Original Research Paper

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"COMPARATIVE EVALUATION OF EFFICACY OF BIOSYNTHESIZED SILVER NANO-PARTICLES, METRONIDAZOLE, GREEN TEA EXTRACT AND COMBINATION OF METRONIDAZOLE AND GREEN TEA EXTRACT WITH SILVER NANOPARTICLES ON MINIMUM INHIBITORY CONCENTRATION AGAINST STRAINS OF *P. GINGIVALIS:* AN IN-VITRO STUDY"

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ABSTRACT

Introduction: Periodontitis is an infectious inflammatory disease caused by the bacteria of the dental plaque and *P. gingivalis* appears to be one of the prime etiological agents in the pathogenesis and progression of the inflammatory events of periodontal disease, this Gram-negative bacterium is an obligately anaerobic rod which forms black-pigmented colonies on blood agar plates. Metronidazole is one of the most widely used antibacterial compounds in the treatment of periodontal disease. Metronidazole seems to be very efficacious and efficiently in inhibiting anaerobic microorganisms in the periodontal pockets. EGCG a phenolic component present in green tea completely inhibits the growth of three strains of *P. gingivalis.* also functions in a similar way to that of chlorhexidine. AgNPs have the potential to decrease the prevalence of periodontal disease and other oral bacterial conditions.

Objective: To assess and compare the efficacy of green biosynthesized silver nanoparticles, metronidazole and green tea extract individually and as a combination against *P. gingivalis.*

Methodology: *P. gingivalis* were cultured under strict anaerobic conditions to test for inhibition against green tea extract, silver nano-particles and metronidazole each and also for the combinaton of the above three. The characterization of the silver nano-particles was done by scanning electron microscopy and UV-Vis spectrophotometry. The efficacy of the samples individually and combined were determined by well diffusion assay and Microbroth dilution assay.

Conclusion: The combination of AgNP, green tea extract and metronidazole has shown to be a possible upgradation to the common antimicrobial regime and also to evade the problem of drug resistance. The combination can be effective adjunct to Scaling and root planing as form of local drug delivery agent.

KEYWORDS : Green tea extract; Metronidazole; Nanoparticles; Minimum inhibitory concentration; *P. gingivalis*; Scanning electron microscopy.

INTRODUCTION:

Periodontitis is a highly prevalent inflammatory disease in tooth supporting tissues, induced by bacteria growing in a biofilm on tooth surfaces. *Porphyromonas gingivalis (P. gingivalis), Treponema denticola (T. denticola), and Tannerella forsythia (T. forsythia)* are among the Gram-negative, mostly anaerobic bacteria found in the biofilm associated with periodontitis.[1] *P. gingivalis* consequently relies on the continual flow of inflammatory serum exudates to receive critical nutrients.[2] The breakdown of C3 by *P. gingivalis* gingipains and *P. intermedia* InpA synergizes attenuation and gives protection to other bacteria that are ordinarily susceptible to complement-mediated lysis.[3] Green tea leaves has cognitive function and positive impact on bone density, caries, periodontal disease, and diabetes.[4] In vitro, green tea polyphenols exhibit antioxidant activity by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions.[5] Metronidazole is one of the most common broad-spectrum antibiotics and is active against most of the periodontal pathogens.[6]

Metallic nanoparticles are the most promising because they have high antibacterial capabilities due to their huge surface area to volume ratio, which is picking researchers attention due to rising microbial resistance to metal ions, antibiotics, and the emergence of resistant strains.[7] Because of its small size, the nanoparticle has a vast surface area to interact with bacterial cells, resulting in a higher proportion of interaction than larger particles.[8] Although there are various studies on periodontal pathogens using green biosynthesized silver nanoparticles, this study is an attempt to assess and compare the efficacy of biosynthesized green tea extract silver nanoparticles, metronidazole and green tea extract individually and in conjugation against *P. gingivalis.*

OBJECTIVE:

- To evaluate the efficacy of silver nano particles against minimum inhibitory concentration of *P. gingivalis* in-vitro.
- To evaluate the efficacy of green tea extract against minimum inhibitory concentration of *P. gingivalis* in-vitro.
- To evaluate the efficacy of metronidazole against minimum inhibitory concentration of *P. gingivalis* in-vitro.
- To evaluate the effect of combination of metronidazole and green tea extract with silver nano-particles against minimum inhibitory concentration of *P. gingivalis* in-vitro and compare it against the individual in-vitro effect of silver nano particles, green tea and metronidazole on *P. gingivalis*.
- To statistically evaluate the intergroup and intragroup effects of the above four groups against *P.gingivalis*

