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# Comparative Effectiveness of Plant Therapy versus Synergistic Activity and Antibiotics in Targeting *Porphyromonas gingivalis*

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## Abstract

Colonisation by periodontopathogen *Porphyromonas gingivalis* is commonly reported with dental plaque. In recent time with increasing drug resistance; control of *P. gingivalis* is better achieved via ethnopharmacology where use of plant bio-actives with antibiotics is norm. In the present study *P. gingivalis* susceptibility towards Amoxycillin (30mcg), Ciprofloxacin (5mcg), Azithromycin (15mcg) and Tetracycline (10mcg) has been recorded along with MIC assay. The present resistance profile has been recorded and resistant drugs were tested in synergy with ethanolic extracts of Ginger, B. *propolis*, Amla, Eucalyptus, and Guava to record comparative change in sole effect and in synergy for better control of infection. In results 15 strains of *P. gingivalis* along with standard strain found to be sensitive towards Ciprofloxacin and Azithromycin and resistant toward Amoxicillin and Tetracycline in totality. Further, extracts of 25 plants recorded for growth inhibition and among them only five plants namely Ginger, *B. propolis*, Amla, *Eucalyptus* and Guava found to be promising antibacterial. Lastly, in synergy study only plant Amla (*Phyllanthus emblica*) with Tetracycline able to showcase better synergy based on significantly high ($P<0.05$) inhibition compared to plant extract and antibiotics sole inhibition data. Conclusively, the success of synergy of Amla (*P. emblica)* with Tetracycline controlling *P. gingivalis* strains was reported in a promising manner and only five plants can be used for better control of periodontitis.

**Key words :** *Porphyromonas gingivalis*, Antibiotics resistance, Plant extract, Synergy, Minimum inhibitory concentration, *P. emblica*, Tetracycline.

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The periodontal pathogen *Porphyromonas gingivalis* reported with increasing antibiotics resistance pattern by as long as 20 years of period; where resistance % ranges from 9 – 10 % in total of strains which is alarming and demands monitoring and control with modern drugs[14] As reported treatment of periodontitis is possible by systemic antibiotics only but its judicious use is recommended to prolong or lowering the antibiotic resistivity as it remains growing global concern[11].

In recent reports, *P. gingivalis* better controlled by systemic antibiotics like Moxifloxacin but resistance already been reported with Amoxicillin, Azithromycin, and Metronidazole[2]. It is reported that adjunctive local and systemic antibiotics treatment temporarily increases the antibiotic resistance of subgingival microorganisms[3] and thus use of synergy drug therapy may be recommended.

Since the introduction of antibiotics in therapies, compounds of treatment have been reduced drastically since few antibiotics available for therapy as approved drugs; resultant antibiotics resistance is on rise that has demanded to shift focus toward combination drug therapy possibly via natural – synthetic combination to overcome resistance or to prolong it especially via learned principles of Ethnopharmacology[1]. Many plant extracts like *Punica granatum*, *Azadirachta indica*, *Commiphora molmol* able to control *P. gingivalis* in synergy with antibiotics which is much more demanded to make modern drugs effective for its future use and to avoid resistance among strains[1]. In the previous study use of essential oil has been proposed to use as new natural agent to control Periodontitis[8]. In a success story, use of plant *Symphytum officinlae* and *Panax ginseng* extract able to control *P. gingivalis* biofilm in combination with Metronidazole is reported and linked with bioactive as acylated Homoserine lactones able to inhibit quorum sensing[6].

According to Kohli *et al*.,[7] ethanolic extracts and plant like *Cocos nucifera* found to be better controlling *P. gingivalis* and further testing via synergy able to overcome bacterial resistance with synthetic drugs.

Looking at these reports, present study designed to screen *P. gingivalis* against prescribed antibiotics there by its resistance could be deciphered. Further by screening 25 ethanolic extract of plants effectiveness of each parameter has been deciphered. Lastly synergistic activity of promising plants with resistant antibiotics has been carried to record the improvement in reversing the resistivity in synthetic drugs in statistical manner.

