MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Antibacterial activity of some plant extracts against *Staphylococcus aureus* and *Streptococcus mutans* isolated from tooth decay

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Received:16/8/2023
Accepted:11/9/2023

Abstract: The relationship between oral disorders and the activity of microbial species that comprise the oral cavity microbiota is well documented. This study was done to determine the antimicrobial properties of the black pepper, green tea and clove extracts against multi-drug resistant bacteria causing caries. Fifty samples were isolated from soft caries of people with dental caries at the dental hospitals of Mansoura University. Identification of these isolates was done by using morphological and biochemical methods. Antibiotic sensitivity profiles of the isolated bacteria were done. Black pepper, green tea and clove extracts were prepared and used for studying their effect against multi-drug resistant bacteria causing caries. *Staphylococcus aureus* was examined using an electron microscope after being exposed to clove extract. Clove extract proved to be the most productive extract, according to our findings.

keywords: Black pepper, Green tea, Clove, *Staphylococcus aureus* , *Streptococcus mutans*

1. Introduction

Oral diseases are regarded as a major public health issue worldwide **[1]**. The relationship between oral disorders and the activity of microbial species that comprise the oral cavity microbiota is well documented **[2]**. *Staphylococcus aureus*, *Streptococcus mutans*, lactobacilli, and other acidogenic Gram-positive bacteria metabolize sucrose to produce organic acids, mainly lactic acid, which dissolve the calcium phosphate in teeth, leading to decalcification and eventual decay **[3]**. Because they create large amounts of lactic acid and can withstand the effects of low pH, *streptococci* overtake all other bacteria in terms of producing dental caries **[4]**. In addition, these bacteria also create extracellular polysaccharides, particularly dextran, leading to the production of plaque around teeth **[5]**. Cases of *S. aureus* and *S. mutans* resistant to antibacterial drugs used in dental care products have been reported **[6]**. The rise in incidence of oral diseases, the increased resistance of pathogenic bacteria to currently used antibiotics, and the financial considerations in developing countries all contribute to the global need for alternative treatment options that are cheap, safe, and effective **[7].**

Natural products created from medicinal plants have been shown to be a rich supply of biologically active molecules and can be used as an excellent alternative option for treating multidrug resistant infections. There is a lot of promise in finding novel bioactive chemicals because there are over 500,000 plant species that exist in the world and only 1% of them have been studied phytochemically **[8]**. Previous reviews have discussed the broad antibacterial properties of plant-derived compounds like essential oils and their role in curing oral problems **[9,10]**.

Green tea contains polyphenols such as catechins, and epigallates. Its antibacterial activity is due to the oxidation of polyphenols catalyzed by polyphenol oxidase enzyme **[11]**. Cloves (*Syzygium aromaticum*) are dried aromatic unopened floral buds, belonging to the family Myrtaceae. Clove has antifungal, antibacterial and antiviral action. It has also been used as an antispasmodic, analgesic and

antiseptic **[12]**. Piperine is an essential alkaloid bioactive component present in black pepper seeds. Piperine has also been found to have a variety of medicinal properties, including antibacterial, antioxidant, chemoprotective, anti-inflammatory, and neuroprotective activity **[13]**.

This study aimed at detecting the antimicrobial activity of piperine, green tea and clove extract against microorganisms causing dental caries.

2. Materials and methods

Collection of samples:

Samples of soft caries were collected from patients with dental caries at the dental hospitals, Department of Oral and Maxillofacial Surgery, Mansoura University. Samples were collected under aseptic conditions and sent to the Microbiology Diagnostics and Infection Control Unit (MDICU), Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University. The samples were cultured on appropriate media, and incubated aerobically at 37^{o}C overnight.

Preparation of plant extracts:

The three herbal plants were dried and pulverized into fine powder. The powdered material was stored in air tight sterile containers and protected from sunlight until required. The seeds were powdered just before preparation. The following method was used for extraction **[14,15]**

1. Ten grams (10g) of every dried powdered plant material was mixed with 100 ml of 95% ethanol solvent in sterile conical flask, covered with foil paper and placed on a rotary shaker for 24 hours.
2. Then it was filtered through Whitman filter paper (No 1).
3. The supernatant was collected and concentrated in vacuum for 15 minutes at 37°C using a Rotary evaporator to make the final volume half of the original volume (stock solution).
4. The concentration was then dissolved in 10 ml of 1% dimethylsulfoxide (DMSO).
5. All extracts were sterilized by filtration through bacterial filter of pore size 0.45µm using positive pressure, then filtrate was kept at 4°C in refrigerator till use.