MATERIALS AND METHODS:

MATERIALS USED:

Preparation of extract-

- Sun dried *Camellia Sinensis* green tea leaves
- Grinder
- Sterile distilled water
- Rotary vacuum evaporator
- Erlenmeyer flask
- Glass rod
- Whiteman's no. 1 filter paper
- Tripod stand
- Gas burner

The microbial strains of the following species were be used-

- *Porphyromonas Gingivalis* (MTCC)

Materials used for green synthesis of silver nano particles-

- Erlenmeyer flask

- Glass rod
- Silver Nitrate solution

Material used for microbial inoculation standard adjustment, culture media and supplements for agar well diffusion & spread plate validation

- 0.5 McFarland standard solution
- Micropipettes and tips
- Gloves
- Mouth mask
- Inoculating loops and swabs
- Sterile cuvettes
- Sterile distilled water
- Petri dishes- glass
- Muller Hinton Agar
- Sterile swabs
- Well borer Mouth mask

Other material-

- Metal spatula
- Sterile cotton
- Butter paper
- Tweezers

Laboratory equipment used:

- Heating mantle
- Incubator
- Centrifuge
- Laminar air flow chamber
- Autoclave
- Microplate reader- LabLife ER-2700
- Biochemical analyser
- Rotary shaker

The study was reviewed and approved by the institutional review board and was carried out in the department of periodontics, Coorg institute of dental sciences, Virajpet.

Preparation of *C. seninsis* leaf extract using Decoction Method:

The leaves of *C. seninsis* was washed and spread over filter paper to remove the wetness of leaf followed by drying at room temperature for an hour. 0.3g of leaves was weighed and sliced into tiny size followed by boiling in 300 mL of sterile distilled water in Erlenmeyer flask for 15 min and was allowed to cool at room temperature. The boiled leaf extract was then filtered twice using Whatman no.1 filter paper and a pale green-yellow in color is obtained which was then transferred to an Eppendorf tube and labelled as **Group 1.** It was then serially diluted to obtain varying concentrations to be used in the test (100%,50%,25%, 12.5%, 6.25%).

Green synthesis of AgNPs:

The AgNPs were synthesized using *C. seninsis* leaf extract as a reducing agent. Briefly, 50 mL of *C. seninsis* leaf extract was mixed with the 100 mL of aqueous solution of 1mM $AgNO_3$ in a 250 mL Erlenmeyer flask at room temperature. The Pale greenish yellow color solution became deeper brown color in the $AgNO_3$. The synthesized AgNPs was then collected by centrifugation (5000 rpm, 25 mins) The air-dried AgNPs was stored in an Eppendorf tube and labelled as **Group 2** and serially diluted (100%,50%,25%,12.5%,6.25%) for further experiment and characterization.

Characterization of synthesized AgNPs:

UV-Visible spectroscopy was carried out by a Shimadzu UV-Vis Spectroscopy (1601) operating at a resolution of 1nm in wavelength range between 365nm-800nm. The size and morphology of AgNPs were examined by using Zeiss Evo10 scanning electron microscope operated at an accelerating voltage of F15.00 kV.

Preparation of Metronidazole Aqueous Suspension:

Metronidazole was purchased from Sigma Aldrich(M3761). 1mg/ml of concentrated metronidazole was prepared using double distilled water which was then serially diluted and transferred into an Eppendorf tube and was labelled as **Group 3** and used for further investigation.

Preparation of MIX group:

Equal proportion of samples (0.33 mg) from each group was pipetted out into a new sterile Eppendorf tube and was labelled as **Group 4**. It was then serially diluted (100%,50%,25%,12.5%,6.25%) for further experiment.

Preparation of bacterial inoculum:

The bacterial culture was maintained on Muller Hinton agar. 24 hr cultures, were prepared by picking a loop full of colonies from the stock plate and adding into MH broth for micro-broth dilution tests and plated on to Muller Hinton agar for well diffusion tests. Following incubation at 37±2°C for 24 hr, McFarland adjustment to 0.5 standard was carried out in either broth or sterile saline for microbroth or well diffusion assays respectively.