Isolation of *P. gingivalis* :

Presence of *P. gingivalis* in plaque already been known and thus used as a source of pathogen isolation. By using Gracey -curette number 5/6 (Hu -Friedy, Chicago, USA) subgingival plaque was sampled from patients visited in dental hospitals in and around Nagpur region, India. Thereafter, blood agar media used as a selective nutrient medium for isolation and inoculated with plaque sample. The plates were incubated under anaerobic conditions for 72-76 hrs. at 37°C. The gram negative, black pigmented colonies with hemolytic zones were selected and confirmed by 16S rRNA analysis as *P. gingivalis*.

*Antibiotic Sensitivity Assay and MIC Assay:*

Antibiotic sensitivity assay carried out with Amoxicillin/Clavulonic acid (AMC) 30 mcg, Ciprofloxacin (CIP) 5 mcg, Azithromycin (AZM) 15 mcg, and Tetracycline (TE) 10 mcg. The required 0.5 Mc Farland O.D. of strains of *P. gingivalis* (N=16) set in Luria Bartani broth used as inoculum. The blood agar plates were inoculated with 100µl of inoculum and further disc of respective antibiotics were placed. The plates were incubated at 37°C for 48-72 hrs. to record the zone of inhibition in millimeter (mm). Further the antibiotics which were found to be resistant, tested for MIC assay using MIC E-strip to confirm present sensitivity using same protocol.

*Antimicrobial Activity of Plant Extract :*

The primary susceptibility of *P. gingivalis* against 25 ethanolic plants extract carried out by using 20 µl of extract (0.2g/ml) concentration in well diffusion assay. During study 0.5 Mc Farland O.D. was set for *P. gingivalis* growing in Luria Bertani broth and inoculated on blood agar plate. The plates were incubated anaerobically for around 48-72 hours at 37°C. The formed zone of inhibition recorded in millimeter(mm) around well and comparatively analyzed.

*Synergistic Assay of Plant extract and Antibiotics :*

In the study 15 *P. gingivalis* plaque isolated strains along with one standard strain *P.gingivalis* ATCC33277 primarily tested for antibiotics sensitivity earlier. Among antibiotics sensitivity assay, antibiotics which were found to be resistant by *P. gingivalis* were further considered for synergistic study. For synergy, those plant extracts which were positive for *P.gingivalis* inhibition considered to be tested with resistant antibiotics as synergy combination.

*Statistical Data Analysis :*

The data of *P.gingivalis* growth inhibition among plant extract, antibiotics and plant plus antibiotics were statistically analyzed by using one way ANOVA setting up P value $< 0.05$ using graph pad prism to calculate significance.

*Isolation of P. gingivalis :*

The blood agar plates inoculated with dental plaque sampled by Gracey-curette found to be positive for anaerobe *P. gingivalis* with black pigmented colonies which are identified by Gram staining and confirmed by 16S rRNA gene sequencing. (Fig. 1).

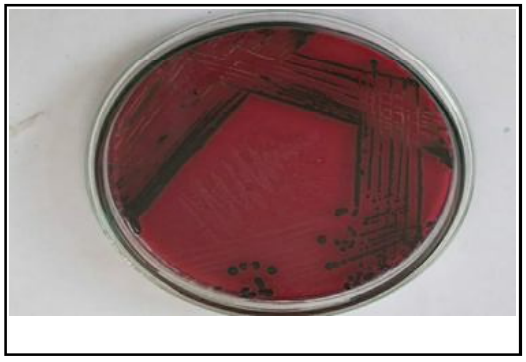


Fig. 1. *P. gingivalis* colonies (black pigmented) growing on Blood agar plates

*Antibiotic Sensitivity Assay and MIC Assay:*

As per antibiotic sensitivity assay total four antibiotics tested against 16 strains of *P. gingivalis*, the promising sensitivity showcased

by strains with Azithromycin and Ciprofloxacin while defined resistant recorded with Tetracycline and Amoxycilin / Clavulonic acid (Table-1) (Fig. 2). Further, only with two resistant drugs Amoxycillin and Tetracycline 0.016 -256 µg/ml concentration MIC assay was assessed and recorded. As per MIC Assay value with standard deviation for Amoxycillin and Tetracycline recorded as 12 ± 4.3 µg and 10 ± 2.9 µg which was found to be statistically equivalent to control *P. gingivalis* therefore P value recorded to be more than > 0.05 *i.e.* P > 0.2539 suggested that both drugs are acting at same dose concentration to control *P. gingivalis*. Here only one strain (P11) found to be completely resistant to Tetracycline even upto 256 µg/ml concentration and rest registered sensitive at given dosage (Fig. 3 Table-2).

