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Original Article

COMPUTATIONAL ANALYSIS, IN SILICO TOXICITY PREDICTION AND *IN VITRO* ANTIMICROBIAL EFFICACY OF *ZINGIBER OFFICINALE* ROSC. EXTRACT AGAINST *PORPHYROMONAS GINGIVALIS*

MINASARI*, FILIA DANA TYASINGSIH, RINI OKTAVIA NASUTION, FIDELIA NAVA SHAKIRA

Department of Oral Biology, and Department of Periodontics, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia
***Corresponding author: Minasari; *Email: minasari@usu.ac.id**

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ABSTRACT

Objective: This study aimed to determine the molecular activity, toxicity prediction and *in vitro* antimicrobial efficacy of *Zingiber officinale* Rosc Extract.

Methods: The molecular docking method was used to evaluate the antibacterial activity of the main compounds in *Zingiber officinale* by examining their interaction with DNA Gyrase IIb and Topoisomerase II. Chemical toxicity analysis was conducted using pK-CSM, SwissADME, and Pro-Tox II methodologies. *Zingiber officinale* rhizome was extracted via maceration, and its phytochemical content was determined. An *in vitro* antibacterial study against *P. gingivalis* was performed by measuring the inhibition zone using digital slide calipers and the disk diffusion method.

Results: The in silico toxicity test of the main components from *Zingiber officinale* revealed that gingerol, shogaols, and paradols have predicted LD50 values of 250 mg/kg, 687 mg/kg, and 2580 mg/kg, respectively, placing them in toxicity classes 3, 4, and 5. Their average similarity is 100% for gingerol and shogaols, and 87.52% for paradols, with prediction accuracies of 100% and 70.97%. Molecular docking indicated that gingerol, shogaols, and paradols inhibit DNA gyrase B and Topoisomerase II, which are involved in bacterial regeneration. The inhibition zones for concentrations of 60%, 40%, 20%, and 10% averaged 22.87 mm, 18.5 mm, 14.5 mm, and 11.31 mm, respectively, with Minimum Inhibitory Concentration (MIC) values of 10% and Minimum Bactericidal Concentration (MBC) values of 40%, showing the highest inhibition zone at 60%.

Conclusion: *Zingiber officinale* rhizome extract showed growth inhibition activity of *Porphyromonas gingivalis* ATCC®33277™.

Keywords: In silico, *In vitro*, Ginger extract, *Porphyromonas gingivalis,* MIC, MBC

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INTRODUCTION

The increasing prevalence of antibiotic resistance poses a significant challenge in treating of infectious diseases. This issue is particularly pertinent in the context of periodontal diseases, where the bacterial pathogen *P. gingivalis* plays a critical role. *P. gingivalis*, a g-negative anaerobic bacterium, is a major etiological agent in chronic periodontitis, an inflammatory condition that can lead to the destruction of the supporting structures of the teeth [1]. The rise of antibiotic-resistant strains of *P. gingivalis* necessitates the exploration of alternative antimicrobial agents with novel mechanisms of action. In this regard, natural products derived from medicinal plants have garnered significant attention due to their potential as sources of diverse bioactive compounds with antimicrobial, anti-inflammatory, and anti-quorum sensing properties. One such plant is *Zingiber officinale* Roscoe, commonly known as ginger, which has a long history of traditional use in various cultures for the treatment of various ailments, including oral and dental diseases [2]. *Zingiber officinale* Roscoe, commonly known as ginger, has a long history of traditional use in various cultures for the treatment of ailments, including oral and dental diseases. Ginger and its phytochemical constituents have demonstrated promising antimicrobial activities against a wide range of microorganisms due to the presence of compounds such as flavonoids, quinones, monoterpenes, sesquiterpenes, b-caryophyllene, camphene, geranial, geranyl acetate, gingerols, alkaloids, steroids/triterpenoids, and phenolics [3].

A range of studies have demonstrated the antimicrobial efficacy of *Zingiber officinale* Rosc extract. Subramani in 2021 found that the extract exhibited significant activity against various bacteria [4], while other study reported its effectiveness against Candida species and *Streptococcus pneumoniae* [5]. Murugesan (2020) identifying its antioxidant and anti-arthritic potential [6]. Lastly, Kaushik (2020) and Al-khazraji (2022) both underscored its antiviral and antibacterial properties, respectively [7, 8]. These studies collectively suggest that *Zingiber officinale Rosc.* extract has significant antimicrobial efficacy [9].

