Research Article **JHMR 2020, 5:39**

Journal of Herbal Medicine Research
(ISSN: 2474-106X)

Evaluation of antimicrobial efficacy of ethanolic extract of Panax ginseng against Periodontal pathogens: An invitro study

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ABSTRACT

The present experimental study was carried out with the aim of evaluating the antibacterial activity of ethanolic extract of Panax ginseng on the most common periodontal pathogens, Porphyromonas gingivalis (Pg) Prevotella intermedia (Pi), Fusobacterium nucleatum (Fn) and Aggregatibacter actinomycetemcomitans (Aa). Ethanolic extract of Panax ginseng was prepared using maceration technique. Thus obtained extract was tested for its antimicrobial efficacy against 4 periodontal pathogens by means of evaluating the Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and time kill curve analysis. MIC was carried out by using serial dilution method. MBC was assessed by incubating the cultures of microorganisms with the same drug concentrations as MIC. Time kill curve (TKC) was analysed by incubating the organisms in anaerobic chamber, to evaluate the timely declination of growth rate. The results indicated that the ethanolic extract of Panax ginseng was both bacteriostatic and bactericidal for Pg and Pi at 0.8 µg/ml and for Fn at 50 µg/ml. But for Aa, it was only bacteriostatic at 25 µg/ml but not bactericidal in nature. Also, Pg showed no growth after 10 minutes, but the growth of Pi, Fn and Aa was not inhibited even after incubation for 2 hours. From this in-vitro study we conclude that the ethanolic extract of panax ginseng showed antimicrobial efficacy against all the test microorganisms, such as Pg, Fn, Pi and Aa. Thus these results can be used to continue with further invitro, invivo research and in day to day clinical treatment of chronic periodontitis.

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How to cite this article:
Amulya. K.S., Jayasheela M., Gayathri G., Mehta D.S. . Evaluation of antimicrobial efficacy of ethanolic extract of Panax ginseng against Periodontal pathogens: An invitro study. Journal of Herbal Medicine Research, 2020,5:39.

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Introduction:

Chronic periodontitis is an infectious disease characterized by progressive attachment loss, bone loss, periodontal pocket formation and/or gingival recession. The periopathogenic bacteria predominantly composed of gram negative anaerobic bacteria such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia* and *Fusobacterium nucleatum* (1).

The main goal of periodontal therapy is to restore the periodontal tissue homeostasis. The conventional periodontal therapy can be broadly categorised as non-surgical and surgical therapy. The traditional non-surgical therapy includes the routine scaling and root planing (SRP). Though SRP is considered “Gold standard”, it has certain limitations such as inability to access deep pockets and furcation and to eliminate tissue invasive pathogens (2). To overcome these limitations, the adjunctive use of systemic or local antimicrobial agents was introduced (3).

Systemic antibiotics have drawbacks of development of resistant strains, superimposed infections and inadequate concentration of active ingredient for sufficient period of time at the site of action. However, local drug delivery agents reach the base of the pocket or furcation areas and acts as a reservoir for adequate antimicrobial effect (4).

Various herbs have been tried as an alternative to the commercially available antimicrobials, such as Neem, Aloe vera, turmeric etc. One among this wide range of herbs is Panax ginseng, popular for its antibacterial, anticancerous, immunomodulatory, antidiabetic properties, along with its beneficial effects on liver, cardiovascular and nervous system (5).

Panax ginseng (family Aaraliaceae) includes Korean, Japanese and American ginseng. It is of 2 types, white ginseng (air drying) and red ginseng (steaming and heating). Due to the enhanced biological activity resulting from the type of processing, red ginseng is more widely used as an herbal medicine than white ginseng (6, 7).

The various bioactive components of ginseng include tetracyclic triterpenoids (ginsenosides), polyacetylenes, polyphenolic compounds, and acidic polysaccharide, of which “ginsenoside” is pharmacologically highly active (8).

Ginseng has bactericidal activity through inhibition of DNA mutagenesis, anti-quorum sensing, anti-adhesive activity, inhibition of pathogen-induced hemagglutination, immune-modulatory functions and demonstrates a protective role against pathogen-induced inflammation. It induces antigenspecific IgM, IgG, and IgA antibody responses, cellular cytotoxicity and promotes natural killer cell activity. It also activates macrophages and enhances their phagocytic activity (9).