Table-1. Antibiotic sensitivity profile of *P. gingivalis* strains (n=15)

| | Amoxicillin (AMC) 30 mcg | Ciprofloxacin (CIP) 5 mcg | Azithromycin (AZM) 15 mcg | Tetracycline 10 mcg |
|---|---|---|---|---|
| Number of values | 13 | 15 | 15 | 15 |
| Minimum | 10.00 | 14.00 | 14.00 | 12.00 |
| 25% Percentile | 10.00 | 22.00 | 16.00 | 13.00 |
| Median | 11.00 | 23.00 | 17.00 | 14.00 |
| 75% Percentile | 15.00 | 25.00 | 18.00 | 16.00 |
| Maximum | 26.00 | 33.00 | 26.00 | 21.00 |
| **Mean** | **12.77±4.640** | **23.40±5.011** | **17.93±3.453** | **14.60±2.293** |
| Std. Error | 1.287 | 1.294 | 0.8916 | 0.5920 |
| **± standard deviation** | | | | |

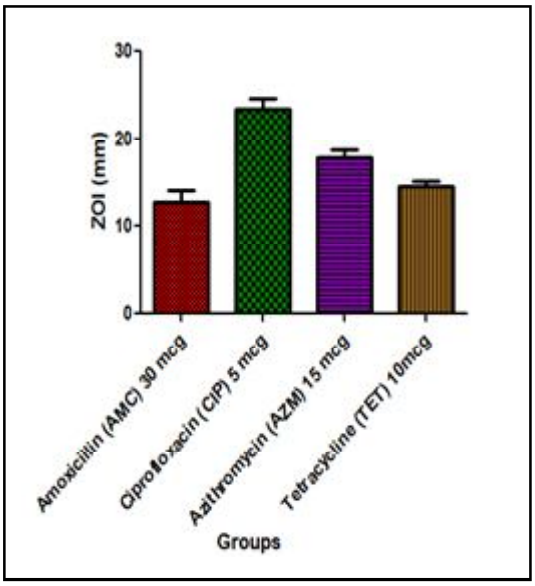
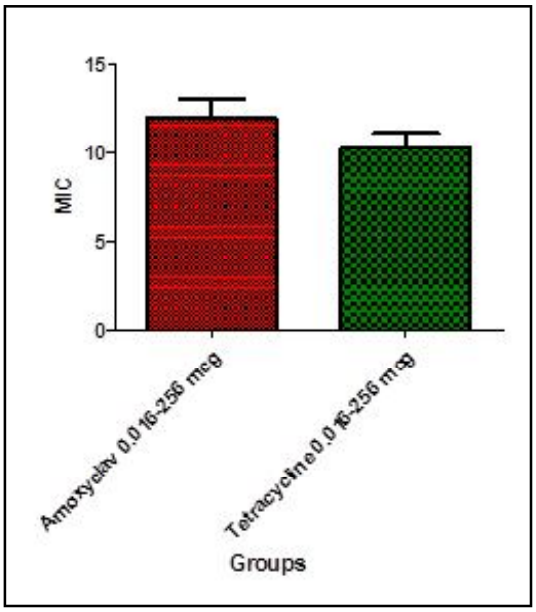


Table-2. Comparative MIC assay of previously resistant antibiotics Amoxyclav and Tetracycline for inhibition concentration using T test (unpaired).

| | Amoxyclav 0.016-256 mcg | Tetracycline 0.016-256 mcg |
|---|---|---|
| Number of values | 15 | 14 |
| Minimum | 0.50 | 6.0 |
| 25% Percentile | 12 | 8.0 |
| Median | 12 | 12 |
| 75% Percentile | 16 | 12 |
| Maximum | 16 | 16 |
| **Mean** | **12±4.3** | **10±2.9** |
| Std. Error | 1.1 | 0.78 |
| **P value : 0.2539 non significant** | | |
| **± standard deviation** | | |