Antibacterial activity of herbal plant extracts against *S. aureus and S. mutans*:

The antibacterial activity of ethanolic plant extracts against *S. aureus and S. mutans* isolates was determined by using the agar well diffusion method according to Hassan et. al, 2009 as follows: **[16]**

1. Muller Hinton Agar medium was prepared and inoculated with *S. aureus, S. mutans* by streaking with sterile nontoxic cotton swab pre moistened with these organism's suspension in three directions over the entire surface of the agar plates to obtain a uniform inoculum. The density of these organism's suspension was equivalent to that of 0.5 MacFarland standard (1.5 x 10^{8} CFU/mL).
2. Sterile cork borer was used to make wells of 6mm in diameter in the agar plate.
3. 150 µl of plant extracts were introduced into each well using sterile Pasteur pipette and allowed to stand for 1 hour at room temperature to allow the plant extracts to diffuse into the medium. The DMSO was used in the same manner as a negative control.
4. The plates were then incubated at 37ºC for 18-24 hours.
5. After incubation the entire diameter of the inhibition zone was measured on all 3 replicates and the average value was tabulated.

Determination of the Minimum inhibitory concentrations (MICs) of the plant extracts using the Microtiter plate technique:

The MICs of the plant extracts were determined by using microtiter plate technique as previously described **[17,18,19]**. *S. aureus* and *S. mutans* isolates (0.5 MacFarland suspension) were dispensed into each well of a sterile 96-well microtiter plate, and 100 µl of the first dilution of the tested extracts (100 mg/ml for clove and green tea) were added into the first row (12 wells). With the exception of the final two tubes, all subsequent serial dilutions involved mixing before transferring 100 mL from the first tube to the second, then 100 mL from the second tube to the third, and so on until 100 mL were placed into the final tube. As a positive control, wells with solely bacterial isolates were employed. Wells

containing sterile uninoculated nutrient broth alone and wells containing each of the three used plant extracts alone were used negative controls. The test plate was incubated for 24 hours at 37°C. Following incubation, the resulting turbidity was measured using a Beckman DU-70 UV-V and optical density readings at 600 nm **[20]**.

Scanning Electron Microscopy (SEM) analysis:

The SEM test was carried out in accordance with the procedure outlined by **[21]**. Discs of a solid media (0.5 cm in diameter) containing bacterial growth were fixed for 24 hours in a refrigerator using a fixative solution that contained 2.5% glutaraldehyde, 2.5% paraformaldehyde, 0.05 M cacodylate buffer, and 0.001 M $CaCl_2$. The samples were then fixed using 1% osmium tetroxide aqueous in water for 1 to 4 hours at room temperature after being rinsed with cacodylate buffer. The materials were dehydrated using a succession of acetone after being washed three times with distilled water. The solid samples were placed on gold-coated carbon tape-covered aluminum stubs and stored in a desiccator with silica gel until SEM analysis **[22]**. The SEM, JSM-5500LV (JEOL, Japan) at a voltage of 20 kV was employed for observation.

Transmission Electron Microscopy (TEM) analysis:

Following overnight incubation, a thin slice was produced, the bacterial colonies were transferred to Eppendorf tubes, three times sterile nanopure water was used to wash the tubes, and finally the tubes were centrifuged to get the pellet (organism). The bacteria were then reconstituted in 1 mL of sterile nanopure water. Bacterial suspension fractions were fixed with 2% glutaraldehyde in 0.1 mol/L sodium phosphate buffer, pH 7.4, at 4°C for 90 minutes. After being postfixed in 1% osmium oxide for 90 minutes at room temperature, cells were stained with 0.25% uranil acetate for 1 hour. The cells were then immersed in Spurr resin and given 24 hours in an oven set to 60 OC for polymerization. Using a Sorvall MT 5000 (Dupont, Boston, MA) ultramicrotome with a diamond knife, resins were sectioned by cutting an 80-nm film at 25°C. The thin slice was seen using a TEM JEOL-JEM 1200 EX II (Jeol Ltd, Tokyo, Japan) at an accelerating voltage of 80 kV while placed on copper grids coated in carbon film. Then, without staining, bacteria were preserved with 20 L of 5% formaldehyde **[23]**. The samples were immediately examined with the TEM.

