catechin on the antifungal activity of antimycotics against Candida albicans, Journal of Antimicrobial Chemotherapy, 53, 2004, 225-9.
[20]. M Hirasawa, K Takada, S Otake. Inhibition of acid production in dental plaque bacteria by green tea catechins. Caries Res; 40, 2006, 265-70.
[21]. J Holt, N Krieg, P Sneath, J Staley, S Williams, Bergey's manual of determinative bacteriology, 9th ed; Williams & Wilkins: Baltimore, MA, USA1994.
[22]. Hot air oven – principle, working mechanism, sterilization control, advantage and disadvantage, http://universe84a.com/hot-air-oven.
[23]. Z Khamverdi, M Azarsina. The Beneficial Effects of Green Tea in Oral Health and Dentistry, Biomedical journal of scientific and technical research, 2019
[24]. M Kilian. The oral microbiome – friend or foe, European Journal of Oral Science, 126, 2018, S1.
[25]. P Koll, R Mandar, H Marcotte, E Leibur, M Mikelsaar, L Hammarstrom. Characterization of oral lactobacilli as potential probiotics for oral health, Oral Microbiology and Immunology, 23, 2008, 139-147.
[26]. P Kumari. Effects of green ten in oral health, International journal of scientific engineering and research, 6, 2018.
[27]. RJ Lamont, DR Demuth, CA Davis, D Malamud, B Rosan. Salivary-Agglutinin-Mediated Adherence of Streptococcus-Mutans to Early Plaque Bacteria, Infection and Immunity, American society for microbiology, 59, 1991, 3446-3450.
[28]. JA Lemon, RG Quivey, H Koo, J Abranches. Streptococcus mutans: a new gram-positive paradigm, Microbiology, 159.
[29]. N Li, C Han, J Chen. Tea preparations protect against DMBA induced oral carcinogenesis in hamsters, Nutrition, Cancer, 35, 1999°, 73–79.
[30]. N Li, Z Sun, C Han, J Chen. The chemopreventive effects of tea on human oral precancerous mucosa lesions, Proceedings of the Society for Experimental Biology and Medicine/, 220 (4), 1999b, 218–224.
[31]. HA Linke, RZ LeGeros. Black tea extract and dental caries formation in hamsters. Int J Food Sci Nutr, 54, 2003, 89-95.
[32]. P Lodhia, K Yaegaki, A Khakbaznejad, T Imai, T Sato, T Tanaka. Effect of green tea on volatile sulfur compounds in mouth air, Journal of Nutritional Science and Vitaminology, 54, 2008, 89-94.
[33]. WJ Loesche. Role of Streptococcus mutans in human dental decay. American society of microbiology, Microbiological review, 50, 1986, 353-380.
[34]. Lu M, Wang Z and Xuan S (2019), Oral Microbiota: A new view of body health, Food Science and Human Wellness, ScienceDirect, 8.
[35]. T Mahmood, N Akhtar, BA Khan. The morphology, characteristics, and medicinal properties of Camellia sinensis' tea, Journal of Medicinal Plants Research, 4(19), 2010.
[36]. L Mao-Jung, DL Joshua, P Saileta, M Xiaofeng. Delivery of Tea Polyphenols to the Oral Cavity by Green Tea Leaves and Black Tea Extract. Cancer Epidemiol. Biomarkers Prev. 13, 2004, 132–137.
[37]. P Marsh. Dental plaque as a biofilm and a microbial community - implications for health and disease. BMC Oral Health, 6, 2006, S14.
[38]. KH Metwalli, SA Khan, MA Jabra-Rizk. Streptococcus mutans, Candida albicans, and the Human Mouth: A stcky Situation, Plos Pathogens 2013.
[39]. M Moezizadeh. Anticariogenic effect of tea: A review of literature, Journal of Dentistry and Oral Hygiene, 5(9), 2013.
[40]. A Moghbel, A Farjzadeh, N Aghel, H Agheli, N Raisi. The Effect of Green Tea on Prevention of Mouth Bacterial Infection, Halitosis, and Plaque Formation on Teeth, Iranian Journal of Toxicology, 5, 2011.
[41]. M Mohan, G Jeevanandan, SM Raja. The role of green tea in oral health - A Review, Asian journal of pharmaceutical and clinical research, (11), 2018, 2-3.
[42]. M Onisi, F Ozaki, F Yoshino, Y Murakami. An experimental evidence of caries preventive activity of non-fluoride component in tea. Koku Eisei Gakkai Zasshi, 31(2), 1981b, 158–162.
[43]. Onisi M, Shimura N, Nakamura C and Sato M (), A field test on the caries preventive effect of tea drinking. J. Dent. Health 31(1) 1981, 13–19.
[44]. T Ooshima, T Minami, W Aono, Y Tamura, S Hamada. Reduction of dental plaque deposition in humans by oolong tea extract. Caries Res, 28, 1994, 146–149.