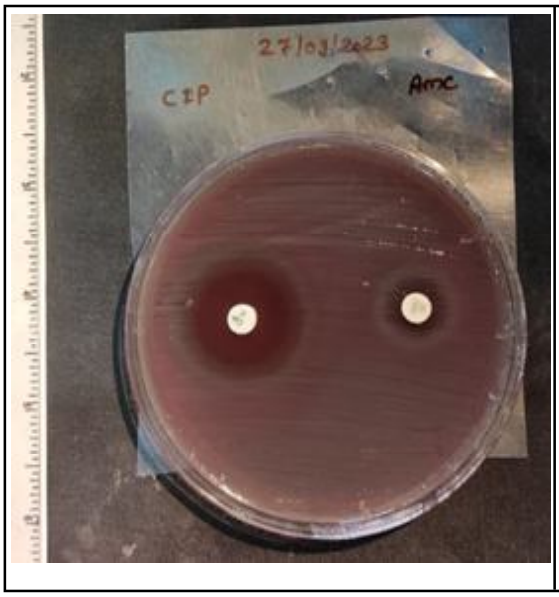




Fig. 2a Antibiotics sensitivity of *P. gingivalis* against Ciprofloxacin and Amoxicillin

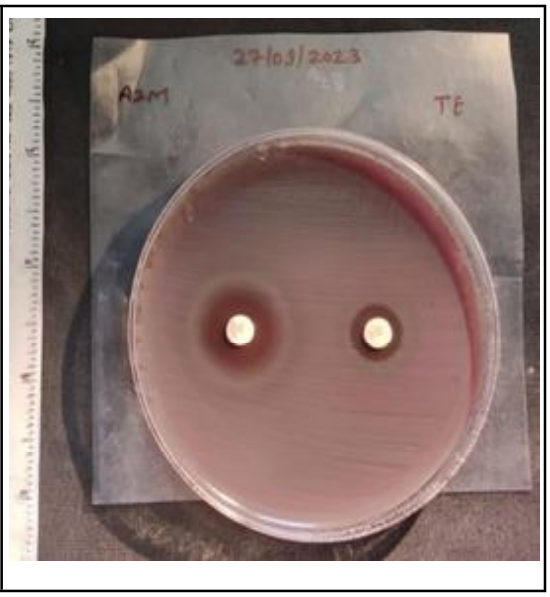



Fig. 2b Antibiotics sensitivity of *P. gingivalis* against Azithromycin and Tetracycline