This study aimed to investigate the antimicrobial efficacy of ginger extract against *P. gingivalis* using a multi-faceted approach that includes molecular docking, in silico toxicity prediction, and *in vitro* antimicrobial assays. Molecular docking studies provide insights into the potential interactions between ginger-derived compounds and bacterial targets, helping to identify the most promising candidates for further investigation. In silico toxicity, prediction offers a preliminary assessment of the safety profile of these compounds, which is crucial for their potential therapeutic application. Finally, *in vitro* antimicrobial assays validate the efficacy of the selected compounds against *P. gingivalis* in a controlled laboratory setting. The combination of these methodologies allows for a comprehensive evaluation of the antimicrobial potential of ginger extract, from theoretical predictions to practical applications. This integrated approach not only facilitates the identification of effective antimicrobial agents but also helps to streamline the drug development process by focusing on compounds with favorable safety profiles. Given the urgent need for new antimicrobial agents to combat antibiotic-resistant pathogens, the findings from this study could have significant implications for the development of alternative therapies for periodontal diseases. By exploring the potential of ginger extract as an antimicrobial agent against *P. gingivalis*, this research contributes to the growing body of evidence supporting the use of natural products in the fight against infectious diseases.

MATERIALS AND METHODS

The research materials included Ginger, distilled water, 5% Brucella Agar Sheep Blood Media, Brain Heart Infusion Broth (BHIB) Media, 96% Ethanol, 70% 0.2% Chlorhexidine gluconate, Dimethyl sulfoxide (DMSO), Vitox supplement, Anaerobic indicator, NaCl 0.45%, and *Porphyromonas gingivalis* ATCC®33277™.

Plant collection and extraction

Ginger was collected from the padang bulan market area in Medan city. The plant was verified by the Herbarium Medanense Office and given approval number 1746/MEDA/2024. A 300 g quantity of dried Ginger rhizome was extracted using a maceration process with a mixture of 70% ethanol. The extraction method necessitated constant agitation at a temperature of 25 °C. Following a 24 h period, the mixture was separated using a filtration process. The operation was replicated twice, resulting in a cumulative tally of the extractions. The gathered specimens were merged and subjected to centrifugation at a speed of 3500 revolutions per minute for a duration of 10 min at ambient temperature. The liquid was subjected to condensation using a rotary evaporator at a temperature of 38 °C, forming the hydroethanolic extract (HESc) [10].

Phytochemical constituent analysis

The extract was screened using the standard qualitative determination procedure for the presence of alkaloids, flavonoids, glycosides, tannins, saponins, triterpenoids, and steroids [11].

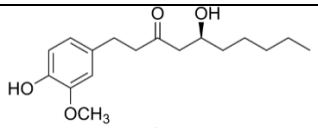
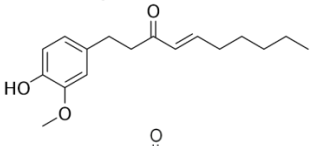
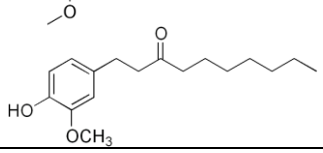
In silico tools

The equipment comprised an HP Laptop equipped with a Windows 11 operating system, 64-bit architecture, 4 GB RAM, 256 GB SSD, and a 14-inch display. This study utilizes a range of software tools for diverse objectives. The mentioned software and databases are as follows: Windows 11 64-bit operating system, Chimera 1.16 for molecular structure visualization, Protein Data Bank for protein structure data access, PubChem for chemical compound information access, and SwissDock for protein-ligand docking simulations.

Preparation of ligands and proteins

DNA gyrase B and topoisomerase II (DNA) are commonly used as protein targets in in silico antibacterial studies because they play crucial roles in bacterial DNA replication, transcription, and repair [12, 13]. The DNA gyrase B and Topoisomerase II (DNA) were acquired from the Protein Data Bank website (*). The PDB file format. Afterward, the UCSF Chimera 1.16 tool was utilized to prepare the sample by removing residues. The test chemicals were synthesized using UCSF Chimera 1.16. This was accomplished by entering the PubChem CID of the ligand, which was previously obtained using the PubChem online service and saved in the mol2 format. Molecular docking entails the interaction between proteins and either test compounds or natural ligands. The docking method was carried out using the SwissDock platform. The docking data was quantified using the Gibbs free energy (ΔG) value [14]. Table 1 lists the precise attributes of these ligands.

Table 1: Ligand name

Name	Formula	Chemical structure
Gingerols	C17H26O4	
shogaols	C17H24O3	
paradols	C17H26O3	

Rendering of docking outcomes

The visualization process was performed using the USCF Chimera 1.16. Protein data and docking results were entered into *.pdb file format. Visualization illustrates the specific type of bond interaction established together with the amino acid that serves as the binding site. The visualization results are presented in *.png file format [15].

Preparation of compound for in silico toxicity prediction

The preparation of each compound to obtain Canonical SMILES was carried out using the Pubchem website (https://pubchem.ncbi.nlm.nih.gov/) [16].

Toxicity prediction of compound with pK-CSM tools

Prediction of compound toxicity using pK-CSM Tools via http://biosig.unimelb.edu.au/pkcsm/prediction, is done by entering Canonical SMILES, then pressing ADMET to get absorption analysis results distribution (VDss, Fraction unbound, BBB permeability, and CNS permeability); metabolism and toxicity [17].