Well diffusion assay:

Muller Hinton agar with a pH of 7.2 ± 0.2 medium was poured into the plates to a uniform depth and refrigerated for solidification. Prior to use, the plates were transferred to the incubator at 37°C for 30 min to clear the moisture content that develops on the agar surface. Overnight culture of test organisms was used and the bacterial suspension was adjusted to McFarland Standard 0.5. The plates were then evenly inoculated with standardized inoculums by means of cotton swab to ensure the confluent growth of the organism. Wells of 6mm in diameter using well cutter were made in the Muller Hinton agar plates and 100 µL(100 µg) of samples (sample concentration mg/mL) from each group was transferred into each well following which the plates were incubated for 24 h at 37 °C and the inhibition zone diameter of each well was measured under a light source on a colony counter with a sterile metal scale. The assay was carried out in triplicate for all the test organisms. Wells with sterile water alone were maintained as a control. Synergistic activity of silver nanoparticles with metronidazole and green tea extract in the ratio of 1:1:1 (MIX-Group 4) was also carried out as per the procedure referred before against *P. gingivalis*.

Determination of minimum inhibitory concentration (MIC)
Microbroth dilution Assay technique:

The 24 hour culture of organisms in MH broth for *P.gingivalis* were adjusted to the McFarland standard 0.5 to yield a cell density of 1.5x 10^8 CFUs/ml and 50 µl was dispensed in each well of a sterile microtiter plate. 100µl of pure inoculum was used as positive growth control and plain broth as negative control. 50 µl of metronidazole along with 50 µl of *P.gingivalis* were added as standard antimicrobial controls. Each concentration from the 4 sample groups along with the inoculated broth was also dispensed to allow for calculation of inhibition. The plates were incubated at 37±2ºC overnight and assessed for turbidity by measuring the optical density at 492nm using a Microplate reader. The well containing the lowest concentration of test sample that showed a reduction in turbidity when compared with the control (without addition of test sample) was regarded as MIC.

STATISTICAL ANALYSIS

Data was analysed using Statistical Package for Social Sciences (SPSS) version 21, IBM Inc. Descriptive data was reported for each variable. Summarized data was presented using Tables and Graphs. Data was normally distributed as tested using the Shaperio-Wilk W test (p-value was less than 0.05). One way ANOVA test was used for comparison of two or more groups. Repeated measures of ANOVA was done for two or more paired reading. Post hoc ana Chi square was used for categorical variables. A level of $p<0.05$ was considered statistically significant.

Well diffusion assay

Table 1: Intra group comparison of well diffusion assay of all four groups at different concentrations

Concentration	Groups	Mean	Std. Deviation	F value	P value
100%	Silver nano particles (AgNPs)	19.333	.5774	1.725	0.220 **NS**
50%		19.667	1.5275		
25%		19.333	1.5275		
12.5%		18.333	1.5275		
6.25%		17.000	1.7321		
100%	Green tea extract	24.00	1.00	3.234	0.060 **NS**
50%		21.33	1.53		
25%		20.00	2.00		
12.5%		21.00	1.00		
6.25%		22.33	1.53		
100%	Metronidazole	26.67	2.52	4.506	**0.024*SIG**
50%		24.33	2.52		

25%		24.00	2.00		
12.5%		23.33	1.53		
6.25%		19.00	2.65		
100%	Mix	27.00	2.65	3.125	0.066 **NS**
50%		25.00	1.00		
25%		23.00	1.00		
12.5%		24.00	1.73		
6.25%		21.00	3.46		

***NS: non-significant, *Sig: significant**

Table 2: Inter group comparison of well diffusion assay activity among four groups at various concentration

Concentrations	Groups	Mean	Std. deviation	F value	P value
100%	AgNPs	19.333	.5774	10.265	**0.004* SIG**
	GTE	24.000	1.0000		
	METRO.	26.667	2.5166		
	MIX	27.000	2.6458		
50%	AgNPs	19.667	1.5275	6.324	**0.017* SIG**
	GTE	21.333	1.5275		
	METRO.	24.333	2.5166		
	MIX	25.000	1.0000		
25%	AgNPs	19.333	1.5275	5.441	**0.025* SIG**
	GTE	20.000	2.0000		
	METRO.	24.000	2.0000		
	MIX	23.000	1.0000		
12.5%	AgNPs	18.333	1.5275	9.128	**0.006* SIG**
	GTE	21.000	1.0000		
	METRO.	23.333	1.5275		
	MIX	24.000	1.7321		
6.25%	AgNPs	17.000	1.7321	2.685	0.117 **NS**
	GTE	22.333	1.5275		
	METRO.	19.000	2.6458		
	MIX	21.000	3.4641		

***NS: non-significant, *Sig: significant**

Microbroth dilution assay technique for determining mic

Table 3: Intra group comparison of groups for MIC against the control at different concentrations