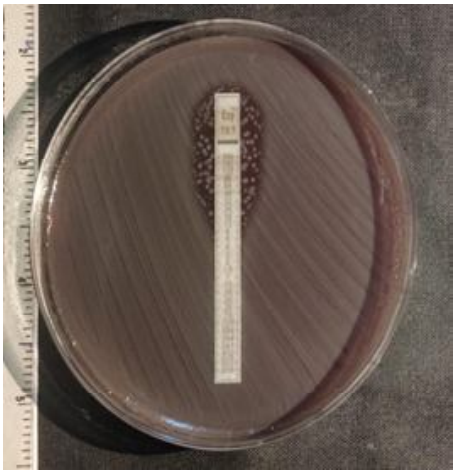


Fig. 3a: *P. gingivalis* found to be with MIC inhibition

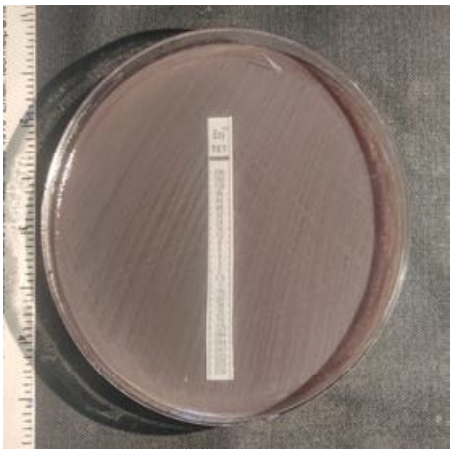


Fig. 3b: *P. gingivalis* (P11) found to be with completely MIC resistance

*Antimicrobial Activity of Plant Extract :*

The 25 plants namely Ginger, Funnel seed, Bettle leaf, Cinnamon, *B.propolis*, Mushroom, Green tea, Mulethi, Amla, Eucalyptus, Guava, Clove, Black pepper, Harar, Bay leaves, Turmeric, Aloevera, Pomegranate, papaya, Jamun, Garlic, Triphala, Giloyseed, White museli and Ashwagandha tested in the present study. Among those- five plants namely Ginger, *B.propolis*, Amla, Eucalyptus and Guava found to be *P. gingivalis* growth controlling with mean zone of inhibition recorded as 20.69±1.79mm, 19.82±3.18mm, 18.18 ±1.60mm, 19.67±1.58mm and 17.82±2.40mm, respectively as shown in Table 3.

As per statistical analysis the mean zone of inhibition of all plant extract found to be equivalent and thus statistically recorded as non-significant (P>0.05) in difference. Still, it has been observed from the sum value data of zone of inhibition (mm in total) recorded in decreasing order with Ginger (269 mm), *B. propolis* (218 mm), Amla (200 mm),Guava (196 mm) and Eucalyptus(177 mm) as given in Table-3 showcased Ginger extract is better controlling as a sole performer.

*Synergistic Activity of Plant Extracts and Antibiotics:*

*Synergy activity of Plant Extracts with Amoxicillin:*

In the present study ethanolic extracts of Ginger found to be better *P.gingivalis* controlling (21 ± 1.7) which is significantly higher ($P < 0.05$) than resistant Amoxicillin (13 ± 4.6 mm) and with synergy (14 ± 6.0).Here it has been observed that plant extract loses its efficiency once synergy made with Amoxicillin; while there is no change in antibiotic activity with synergy (Table 4).

Table-3. *P. gingivalis* growth inhibition by ethanolic plant extract recorded as zone of inhibition

| | Ginger | *B. propolis* | Amla | Eucalyptus | Guava |
|---|---|---|---|---|---|
| Number of values | 13 | 11 | 11 | 9 | 11 |
| Minimum | 18.00 | 16.00 | 16.00 | 18.00 | 14.00 |
| 25% Percentile | 19.50 | 16.00 | 16.00 | 18.00 | 16.00 |
| Median | 20.00 | 20.00 | 18.00 | 20.00 | 18.00 |
| 75% Percentile | 22.00 | 22.00 | 20.00 | 21.00 | 20.00 |
| Maximum | 24.00 | 25.00 | 20.00 | 22.00 | 22.00 |
| Mean | 20.69 ± 1.79 | 19.82 ± 3.18 | 18.18 ± 1.60 | 19.67 ± 1.58 | 17.82 ± 2.40 |
| P>0.05 non-significant | | | | | |
| Std. Error | 0.4985 | 0.9612 | 0.4828 | 0.5270 | 0.7239 |
| Sum | 269.0 | 218.0 | 200.0 | 177.0 | 196.0 |

Table-4. Statistical analysis of plant extract, amoxicillin and Synergy against *P. gingivalis*

| Ginger (EG) | Amoxicillin | EG+AMC |
|---|---|---|
| 21 ± 1.7[a] | 13 ± 4.6[bd] | 14 ± 6.0[cd] |
| B. *propolis* (EP) | Amoxicillin | EP + AMC |
| 20 ± 3.2[a] | 13 ± 4.6[bc] | 16 ± 6.5[ac] |
| Amla (EA) | Amoxicillin | EA + AMC |
| 18 ± 1.6[a] | 13 ± 4.6[bc] | 15 ± 3.5[ac] |
| Eucalyptus (EE) | Amoxicillin | EE + AMC |
| 20 ± 1.6[a] | 13 ± 4.6[bd] | 15 ± 4.7[cd] |
| Guava (EU) | Amoxicillin | EU + AMC |
| 18 ± 2.4[a] | 13 ± 4.6[bc] | 15 ± 4.3[ac] |
| ± Standard deviation; Superscript showing same letter in a row suggest non-significance (P>0.05) | | |

Ethanolic extract of *B. propolis* recorded with higher zone of inhibition (20 ± 3.2) as compared to Amoxicillin (13 ± 4.6) and with synergy (16 ± 6.5) that has found to be statistically significant ($P < 0.05$) with Amoxicillin data only while no significant difference ($P > 0.05$)recorded with synergy (Table-4).