Minimum inhibitory concentrations (MIC) test confirms the susceptibility / resistance of microorganisms to an antimicrobial agent and Minimum bactericidal concentration (MBC) test determines the minimum concentration of an antimicrobial agent, to achieve bactericidal effect. Both these tests can be good but relatively expensive tools to rank new antimicrobial agents by potency for screening purposes. The Time-kill curve monitors bacterial growth and death over a wide range of antimicrobial concentrations over time and hence it helps in better understanding of antimicrobial agent and could be used to optimize dosing strategies and help to prevent treatment failures (10).

To the best of our knowledge, there were no studies to evaluate the antimicrobial efficacy of ethanolic extract of ginseng on periodontal pathogens. Hence, the aim of present study was to evaluate the MIC, MBC and time kill curve analysis of ethanolic extracts of red *Panax ginseng* against key periodontal pathogens: *Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Fusobacterium nucleatum (Fn),* and *Aggregatibacter actinomycetemcomitans (Aa)*.

Figure 1: Panax Ginseng Powder

Figure 2: Ethanolic Extract Of Panax Ginseng

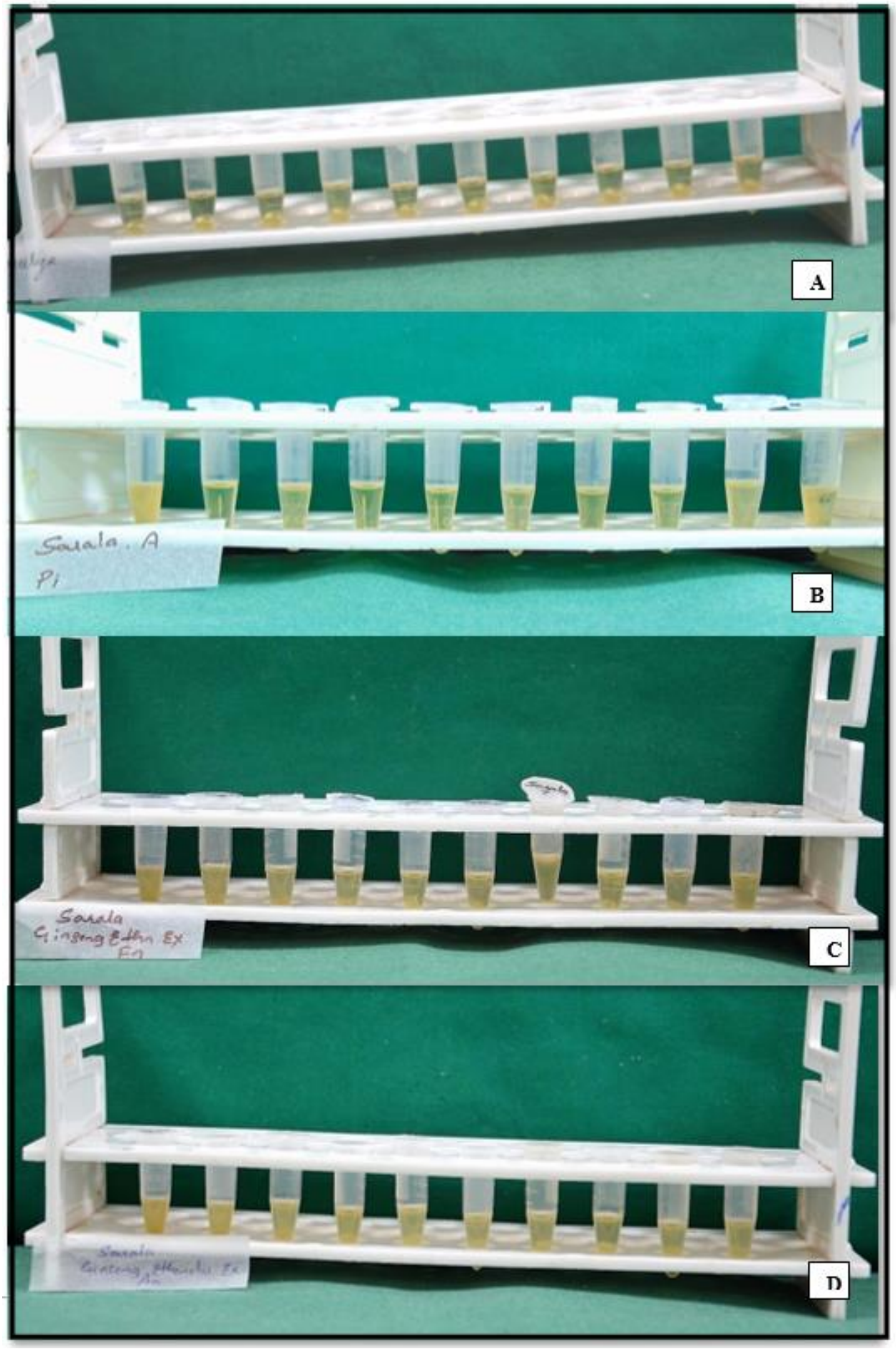


Figure 3: Minimum Inhibitory Concentration. A: Porphyromonas gingivalis; B: Prevotella intermedia; C: Fusobacterium nucleatum; D: Aggregatibacter actinomycetemcomitans

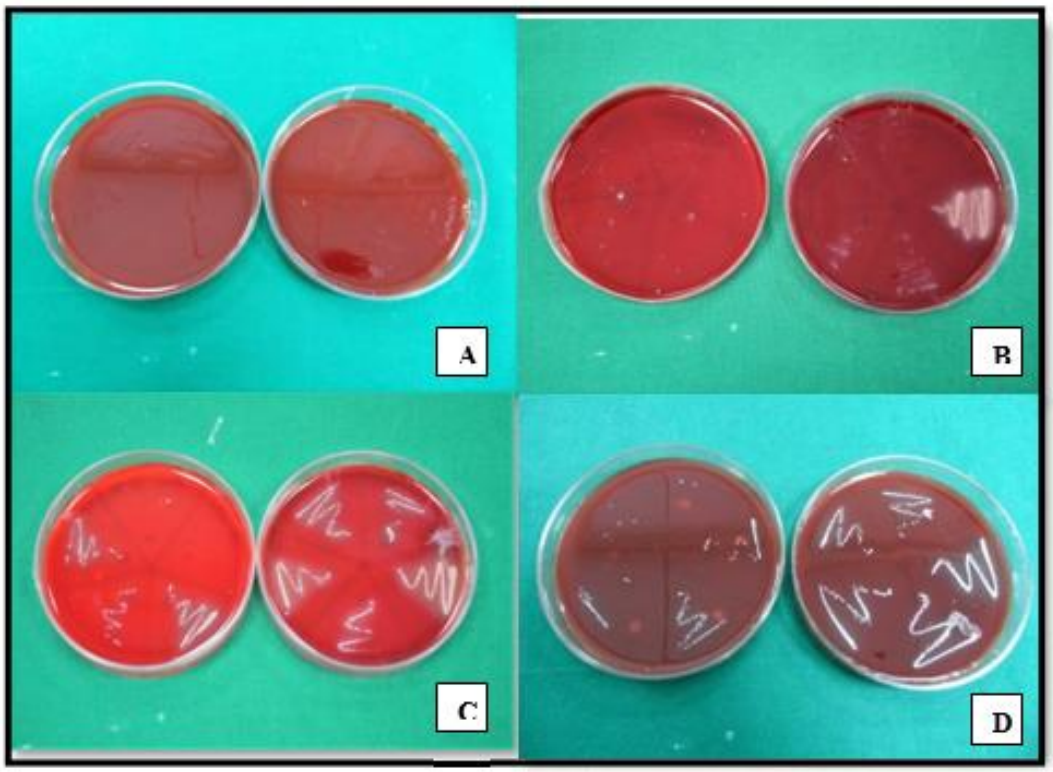


Figure 4: Minimum Bactericidal Concentration. A: Porphyromonas gingivalis; B: Prevotella intermedia; C: Fusobacterium nucleatum; D: Aggregatibacter actinomycetemcomitans