Concentration	Group	Mean	Std Deviation	F value	P value
100%	Green tea extract	0.230	0.021	301.100	**0.001* SIG**
50%		0.349	0.028		
6.25%		0.460	0.035		
3.125%		0.648	0.008		
1.562%		0.778	0.007		
0.781%		0.814	0.014		
100%	Metronidazole	0.101	0.004	5375.407	**0.001* SIG**
50%		0.107	0.004		
6.25%		0.541	0.006		
3.125%		0.683	0.006		
1.562%		0.714	0.008		
0.781%		0.818	0.007		
100%	Silver nanoparticles (AgNP)	0.934	0.026	34.206	**0.001* SIG**
50%		0.983	0.018		
6.25%		1.066	0.013		
3.125%		0.862	0.025		
1.562%		0.8605	0.019		
0.781%		0.891	0.015		
100%	Mix	0.191	0.015	1376.652	**0.001* SIG**
50%		0.221	0.008		
6.25%		0.294	0.010		
3.125%		0.361	0.016		
1.562%		0.488	0.004		
0.781%		0.739	0.002		
Positive control		0.865	0.007		

***NS: non-significant, *Sig: significant**

Table 4: Inter group comparison of groups for MIC against the control at different concentrations

Groups	Concentration	Mean	Std. Deviation	F value	p value
Silver nanoparticles (AgNP)	100%	0.93	0.03	950.189	**0.0001 * SIG**
	50%	0.98	0.02		
	6.25%	1.07	0.01		
	3.125%	0.86	0.03		
	1.562%	0.86	0.02		
	0.781%	0.89	0.02		
MIX	100%	0.19	0.01		
	50%	0.22	0.01		
	6.25%	0.29	0.01		
	3.125%	0.36	0.02		
	1.562%	0.49	0.00		
	0.781%	0.74	0.00		
Green Tea Extract	100%	0.23	0.02		
	50%	0.35	0.03		
	6.25%	0.46	0.04		
	3.125%	0.65	0.01		
	1.562%	0.78	0.01		
	0.781%	0.81	0.01		
Metronidazole	100%	0.10	0.00		
	50%	0.11	0.00		
	6.25%	0.54	0.01		
	3.125%	0.68	0.01		
	1.562%	0.71	0.01		
	0.781%	0.82	0.01		
	Positive control	0.87	0.01		

***NS: non-significant, *Sig: significant**

RESULTS

Intra-group comparison of well diffusion assay activity of all the four groups showed the difference failed to reach the level of significance in all the groups except in the metronidazole group. (Table 1) However inter-group comparison of the activity showed significant difference in all the concentrations of samples except 6.25%. (Table 2)

MIC was determined using microbroth dilution assay. Since the well diffusion assay showed inhibition zone upto 6.25% in all the groups it was decided to serially dilute the concentration four times from 6.25%. (100%,50%,6.25%.3.125%,1.5625% & 0.78175%) The values of inhibition obtained showed a significant dose dependent correlation and were variable.

Intra-group and inter-group comparison showed significant reduction in turbidity in all the concentration of three groups except in silver nano particles when compared against the positive control. (Table 3&4)

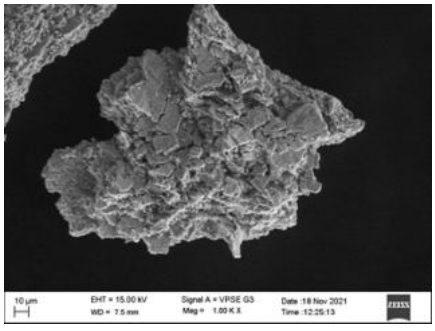

Figure 1: The scanning electron microscopy image of green synthesized AgNPs at 10 µm scale

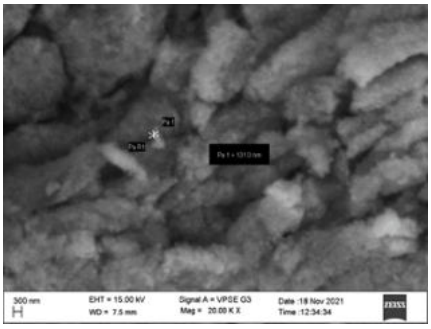

Figure 2: The scanning electron microscopy image of green synthesized AgNPs at 300 nm scale