Amla extract once again able to well control *P.gingivalis*(18 ±1.6) compared to resistant Amoxicillin (13 ± 4.6) with significant difference($P < 0.05$) but remained non-significant in difference ($P>0.05$) with synergy (15±3.5) (Table-4).

Ethanolic extract of Eucalyptus (20 ± 1.6) did control the *P. gingivalis* growth prominently and proving better compared to Amoxicillin (13 ± 4.6) and synergy (15 ± 4.7) with significant difference ($P<0.05$) while synergistic action reduced the original plant extract activity.

In case of ethanolic extract of Guava; inhibition of only plant extract (18 ± 2.4) and of synergy (15 ± 4.3) found to be similar ($P> 0.05$) and better than Amoxicillin ($P<0.05$) in zone of inhibition. In the overall study, it has been observed that antibiotic activity did not improve once checked with synergies of five plants but sum value recorded to be on higher side but lower than only plant extract (Table-4).

*Synergistic activity of plant extract with Tetracycline :*

In the present study ethanolic extract of ginger found to be better controlling *P. gingivalis* strains (21 ± 1.7) which was significantly (P<0.05) higher than Tetracycline (15 ± 2.3) and synergy also (16 ± 3.8) indicated sole use of ginger extract is better than synergy (Table-5).

Ethanolic extract of *B.propolis* able to control better (20 ± 3.2) than Tetracycline (15 ± 2.3) and synergy with significance difference (P<0.05) indicated that synergy did not improve the inhibition once used with tetracycline (Table-5).

In case of ethanolic extract of Amla profound increase in synergy inhibition against *P. gingivalis* recorded (22 ± 1.7) which was significantly (P< 0.05) higher than Tetracycline (15 ± 2.3) and ethanolic Amla extract (18 ± 1.6); which was for the first time indicated in the study that Amla and Tetracycline synergy will assist in increasing sensitivity towards tetracycline and thus can be investigated in detail to decipher its ability to control resistant by involving the synergism (Table-5).

Ethanolic extract of Eucalyptus found to be once again promising to control *P.gingivalis* (20 ± 1.6) as compared to tetracycline(1.5 ±1.6) and synergy (18 ±1.6) with significant difference (P<0.05).Here once again synergy did not able to improve the tetracycline activity indicated relatively similar inhibition (Table-5).

In an ethanolic extract of guava the growth inhibition of *P. gingivalis* found to be 18 ± 2.4 while tetracycline and synergy inhibition found to be 15 ± 2.3 and 17 ± 4.5mm, respectively indicated no significant difference (P>0.05) in all set tested (Table-5).

Table 5: Statistical analysis of plant extract, tetracycline, and Synergy against *P. gingivalis*

| Ginger (EG) | Amoxicillin | EG+AMC |
|---|---|---|
| Ginger (EG) | Tetracycline | EG+TET |
| $21 \pm 1.7^{a}$ | $15 \pm 2.3^{bd}$ | $16 \pm 3.8^{cd}$ |
| B. propolis (EP) | Tetracycline | EP + TET |
| $20 \pm 3.2^{a}$ | $15 \pm 2.3^{bd}$ | $14 \pm 3.0^{cd}$ |
| Amla (EA) | Tetracycline | EA + TET |
| $18 \pm 1.6^{a}$ | $15 \pm 2.3^{b}$ | $22 \pm 1.7^{c}$ |
| Eucalyptus (EE) | Tetracycline | EE + TET |
| $20 \pm 1.6^{a}$ | $15 \pm 1.6^{bc}$ | $18 \pm 1.6^{ac}$ |
| Guava (EU) | Tetracycline | EU + TET |
| $18 \pm 2.4^{a}$ | $15 \pm 2.3^{a}$ | $17 \pm 4.5^{a}$ |
| ± Standard deviation; Superscript showing same letter in a row suggest non-significance (P>0.05) | | |