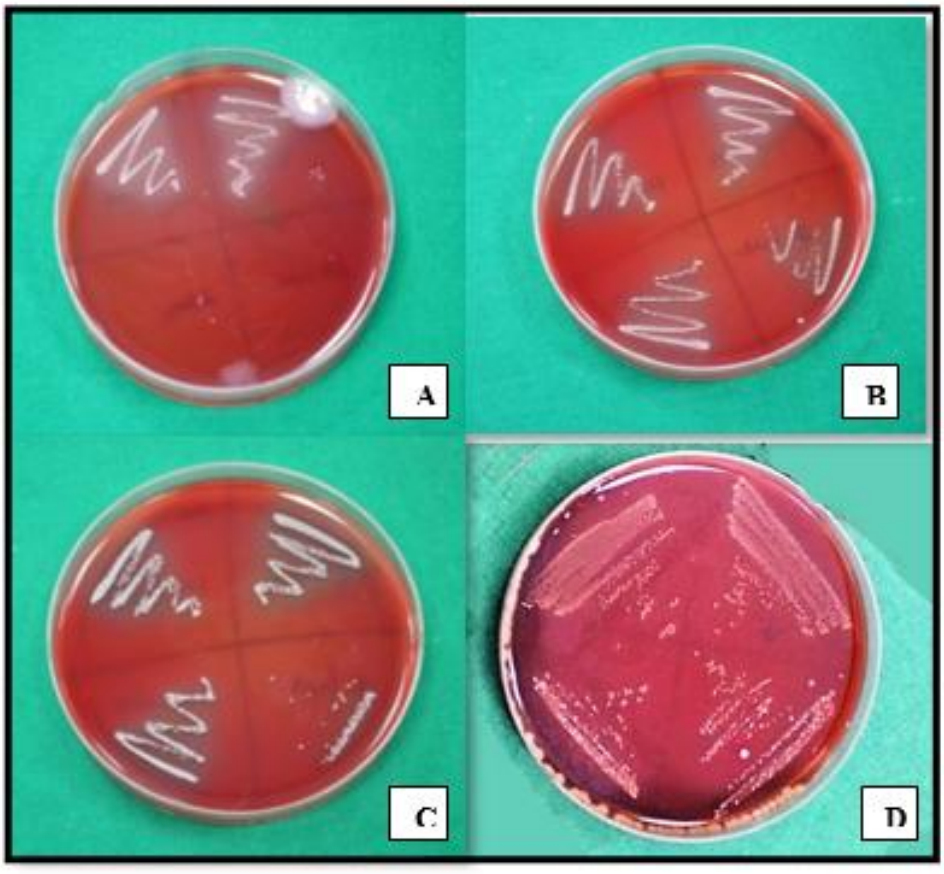


Figure 5: Time Kill Curve Analysis. A: Porphyromonas gingivalis; B: Prevotella intermedia; C: Fusobacterium nucleatum; D: Aggregatibacter actinomycetemcomitans