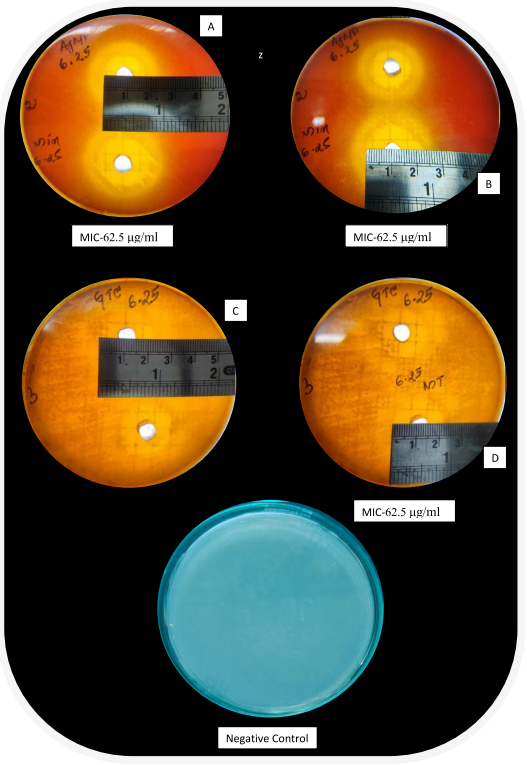

Figure 3: Results of well diffusion method to assess activity of AgNP (A), Mix (B), GTE (C) and Metronidazole(D) groups against *P. Gingivalis*

DISCUSSION:

Over the past few decades, the efficiency of plants has been established, even against drug-resistant species, and it is thought that the receptors for plant-derived antimicrobials are distinct from those for currently available synthetic antimicrobials, which opens up an intriguing avenue to research.[10] **Sakanaka S *et al*** [11] also found out that at concentrations of 250 or 500 g/ml, EGCG totally inhibits the growth of three strains of *P. gingivalis*. It suppressed the enzymatic activities of *P. gingivalis* in a similar way to Chlorhexidine, Doxycycline, and non-antimicrobial chemically modified tetracycline derivatives.

In our study the microbroth assay carried out for green tea extract against *P. gingivalis* showed dose dependent power to inhibit the growth. Since the turbidity reading after 24 hrs at 1.562% was 0.778 which is almost same as the growth of inoculum (Control) at 24hrs (0.87) the MIC of Green tea extract group was concluded to be at 3.125%. (Table 4)

Encapsulating antimicrobial medicines in nanoparticle systems appears as a novel and promising alternative that improves therapeutic efficacy while reducing drug side effects.[12] Silver is an oxygen charged aqueous media which catalyzes the complete destructive oxidation of microorganisms[13] Due to their thin, less rigid, and porous cell walls, gram negative microbes are shown to be more vulnerable to the antibacterial activity of silver nanoparticles than gram positive germs.[14,15] **Shirisha P et al**[16] evaluated four bacterial species *F. nucleatum, P. gingivalis, A. actinomycetumcomitans*, and *P. intermedia* with silver nanoparticles made from *ocimum extract*, and the MIC was determined to be in the µg/ml range. In our study though the well diffusion assay showed significant zone of inhibition in the well diffusion assay, none of the concentrations used showed any statistically significant effect against the growth. (Table 1&2) Similarly, during the assessment of turbidity using microbroth analysis none of the concentrations of AgNP were effective in controlling the growth of the bacteria when compared to the control group. (Table 3&4). The probable reason for this result would be because of the variation in the biochemical or physical properties of these nanoparticles.

Metronidazole group also showed inhibition zones in well diffusion assay and statistical analysis also showed to be significant. Microbroth assay also showed significant dose dependent inhibition of metronidazole when compared to positive control. Another study done by **Fredy Gamboa et al**[17] also found out that 30 isolated strains of *P. gingivalis* was sensitive to metronidazole with MIC values ranging from 0015-4ug/ml. **Carlos-Martín Ardila & Jader-Alexander Bedoya-García**[18] where antibiotic susceptibility to amoxicillin, metronidazole, azithromycin, and moxifloxacin was assessed using an E-test on subgingival samples from 76 patients. They discovered that amoxicillin (24.6%), azithromycin (21.3%), and metronidazole resistance was present in *P. gingivalis* samples (24.6 %).