[45]. S Otake, M Makimura, T Kuroki, Y Nishihara, M Hirasawa . Anticaries effects of polyphenolic compounds from Japanese green tea. Caries Res, 25, 1991, 438-443.
[46]. MA Pfaller, DJ Diekema, RN Jones, SA Messer, RJ Hollis. Sentry Participants Group, Trends in antifungal susceptibility of Candida spp. Isolated from pediatric and adult patients with bloodstream infections: SENTRY antimicrobial surveillance program, 1997 to 2000, Journal of Clinical Microbiology, 40, 2002, 852-856.
[47]. Ramesh K, Rajesh E, Devi GN and Jimson S (2016), Green tea in oral health - A Review, Biomedical & Pharmacology Journal, Vol. 9(2), 2016, 851-852.
[48]. Ramesh, E Rajesh, GN Devi, S Jimson. Green Tea in Oral Health – A Review, Biomedical and Pharmacology Journal, 2016.
[49]. U Ranganathan, CH Akhila. Streptococcus mutans: has it become prime perperator for oral manifestations, Journal of Microbiology and eaperimentation, 2019.
[50]. S Rassameemasmaung, P Phusudsawang, V Sangalungkarn. Effect of green tea mouthwash on oral malodor. ISRN 2013, 975148.
[51]. M Sakamoto, M Umeda, Y Benno. Molecular analysis of human oral microbiota. J. Periodont. Res, 40, 2005, 277-285.
[52]. S Sakanaka, T Sato, M Kim, T Yamamoto. Inhibitory Effects of Green Tea Polyphenols on Glucan Synthesis and Cellular Adherence of Cariogenic Streptococci, Agricultural and Biological. Chemistry, 54 (11), 1990, 2925-2929.
[53]. A Sapkota. Autoclave – definition, parts, principle, procedure, types and uses, Microbe notes, 2020.
[54]. A Sapkota. Instruments used in microbiology lab with principle and uses, microbe notes, 2020.
[55]. A Sapkota. Laminar flow hood/cabinet – definition, parts, principle, uses, types, Microbe notes, 2020.
[56]. A Sapkota. Nutrient agar-principle, composition, preparation, results, uses, Microbe notes, 2020.
[57]. S Sarkar, A Chowdhury, S Das, B Chakraborty, P Mandal, M Chowdhury. Major tea processing particles in India, Original research article, International Journal of Bioassays, 2016.

[58]. S Sehgal. Dental Caries are formed, 2019. https://medium.com/radiantstardental/how-dental-caries-are-formed.
[59]. MG Shepherd. The pathogenesis and host defence mechanisms of oral candidosis, N Z Dent J, 82, 1986, 78-81.
[60]. V Singh, Kuldeep, A Bajaj, M Kalra. Green tea: enhancing oral health, Journal of Dental Sciences University, 1, 2016.
[61]. JM Song, KH Lee, BLSeong. Antiviral effect of catechins in green tea on influenza virus, Antiviral Res, 68, 2005, 66-74.
[62]. S Soni, P Jain, VK Chaudhary. Beneficial effects of polyphenolic beverage on oral and Periodontal health, Journal of contemporary dental sciences, 1 (1) 33-41.
[63]. A Staskova, R Nemcova, S Lauko, A Jenca. Oral Microbiota from the Stomatology Perpective, Open access peer-reviewed chapter, 2019.
[64]. JM Tanzer, J Livingston, AM Thompson. The microbiology of primary dental caries in humans, J. Dent. Educ, 65, 2001, 1028-1037.
[65]. Trisha, Microscopy and properties of microscope, Biology discussion,http://www.biologydiscussion.com/microscope/microscopy-&-properties-of- microscope.
[66]. WG Wade. The oral microbiome in health and disease, Pharmacological research, ScienceDirect, 69(1), 2013, 137-143.
[67]. JH Weisburger, A Rivenson, Caliaga. Effect of tea extracts, polyphenols, and epigallocatechin gallate on azoxymethane-induced colon cancer, proceedings of the society for experimental biology and medicine, 217, 1998, 104-108.
[68]. CD Wu, GX Wei. Tea as a functional food for oral health, Nutrition, 18, 2002, 443-444.
[69]. M Yoshiharu, K Kazuko, H Yukio, T Toshio. Anti-demineralizing potential of bottled sugar-free green tea beverages in vitro, Oral Sci. Int, 6, 2009, 21-26.
[70]. H Yu, T Oho, S Tagomori, T Morioka, Anticariogenic effects of green tea, Fukuoka Igaku Zasshi, 83, 1992, 174-180.
[71]. Zelnicek T (2016), Streptococcus mutans – Tooth Decay, Microwiki,http://microbewiki.kenyon.edu/index.php/streptococcus-mutans-Tooth-Decay.
[72]. J Zhang, S Kashket. Inhibition of salivary amylase by black and green teas and their effects on the intraoral hydrolysis of starch. Caries Research, 32, 1998, 233-238.