Table 1: minimum inhibitory concentration

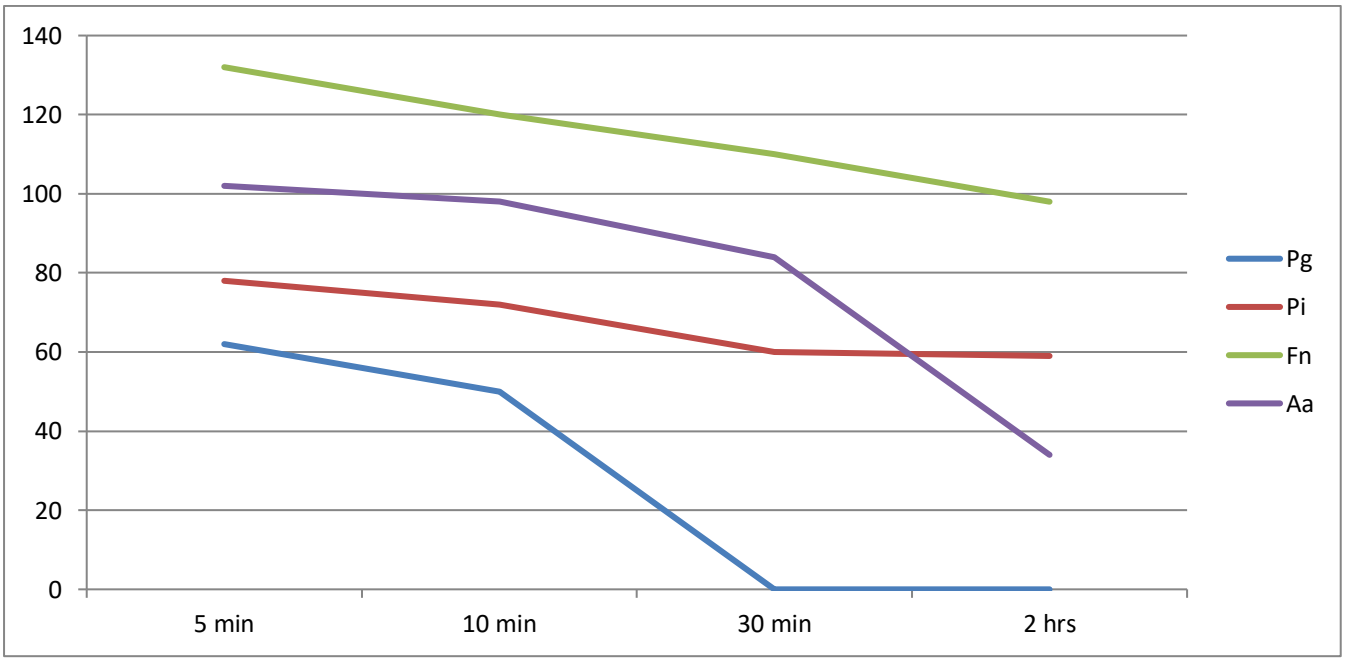
Sl.No	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
	Ginseng ethanolic extract										
1.	Pg	S	S	S	S	S	S	S	S	R	R
2.	Pi	S	S	S	S	S	S	S	S	R	R
3.	Fn	S	S	R	R	R	R	R	R	R	R
4.	Aa	S	S	S	R	R	R	R	R	R	R

Table 2: Minimum bactericidal capacity

Sl.No	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
	Ginseng ethanolic extract										
1.	Pg	NG	NG	NG	NG	NG	NG	NG	NG	18	34
2.	Pi	NG	NG	NG	NG	NG	NG	NG	NG	40	54
3.	Fn	NG	NG	25	38	49	58	62	78	96	102
4.	Aa	03	04	08	28	36	52	69	80	89	92

Table 3: Time Kill Curve Analysis

Sl.No	Samples	05 min	10 min	30 min	2 hrs
	Ginseng ethanolic extract				
1.	Pg	62	50	NG	NG
2.	Pi	78	72	60	59
3.	Fn	132	120	110	98
4.	Aa	102	98	84	34



Graph 1: Time kill curve analysis

MATERIALS AND METHODS:

Preparation of Alcoholic and Aqueous extracts:

100% pure Korean red Ginseng powder was obtained from Biotrex Neutraceuticals, Ahmedabad, Gujarat, India (figure 1). It was certified to be free from any form of bacteria, yeast, or mold, by the manufacturer after microbial analysis. The ethanolic extract was prepared using the maceration technique, wherein 200 gm of coarsely powdered crude compound was macerated with 500 ml of 100% ethanol for 24 hours and then subjected to filtration with Whitman filter paper. The filtrate so obtained was reduced at room temperature to obtain the residue of Panax ginseng, which was semi solid in nature (figure 2).

Bacterial Strains:

Bacterial strains used in this study were American type culture collection (ATCC), Manassas, VA, USA. The tested bacterial strains in this study were *Porphyromonas gingivalis* ATCC 33277, *Prevotella intermedia* ATCC 25611, *Fusobacterium nucleatum* ATCC 25586 and *Aggregatibacter actinomycetocomitans* ATCC 29523.

Procedure*:*

MIC Test: (figure 3)

- 9 dilutions of each drug have to be done with Thioglycollate broth for MIC.
- In the initial tube 20 microliter of drug was added into the 380 microliter of Thioglycollate broth.
- For dilutions 200microliter of Thioglycollate broth was added into the next 9 tubes separately.
- Then from the initial tube 200 microliter was transferred to the first tube containing 200 microliter of Thioglycollate broth. This was regarded as 10^{-1} dilution
- From 10^{-1} diluted tube 200 microliter was transferred to second tube to make 10^{-2} dilution.
- The serial dilution was repeated up to 10^{-9} dilution for each drug.
- 5 microliter was taken from the maintained stock cultures of required organisms and added into 2ml of Thioglycollate broth.
- 200 microliter of cultured suspension was added to each serially diluted tube.
- The tubes were incubated in anaerobic jar at 37°C, for 48 – 72 hrs, to observe turbidity.