3. Results and Discussion

In this study, fifty clinical samples were obtained from the patients admitted to Mansoura University dental Hospitals. Out of the fifty samples, 59 clinical isolates were isolated **(Table 1)**.

Table 1: Number of dental caries associated bacteria isolated from patients.

Microorganisms	**Number of isolates (N=59)**
Streptococcus mutans Streptococcus sobrinus	14 4
Staphylococcus aureus Staphylococcus epidermidis	25 5
Lactobacillus	9
Candida albicans	2

As shown in **Table (1)** the most common bacteria associated with dental caries were *S. aureus* followed by *S. mutans* and.This is in agreement with**,** who said that the most representative human cariogenic microbes included *S. mitis, S. mutans, S. aureus, Lactobacillus* **[24]**.

Antibacterial activity of the plant extracts against *S.* aureus and S. mutans isolates

In the current study three ethanolic crude extracts derived from different parts of three herbal plants species traditionally used in Egypt and belonging to different families were screened for their antibacterial activity against clinical *S. aureus* and *S. mutans* isolates.

The diameter of the inhibition zones of ethanolic crude extracts are tabulated in **Table (2)** and shown in **Photo (1).** Of all extracts, the ethanolic extract of clove was the most active one with inhibition zone diameters ranging between 20mm-25mm followed by green tea with inhibition zone diameters ranging between 0mm-20mm. However, black pepper had no effect. These results are supported by**,** who reported that maximum antibacterial efficacy was shown by clove(30mm), and minimal inhibition was shown by green tea (2mm) **[24]**.

Table (2): Antimicrobial activity of ethanolic plant extracts against clinical *S. aureus* isolates

Resistant *S. aureus* isolatescode	Diameter of inhibition zone (mm) of different ethanolic plant extracts		
	Green Tea	Clove	Black Pepper
2	0	20	0
14	15	20	0
16	10	25	0
21	15	20	0
28	0	20	0
34	12	25	0
37	20	25	0
40	0	20	5
46	5	25	0
51	10	20	0

Photo (1): Inhibition zones of different ethanolic plant extracts against *S. aureus* isolates where 1: Green tea; 2: clove; *3*: Black pepper; and 4: DMSO.

Table (3): Antimicrobial activity of ethanolic plant extracts against clinical *S. mutans* isolates

Resistant *Streptococcus mutans* isolates code	Diameter of inhibition zone (mm) of different ethanolic plant extracts		
	Green Tea	Clove	Black Pepper
19	10	10	0
27	15	13	0
36	10	10	0

Table (3) lists the widths of the inhibition zones of ethanolic crude extracts. The most active extract was the ethanolic extract of green tea, which induced inhibition zone diameters between 10mm and 15mm. Clove caused inhibition zone diameters between 10mm and 13mm. Black pepper, however, had no impact. According to **[24]**, black pepper extract can weakly to moderately suppress *S. mutans* growth at each test dose. This contradicts our findings. However, at the prescribed dose, the black pepper extract exhibited no antibacterial action against the isolates of *S. mutans* or *S. aureus*. Our findings contradict those of who claimed that black pepper extract (Piper nigrum L.) can weakly to moderately suppress the development of *S. mutans* at each dose of the test **[25]**. In their study the inhibition zone diameters of 6.25%, 12.5%, and 25% of black pepper were 12.4 mm, 13.7 mm, and 14.9 mm, *respectively*. Additionally, our results also disagree with**,** who claimed that black pepper extract had anti-bacterial action against *S. aureus* with inhibitory zone diameter of >10mm **[26]**.

Minimum inhibitory concentrations (MICs) of the effective plants extract against *S. aureus* and *S. mutans* clinical isolates

Table (4): Minimum inhibitory concentration (MICs) of clove extract against clinical isolates *S. aureus* and *S. mutans*.

Serial Conc. of clove extract (mg/ml)	Optical density readings of clove extract at 360-450nm	
	S. aureus	*S. mutans*
Negative control	0.0968	0.1255
Positive control	1.3994	1.0755
100	0.7124	0.6112
50	0.8323	0.6913
25	0.9511	0.7778
12.5	1.0242	0.8491
6.25	1.1232	0.9077
3.125	1.1551	1.0611
1.563	1.2966	1.0756
0.781	1.3995	1.0757
0.391	1.3996	1.0759
0.195	1.3999	1.0760
MIC Value	1.563	3.125

Table (5): Minimum inhibitory concentrations (MICs) of green tea extract against clinical isolates *S. aureus and S. mutans*.