As we know interaction of human with many bacterial pathogens remains complex where the association are beneficial, mutual, and pathogenic also. In case of dental plaque which is a complex biofilm forms on the hard tissues (teeth) in oral cavity by bacterial consortium proving to be detrimental in many cases. Till date, as many as 500 bacterial species involved in dental plaque by colonization, adhesion and interaction leads to periodontal disease[16]. Among pathogens, periodontopathogen *Porphyromonas gingivalis* involved in periodontitis and reported to colonize with other pathogens to bring about dental plaque[18]. It has been observed that crowded dental areas maintained by good oral hygiene remains more

susceptible to *P. gingivalis* colonization[13]. As per recent trend bacterial infection and their developing antibiotic resistance which may be inherent or acquired can be controlled by research products developed by ethnopharmacology. Here use of plant bio-actives with resistant antibiotics may increase effectiveness when used in combination and thus demand more research[1].

In the present study we have investigated 15 *P. gingivalis* isolated from dental plaque reamined resistant to the Tetracycline and Amoxicillin and sensitive towards Ciprofloxacin, and Azithromycin indicated drug resistance is selective in nature. According to Sanai *et al*.,[17]. black pigmented *P. gingivalis* found to be positive in expression of tet (Q) and erm (F) able to bring about tetracycline resistance indicated that present strains of *P.gingivalis* may be able to express same genes for recorded resistance.The possible resistance to Tetracycline may be because of its extensive use as adjunctive to conventional periodontal therapy[15].

Further, drug Amoxicillin found to be less effective to control *P. gingivalis* but possible to improve its effectiveness by synergy as reported earlier by Abullais Saquib et al[1].Since clinical reports already confirmed that strains of *P. gingivalis* able to grow in presence of amoxycillin with > 40 % strains count[10] which is in coherent to the present study also. In other report with more than 150 *P. gingivalis* interestingly all strains found to be Amoxycillin/ Clavulonic acid sensitive which indicates variable pattern of antimicrobial resistance exist among strains and locations; thus, screening of pathogens must be recommended before therapy via antibiogram assay[2].

In the present study mass screening of 25 ethanolic extracts of plants done for *P. gingivalis* sensitivity; where five plants found to be promising in controlling *P. gingivalis* which are Ginger, *B. propolis*, Amla, Guava, and Eucalyptus only. Use of plant extract in controlling periodontitis has been suggested in many studies earlier either solely or in synergy giving strong support for plant bioactive role in future control of periodontitis[1,5,12]. In the present study two resistant antibiotics Amoxycillin / Clavulonic acid and Tetracycline found to be become effective only on a higher dosage which was confirmed by MIC indicated that if we can use plant synergy with these regularly advised drugs, the possible resistant may be brought down or can be controlled once used with low dose antibiotics also. In the present study synergy with these two drugs was performed and reported that sole use of plant extract majorly able to bring about significantly ($P<0.05$) increased growth inhibition against *P. gingivalis* once compared with antibiotics (Tetracycline and Amoxycillin / Clavulonic acid) and with synergy sets. Here, among all synergy groups, Amla extract (*Phyllanthus emblica*) only able to increase growth inhibition in synergy with Tetracycline but not with Amoxycillin indicated selective activity of *P. emblica* for effectiveness. In the earlier studies plant extract Amla (*P.emblica)* already proven its proficiency to control range of oral pathogens including *P. gingivalis* once used as a sole source or in synergy which strongly supports our study also[5,9,12]. Overall, data suggested that selective plant extracts are promising to control *P. gingivalis* and among them use of Amla with Tetracycline could be

better synergy for future applications in periodontitis control.