MBC Test: (figure 4)

- Same concentrations of MIC were taken and incubated for 24 hrs then the colony count was taken.
- MBC is done to see whether there was bactericostatic or bactericidal effect of the extract (Drug) against the organism.
- Absence of bacterial growth shows bactericidal effect and presence of bacterial growth, as measured by calculating number of bacterial colonies present in the culture plate, shows the bacteriostatic effect of the drug.

Note: For facultative anaerobes, tubes were incubated in CO_2 jar at 37°C for 48-72 hrs. For strict anaerobes, tubes were incubated in anaerobic jars for 48-72 hrs.

Time Kill Curve Assay: (figure 5)

Equal quantity of the broth with organism and compound was mixed then immediately it was plated, this was noted as 0 hrs. Tubes were kept in CO2 jar till further time slot. And every 5mins, 10mins, 30mins and 2hrs, it was cultured or plated, and incubated according to the growth requirement, i.e., in CO2 Jar and anaerobic jar. After 48-72 hrs of incubation the plates were removed and the percentage of dead cells is calculated relatively to the growth control by determining the number of living cells (CFU/mL) of each tube by following the agar plate count method.

RESULTS:

MIC: (figure 3, table 1)

The growth of Pg and Pi was inhibited till 0.8 µg/ml. Similarly, the growth of Fn was inhibited

till 50 µg/ml and Aa, till 25 µg/ml. Thus, ethanolic extract of ginseng was bacteriostatic for the specimen bacteria at the above mentioned concentrations respectively, and these concentrations are considered as MIC of the respective bacteria.

MBC: (figure 4, table 2)

There was no growth of Pg and Pi till 0.8 µg/ml and no growth of Fn till 50 µg/ml. But, there was growth of Aa in all the concentrations. Thus, ethanolic extract of ginseng was bactericidal for Pg, Pi and Fn, at the above mentioned concentrations respectively, but regarding Aa, it was only bacteriostatic.

TKC: (figure 4, table 3, graph 1)

Ethanolic extract of ginseng against Pg, showed no growth after 10 minutes, whereas the growth of Pi, Fn and Aa was not inhibited even after incubation for 2 hours.

DISCUSSION:

Panax ginseng, an alternative phytomedicine, whose beneficial effects on immune disorders; diabetes; liver diseases, neuronal, cardiovascular, and infectious diseases were known for centuries (5).

P. ginseng can be used as either white ginseng (air drying) or red ginseng (steaming and heating) based on the method of processing (7). According to Peng xue et al, the less polar ginsenosides were present in higher concentrations in red ginseng, exhibited higher antimicrobial properties and disrupts bacterial cell membrane integrity more rapidly (11). It was also found that Ginsenoside Rg3, a potent antibacterial substance, was produced by heating ginseng at 100 °C for 4 and 8 h, which was absent in non-heated ginseng (12). Hence, owing to its superior antibacterial activity, heat transformed red ginseng was used in this study.

The antioxidant activities (total phenolics and flavonoids) of aqueous, methanolic and ethanolic extracts of ginseng leaves were studied by Kang, O.J et al. It was found that the total phenols and flavanoids were significantly higher ($p < 0.05$) in ethanolic extract (600.57 mg/100 g and 1701 mg/100 g) than methanolic (374.43 and 1512.64 mg/100 g) and aqueous extracts (248.30 and 680.05 mg/100 g). This suggests that ethanolic extract of ginseng leaves has the most effective antioxidant capacity compared to the methanolic and aqueous extracts. Hence, ethanolic extract of *Ginseng* was used in this study (13). The ethanolic extract also showed the highest hydroxyl radical scavenging and ferrous ion chelating activity, thus having maximum antioxidant capacity (14).

Previously, the MIC and MBC values of various genosides of aqueous extract of panax ginseng were determined for organisms like *F. nucleatum*, *C. perfringens*, and *P. gingivalis.* The ginsenoside-Rg5 exhibited the minimum MIC and MBC among all of the ginsenosides and the less polar ginsenoside HTS-4 fraction showed maximum activity against the above mentioned anaerobic bacteria (11). But, in our study, ethanolic extract of P. Ginseng showed antibacterial activity against F.n at 50 µg/ml concentration and at 0.8 µg/ml for P.g.