Serial Conc. of green tea extract (mg/ml)	Optical density readings of green tea extract at 360-450nm	
	S. aureus	*S. mutans*
Negative control	0.0658	0.1544
Positive control	1.1532	1.0546
100	0.8823	0.6112
50	0.9213	0.6945
25	0.996	0.754
12.5	1.077	0.778
6.25	1.154	0.818
3.125	1.155	0.986
1.563	1.516	1.055
0.781	1.157	1.056
0.391	1.158	1.057
0.195	1.159	1.058
MIC Value	12.5	3.125

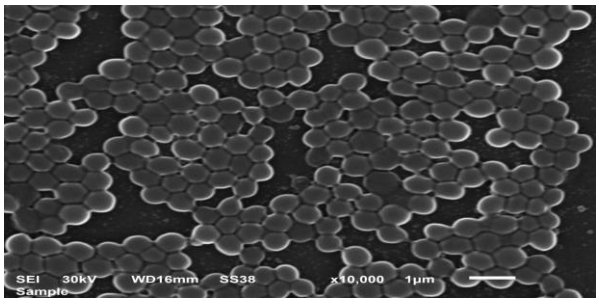
The most effective herbal plant extracts against *S. aureus* and *S. mutans* isolates were: clove and green tea extracts. Values of MICs

were determined by using the microtitration plate and were shown in **Tables (4 & 5)**. The results demonstrated that the MICs of clove for *S. aureus and S. mutans* were 1.563 and 3.125 mg/ml respectively and green tea were 12.5 and 3.125mg/ml respectively. the highest MIC was observed in clove extract for *S. aureus*, followed by green tea extract. while the highest MIC was observed in green tea extract for *S. mutans*, followed by clove extract. The results indicated that there is decrease in the growth of *S. aureus and S. mutans* with the increase of plant extracts concentration and vice versa.

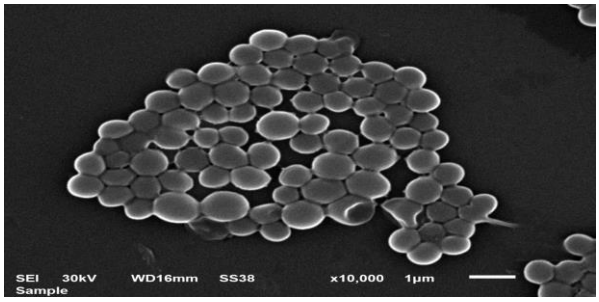
Our results are in agreement with who recorded that the MICs of *Szyigium aromaticum* against *S.aureus* ranged from 5 to 20 mg/mL **[27].** On the other hand, our results disagree with, who reported that catechins are inhibitory for *S. mutans* with MICs ranging between 50 and 1000 mg/ml **[28]**, and also disagree with, who recorded that green tea extract showed insufficient antibacterial activity **[29]**.

***Electron* microscopic examination of plant susceptible *S. aureus* isolate**

The most effective medicinal plant extract against MDR *S. aureus* isolate was clove. This isolate was examined under electron microscope before and after treatment with plant extract.



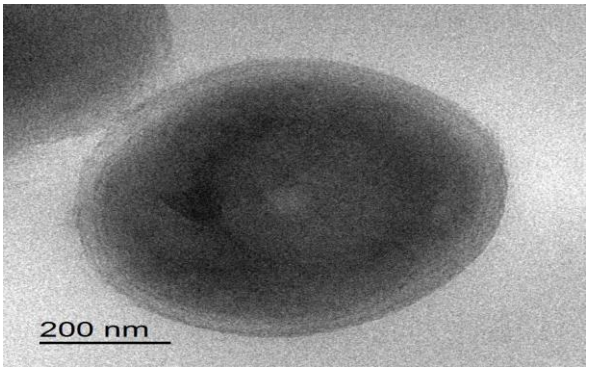
(A) Before treatment



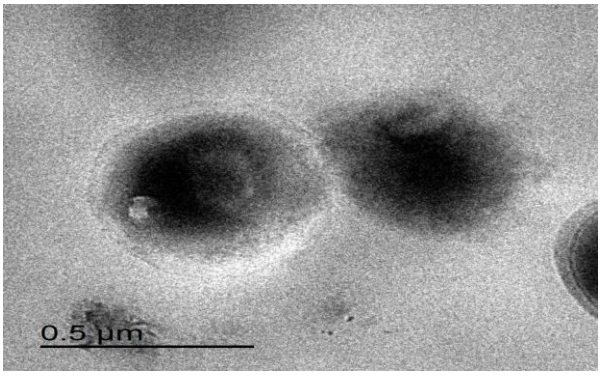
(B) After treatment

Photo (2): Scanning Electron microscopic examination of plant susceptible *S. aureus* isolate

As shown in **photo (2)** (A & B), there is shrinkage of bacterial cells, damage on the cell surface that became irregular, adhesion and aggregation of injured cells or cellular debris in *S. aureus* cells treated with extract. The sizes and location of these cells varied. However, the morphology of the bacterial cells in the untreated control group was regular and spherical.