The dental Plaque is a complex infection brought about colonisation by more than 500 bacterial pathogens. Among them *P. gingivalis* found to be major pathogens and demand better therapy to reduce its drug resistance burden. In the present study, it has been observed that many strains of them were sensitive to prescribed antibiotics like Ciprofloxacin and Azithromycin; whereas resistance recorded with two antibiotics Amoxycillin and Tetracycline. Further when we have tested 25 plant ethanolic extract; only five plants found to be better controlling *P. gingivalis* than antibiotics especially when checked with resistant drug inhibition.In the conclusion part, when synergistic activity of these five plants with Amoxicillin and Tetracycline was tested; no synergetic effect was observed with extracts of Ginger, B. propolis, Eucalyptus, Guava with Tetracycline and Amoxycillin. Only Amla extract with Tetracycline able to showcase increased inhibition as synergy. Hence, using this combination can result in a reduction in the minimum dose required for effective antimicrobial effects which is interesting because it may decrease both the risk of side effects and the costs of treatment of oral infection. The present results are promising and may enhance the use of natural extracts instead of antibiotics. Further researches are needed to understand the mechanisms involved in these synergistic effects which are still poorly understood.

References :


1. Abullais Saquib, S., Abdullah Al Qahtani, N., Ahmad, I., Arora, S., Mohammed Asif, S., Ahmed Javali, M., and N. Nisar, (2021). *Journal of infection in developing countries*, *15*(11): 1685–1693.
2. Ardila, C. M., and J. A. Bedoya-García, (2020). *Journal of global antimicrobial resistance*, *22*: 215–218.
3. Ardila, C. M., J. A. Bedoya-García, and D. E. Arrubla-Escobar, (2023). *Oral diseases*, *29*(7): 2501–2511.
4. Ardila, C. M., M. I. Granada, and I. C. Guzmán, (2010). *Journal of periodontal research*, *45*(4): 557–563.
5. Gao, Q., X. Li, H. Huang, Y. Guan, Q. Mi, and J. Yao, (2018). *Current microbiology*, *75*(5): 604-610.
6. Ibrahim, S. M., A. S. Al-Mizraqchi, and J. Haider, (2023). *Antibiotics (Basel, Switzerland)*, *12*(8): 1288.
7. Kohli, D., S. M. Hugar, K. G. Bhat, P. P. Shah, M. V. Mundada, and C. M. Badakar (2018). *Journal of the Indian Society of Pedodontics and Preventive Dentistry*, *36*(2): 142–150.
8. Lakhdar, L., M. Hmamouchi, S. Rida, and O. Ennibi, (2012). *Odonto-stomatologie-tropicale = Tropical dental journal*, *35*(140): 38–46.
9. Li, G., Q. Yu, M. Li, D. Zhang, J. Yu, X. Yu, ... and H. Huang, (2023). *Food & Function*, *14*(17): 7738-7759.
10. Loyola-Rodriguez, J.P., J.O. Garcia-Cortes, R.E. Martinez-Martinez, N. Patiño-Marin, G. A. Martinez-Castañon, N. V. Zavala-Alonso and A. Amano, (2014). *Australian dental journal*, *59*(4): 497–503.
11. Ng, E., J. R. H. Tay, S. K. Boey, M. L. Laine, S. Ivanovski, and C. J. Seneviratne, (2023). *Critical reviews in microbiology*, 1–12. *Advance online publication.*
12. Pathak, A., A. Sardar, V. Kadam, B. Rekadwad, and S. M. Karuppayil, (2012).

Efficacy of some medicinal plants against human dental pathogens.

13. Rafiei, E., H. Zandi, N. Joshan, F.R. Maybodi and R. Fallah, (2022). *American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics*, *161*(3): 375–380.
14. Rams, T. E., J. D. Sautter, and A. J. Van Winkelhoff, (2023). *Antibiotics (Basel, Switzerland)*, *12*(11): 1584.
15. Rodrigues, R. M., C. Gonçalves, R. Souto, E. J. Feres-Filho, M. Uzeda, and A. P. Colombo (2004). *Journal of clinical periodontology*, *31*(6): 420–427.
16. Rosan, B. and R.J. Lamont, (2000). Dental plaque formation. *Microbes and infection*, *2*(13): 1599–1607.
17. Sanai, Y., G. R. Persson, J. R. Starr, H. S. Luis, M. Bernardo, J. Leitao, and M. C. Roberts, (2002). *Journal of clinical periodontology*, *29*(10): 929–934.
18. Souccar, N. M., M. Chakhtoura, J. G. Ghafari, and A. M. Abdelnoor, (2010). *Journal of infection in developing countries*, *4*(6): 362–366.