The ginsenosides composition of heated ginseng extract and its antimicrobial activity [MIC & MBC] was studied for *Bacillus cereus* and *Staphylococcus aureus.* Ginseng heated at 100 °C, showed maximum antimicrobial activity against B. cereus and S. aureus, which were completely inhibited after 2 and 8 hrs culture respectively. It was also found that Ginsenoside Rg_3, a potent antibacterial substance, which was absent in non-heated ginseng, was produced by heating ginseng at 100 °C for 4 hrs (12).

Panax ginseng was also used for the herbal bio-synthesis of gold and silver nanoparticles (AgNPs) and the process was considered as rapid, ecofriendly and economical. Thus prepared AgNPs showed antimicrobial properties at 3 µg concentration against many pathogenic strains such as, *Escherichia coli*, *Salmonella enterica*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Bacillus anthracis* and *Bacillus cereus*. In addition, they showed complete inhibition of

biofilm formation by *S.aureus* and *Pseudomonas aeruginosa* at 4 µg/ml concentration (15).

Disc diffusion test and growth curve analysis also showed that ginseng significantly inhibited the growth of *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Haemophilus influenza* (16).

Ginseng also inhibited growth, quorum sensing properties, virulence factors and biofilm forming capacity of *P. Aeruginosa. T*his shows that ginseng enhances bacterial clearance (17, 18), thus helpful in treatment of periodontal disease, which occurs due to microbial plaque, initiated as biofilm formation on tooth surface.

It was found that genosides obtained from the roots of *Panax ginseng* showed a strong inhibitory activity (minimum inhibitory concentration 0.25 mg/mL) and suppresses the ability of *Porphyromonas gingivalis* to agglutinate with erythrocytes (19). It was also found that the acidic polysaccharide from *P. ginseng*, PG-F2 showed antiadhesive effects against *Aa*, *Propionibacterium acnes*, and *Staphylococcus aureus* with MIC of 0.25–0.5 mg/mL. Whereas, there was no inhibitory effect of PG-F2 on *Lactobacillus acidophilus*, *Escherichia coli*, or *Staphylococcus epidermidis*. This suggests that PG-F2 shows selective antiadhesive and antibacterial effects against pathogenic bacteria, but not on beneficial or non pathogenic bacteria (20).

This is very similar to our study where, ethanolic extract of P. Ginseng was used to calculate MIC and MBC against Pg and was found that it has effective antibacterial activity at a concentration of 0.8 µgm/ml.

CONCLUSION

From the present study it can be concluded that Panax ginseng has a potent antimicrobial activity over the common periodontal pathogens. The ethanolic extract showed potent antibacterial activity and a concentration dependent killing property.

There are certain limitations of this study, such as:

- Invitro results cannot be directly implicated into clinical research, due to difference in the laboratory and intraoral conditions (biofilm mode of bacterial existence).
- This invitro study was conducted against only 4 periodontal pathogens. Analysing the antibacterial efficacy of the drug against various other periodontal pathogens is necessary before initiating the clinical trials.
- The drug release profile has to be evaluated to determine the duration of drug usage. But, again, the laboratory drug release duration differ from invivo drug release, due to interference of saliva and GCF.

Future prospective:

This study was intended for development of novel, more potent and selective therapeutic agent based on the values MIC, MBC and time kill curve analysis values obtained from the present study.

The MIC, MBC and time kill curve analysis values obtained from the in vitro study can be implemented to formulate various local drug delivery systems and can be used in further clinical studies to evaluate the efficacy of the drug. This would not only serve as an adjunct to SRP for effective treatment outcomes but also provide new therapeutic options in patients who are at greater risk for periodontal destruction, without the potential side effects of traditionally used antimicrobial therapy.

Acknowledgement:

The author would like to acknowledge the contributions of Department of Microbiology, Maratha Mandal Dental college, Belgaum, Karnataka, India and Bapuji College of Pharmacy, Davangere, Karnataka, India, for their timely help in conducting this study.

Financial support and sponsorship:

This was a self funded study, conducted with research orientation and academic purpose. No financial support was provided by any organisation and no grants received for the same.

Conflicts of Interest:

No conflicts of interest.

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