(A) Before treatment



(B) After treatment

Photo (3): Transmission Electron Microscopic examination of plant susceptible *S. aureus* isolate

As shown in **photo (3)**, the cell membrane and the cell wall deformation were evident in the results, typical cellular forms are lost, cell walls and membranes separated, the cells got larger, and lost their regular shapes.

The electron microscope results were in agreement with, which claimed that *S. aureus* cells treated with extract under SEM showed adhesion and aggregation of injured cells or cellular debris. Furthermore, the size and dispersion of these cells were not constant. Also, under TEM many indications of cell membrane and cell wall deformation may be noticed such as loss of normal cellular forms, separation of cell wall and membrane, large cell sizes, and loss of regular cellular shapes were the most obvious deformation indicators **[30]**.

4. Conclusion

Due to the presence of bioactive chemicals that can hinder the growth of bacteria causing oral illness, the use of medicinal and edible plants (such as clove and green tea extracts) for the formulation of toothpaste should be of interest in oral care products. The use of herbal toothpaste may lower the treatment costs and protect against the negative effects of oral care products containing synthetic ingredients.

4. References

1. Petersen, P.E., Bourgeois, D., Ogawa, H., Estupinan-Day, S. and Ndiaye, C. (2005): The global burden of oral diseases and risks to oral health. Bulletin of the world health organization, **83**, pp.661-669.
2. Jenkinson, H.F. and lamont, R.J. (2005): Oral microbial communities in sickness and in activity health. Trends in microbiology, **13(12)**, pp.589-595.
3. Loesche, W. (2007): Dental caries and periodontitis: contrasting two infections that have medical implications. Infectious disease clinics of North America, **21(2),** pp.471-502.
4. Matsumoto-Nakano, M. (2018): Role of Streptococcus mutans surface proteins for biofilm formation. Japanese Dental Science Review, **54(1)**, pp.22-29.
5. Featherstone, J.D.B. (2004): The continuum of dental caries—evidence for a dynamic disease process. *Journal of dental* research, **83**(1_suppl), pp.39-42.
6. Deng, D.M., Ten Cate, J.M. and Crielaard, W. (2007): The adaptive response of Streptococcus mutans towards oral care products: involvement of the ClpP serine protease. European *journal of oral sciences*, **115(5)**, pp.363-370.
7. Badria, F.A. and Zidan, O.A. (2004): Natural products for dental caries prevention. *Journal of Medicinal Food*, **7(3),** pp.381-384.
8. Hobson, C., Chan, A. N., & Wright, G. D. (2021). The antibiotic resistome: a guide for the discovery of natural products as antimicrobial agents. Chemical Reviews, **121(6),** 3464-3494.
9. Valdivieso-Ugarte, M., Gomez-Llorente, C., Plaza-Díaz, J., and Gil, Á. (2019). Antimicrobial, antioxidant, and immunomodulatory properties of essential oils: A systematic review. Nutrients, **11(11)**, 2786.
10. Hou, T., Sana, S. S., Li, H., Xing, Y., Nanda, A., Netala, V. R., and Zhang, Z. (2022). Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. Food Bioscience, 47, 101716.
11. Moon, S.E., Kim, H.Y. and Cha, J.D. (2011): Synergistic effect between clove oil and its major compounds and antibiotics against oral bacteria. Archives of oral biology, **56(9),** pp.907-916.
12. Mittal, R. and Gupta, R.L. (2000): In vitro antioxidant activity of piperine. Methods and findings in experimental and clinical pharmacology, **22(5)**, pp.271-274.
13. Kanth, M.R., Prakash, A.R., Sreenath, G., Reddy, V.S. and Huldah, S. (2016): Efficacy of specific plant products on microorganisms causing dental caries. *Journal of Clinical and Diagnostic* Research, **10(12)**, p.ZM01.
14. Handa, S.S.; Khanuja, S.P.S.; Longo, G. and Rakesh, D.D. (2008): Extraction technologies for medicinal are aromatic plants, United Nations Industrial Development Organization and the International Centre for Science and High Technology, ICS-UNIDO, Italy.
15. Al-Daihan, S.; Al-Faham, M.; Al-shawi, N.; Almayman, R..; Brnawi, A.; Zargar, S. and Shafi Bhat, R. (2013): Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. *J. King Saudi Uni. Sc*., **25**: 115-120.
16. Hassan, A., Rahman, S., Deeba, F. and Mahmud, S. (2009): Antimicrobial activity of some plant extracts having hepatoprotective effects. *Journal of Medicinal Plants* Research, **3(1)**, pp.020-023.
17. Abu-Shanab, B., ADWAN, G.M., Abu-Safiya, D., Jarrar, N. and Adwan, K. (2004): Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. *Turkish Journal of Biology*, **28(2)**, pp.99-102.
18. Abou-Elkhair, E., Fadda, H., & Abu-Mohsen, U. (2010): Antibacterial activity