Therefore, modification of the common antibacterial regime for gram negative anerobic was tried in our study where a combination of green synthesized AgNP, crude green tea extract with metronidazole was evaluated against *P. gingivalis.* Mean zone of inhibition was relatively higher in group 4 (MIX) than other group. The maximum effect was showed by MIX group and minimum by AgNP which were statistically significant too. The best mean turbidity was also seen in MIX group compared to other groups against *P. ginigivalis.* This can be attributed to the synergistic effect of antibacterial properties of metronidazole and green tea along with the increased drug carrying and delivering properties of silver nanoparticles against micro-organisms. To our knowledge this is the first study to investigate the effect of combination of green tea extract, silver nanoparticles and metronidazole against *P. gingivalis.*

CONCLUSION:

All the groups tested against *P. gingivalis* showed significant inhibitory potential with varying concentrations of the samples except AgNPs. Out of all the groups the inhibitory property of the combination of all the test samples (AgNP + Green tea extract + Metronidazole) proved to be significantly superior to each of the individual group when compared separately.

Therefore, it can be concluded that the combination of all these can be effective upgradation to the common antimicrobial regime and also to evade the problem of drug resistance.

The combination can be effective adjunct to Scaling and root planing as form of local drug delivery agent.

REFERENCES:

1. Holt SC, Ebersole JL. Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia: The 'red complex', a prototype polybacterial pathogenic consortium in periodontitis. Vol. 38, Periodontology 2000. Periodontol 2000; 2005. p. 72–122.
2. Darveau RP, Hajishengallis G, Curtis MA. Porphyromonas gingivalis as a potential community activist for disease. J Dent Res. 2012 Sep;91(9):816–20.
3. Potempa M, Potempa J, Kantyka T, Nguyen KA, Wawrzonek K, Manandhar SP, et al. Interpain A, a cysteine proteinase from Prevotella intermedia, inhibits complement by degrading complement factor C3. PLoS Pathog. 2009 Feb;5(2).
4. McKay DL, Blumberg JB. The Role of Tea in Human Health: An Update. J Am Coll Nutr. 2002 Feb 1;21(1):1–13.
5. Łuczaj W, Waszkiewicz E, Skrzydlewska E, Roszkowska-Jakimiec W. Green tea protection against age-dependent ethanol-induced oxidative stress. J Toxicol Environ Heal - Part A. 2004;67(7):595–606.
6. Jeffcoat MK, Bray KS, Ciancio SG, Dentino AR, Fine DH, Gordon JM, et al. Adjunctive Use of a Subgingival Controlled-Release Chlorhexidine Chip Reduces Probing Depth and Improves Attachment Level Compared With Scaling and Root Planing Alone. J Periodontol. 1998 Sep;69(9):989–97.
7. Gong P, Wang K, Tan W, et al. Preparation and antibacterial activity of Fe & Ag nanoparticles. Nanot. 2007 Jul 18;18(28):285604.
8. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Appl Environ Microbiol. 2007 Mar;73(6):1712–20.
9. Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. Vol. 100, Journal of Ethnopharmacology. 2005. p. 80–4.
10. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol. 2001;74(2):113–23.
11. Sakanaka S, Aizawa M, Kim M, Yamamoto T. Inhibitory Effects of Green Tea Polyphenols on Growth and Cellular Adherence of an Oral Bacterium, Porphyromonas gingivalis. Biosci Biotechnol Biochem. 1996;60(5):745–9.
12. Zhang L, Pornpattananangkul D, Hu C-M, Huang C-M. Development of Nanoparticles for Antimicrobial Drug Delivery. Curr Med Chem. 2010 Jan 18;17(6):585–94.
13. Davies RL, Etris SF. The development and functions of silver in water purification and disease control. Catal Today. 1997;36(1):107–14.
14. Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. J Nanoparticle Res. 2011 Jul;13(7):2981–8.
15. Murray RG, Steed P, Elson HE. The location of the mucopeptide in sections of the cell wall of escherichia coli and other gram-negative bacteria. Can j microbiol. 1965;11:547–60.
16. P Sirisha, GV Gayathri, SM Dhoom, KS Amulya. Antimicrobial Effect of Silver Nanoparticles Synthesised with Ocimum Sanctum Leaf Extract on Periodontal Pathogens. J Oral Heal Dent Sci. 2017;1(1):106.
17. Gamboa F, Acosta A, García DA, Velosa J, Araya N, Ledergerber R. Occurrence of porphyromonas gingivalis and its antibacterial susceptibility to metronidazole and tetracycline in patients with chronic periodontitis. Acta Odontol Latinoam. 2014;27(3):137–44.
18. Ardila CM, Bedoya-García JA. Antimicrobial resistance of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythia in periodontitis patients. J Glob Antimicrob Resist. 2020 Sep 1;22:215–8.