and Phytochemical analysis of some medicinal plants from Gaza Strip-Palestine. *Journal of Al-Azhar University-Gaza, ICBAS Special Issue*, **12**, 45-54.
19. Radojevic, I.D., Stankovic, M.S., Stefanovic, O.D., Topuzovic, M.D., Comic, L.R. and Ostojic, A.M. (2012): Great horsetail (Equisetum telmateia Ehrh.): active substances content and *biological effects. EXCLI journal*, **11**, p.59.
20. Wiegand, I., Hilpert, K. and Hancock, R.E. (2008): Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols, **3(2),** pp.163-175.
21. Alves, E., Lucas, G.C., Pozza, E.A. and de Carvalho Alves, M. (2013): Scanning electron microscopy for fungal sample examination. Laboratory protocols in fungal biology: current methods in fungal biology, pp.133-150.
22. Daroodi, Z., Taheri, P. and Tarighi, S. (2021): Direct antagonistic activity and tomato resistance induction of the endophytic fungus Acrophialophora jodhpurensis against Rhizoctonia solani. Biological Control, 160, p.104696.
23. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Tapia-Ramirez J and Yacaman MJ (2005): The bactericidal effect of silver nanoparticles. Nanotechnology. **16**:2346-2353.
24. KANTH, M.R., PRAKASH, A., Sreenath, G., REDDY, V.S. and Huldah, S. (2016): Efficacy of Specific Plant Products on Dental Caries Causing Microorganisms. *Journal of Clinical & Diagnostic* Research, **10(12).**
25. Hakim, R.F. and Alwi, F., (2020): Effect of Black Pepper Extract (Piper nigrum L) towards Streptococcus Mutans Growth: Pengaruh Ekstrak Lada Hitam (Piper nigrum L) terhadap Pertumbuhan *Streptococcus Mutans. Dentika: Dental Journal*, **23(1**), pp.13-17
26. Pundir, R.K. and Jain, P. (2010): Comparative studies on the antimicrobial activity of black pepper (Piper nigrum) and turmeric (Curcuma longa) extracts. *Int J Appl Biol Pharm Technol*, **1(2),** pp.492-500.
27. Oluwasina, O.O., Ezenwosu, I.V., Ogidi, C.O. and Oyetayo, V.O. (2019): Antimicrobial potential of toothpaste formulated from extracts of Syzygium aromaticum, Dennettia tripetala and Jatropha curcas latex against some oral pathogenic microorganisms. AMB Express, **9**, pp.1-13.
28. Taylor, P.W., Hamilton-Miller, J.M. and Stapleton, P.D. (2005): Antimicrobial properties of green tea catechins. Food science and technology bulletin, 2, p.71.
29. Bazzaz, B.S.F., Sarabandi, S., Khameneh, B. and Hosseinzadeh, H. (2016): Effect of catechins, green tea extract and methylxanthines in combination with gentamicin against Staphylococcus aureus and Pseudomonas aeruginosa: combination therapy against resistant bacteria. *Journal of pharmacopuncture*, **19(4)**, p.312.
30. El-Maati, M. F. A., Mahgoub, S. A., Labib, S. M., Al-Gaby, A. M. A., & Ramadan, M. F. (2016): Phenolic extracts of clove (Syzygium aromaticum) with novel antioxidant and antibacterial activities. *European Journal of Integrative Medicine*, **8(4),** 494–504.