Toxicity prediction of compound with Pro-Tox II

Prediction of compound toxicity with Pro-Tox II is accessed via https://tox-new.charite.de/protox_II/, then press Tox Prediction and enter Canonical SMILES, tick all toxicity parameters, then Start Tox-Prediction to get the results of the toxicity analysis of the compound (LD50, Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, Cytotoxicity, AhR, AR, AR-LBD, Aromatase, ER, ER-LBD, PPAR-Gamma, nrf2/ARE, HSE, MMP, Phosphoprotein tumor suppressor, and ATAD 5) [18].

Antibacterial evaluation procedure

A total of 100 µl of *P. gingivalis* suspension was added to each inoculum tube containing 2 ml of Brain Heart Infusion Broth (BHI-B) media and then vortexed. After that, ginger extract at concentrations of 60%, 40%, 20%, and 10% was added to the bacterial suspension solution. All tubes were incubated at 37 °C for 48 h in an obligate anaerobic atmosphere in the anaerobic jar and then observed.

The turbidity of the incubation solution was observed to determine the Minimum Inhibitory Concentration (MIC) [19], which is the lowest concentration at which there is still growth of bacterial colonies but less than the negative control. Next, the culture fluid resulting from incubation was streaked onto 5% Brucella sheep blood agar solid media using an inoculating loop blue with a full streak and then incubated at 37 °C for 48 h. Bacterial colonies were then counted using the Standard Plate Count (SPC) method. Each petri dish with the colonies that appeared was marked with a marker from the back of the petri dish and then counted. This method is used to determine the number of bacterial colonies at each concentration. The lowest concentration that does not indicate the presence of bacterial colonies is the Minimum Bactericidal Concentration (MBC).

Statistical analysis

The data was obtained and analyzed using the Statistics for the Social Sciences (SPSS) application. If the data is normally

distributed, it will then be tested using one-way ANOVA and post hoc (LSD). If the data is not normally distributed, it will then be tested using Kruskal Wallis and Mann Whitney. Data were managed at a significance of $p \leq 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

The present investigation involved the qualitative phytochemical content examination. The results are shown in table 2.

Table 2: Phytochemical screening result ginger

No.	Content	Reagent	Dried sample	Extract
1	flavonoids	HCL(c), Mg powder. Amyl alcohol	+	+
2	alkaloids	Mayer	+	+
		Bouchardat	+	+
		Dragendorf	+	+
3	saponins	Foam test	+	+
4	tannins	FeCl3	+	+
6	terpenoid	Liberman Burchard	+	+

The phytochemical screening of the dried sample and its extract of Ginger revealed the presence of multiple bioactive compounds, including flavonoids, alkaloids, saponins, tannins, and terpenoids. These findings are consistent with previous studies on similar plant extracts, which have also demonstrated the presence of these compounds and their associated biological activities [20].

Docking visualization

In this study, antibacterial molecular docking of *Zingiber officinale* Rosc extract were evaluated. The docking Affinity Score visualization results for gingerols, shogaols, and paradols against DNA gyrase B and human topoisomerase IIα are summarized in table 2 and 3.

Table 3: Docking affinity scores on DNA gyrase B

Ligand	Protein	ΔG (kkal/mol)	Amino acid residue
Gingerols	DNA gyrase B	-7.0	Chain A: VAL43 ASP45 ASN46 ALA47 ASP49 GLU50 VAL71 GLN72 ASP73 GLY75 ARG76 GLY77 ILE78 PRO79 ILE94 MET95 VAL120 lEU132 THR165 MET166 VAL167
Shogaols	DNA gyrase B	-7.1	Chain A: VAL43 ASP45 ASN46 ALA47 ASP49 GLU50 ALA53 VAL71 GLN72 ASP73 GLY75 ARG76 GLY77 ILE78 PRO79 ILE94 MET95 VAL97 HIS99 GLY119 VAL120 ARG136 GLY164 THR165 MET166 VAL167
Paradols	DNA gyrase B	-7.0	Chain A: GLU42 VAL43 ASN46 ALA47 ASP49 GLU50 ALA53 VAL71 GLN72 ASP73 GLY75 ARG76 GLY77 ILE78 PRO79 ILE94 MET95 VAL97 HIS99 GLY119 VAL120 SER121 ARG136 GLY164 THR165 MET166 VAL167

Table 4: Docking affinity scores on human topoisomerase IIα

Ligand	Protein	ΔG (kkal/mol)	Amino acid residue
Gingerols	human topoisomerase IIα	-7.2	Chain A: GLN542 ASP543 GLN544 SER547 ILE577 TYR590 SER591 lEU592 PRO593 PHE668 ARG672 ARG673 lYS676 GLU682 TYR684 lEU685 TYR686 GLU702 lEU705 ASN708 SER709 ASN711 GLU712 ARG713 ILE715 PRO716 SER717 lEU722 lYS723 PRO724 ARG727 lYS728 lYS827 lEU829 GLU837 PRO838 GLU839 TRP840 PHE1003 ASP1004 HIS1005 VAL1006 GLY1007 CYS1008
Shogaols	human topoisomerase IIα	-7.3	Chain A: MET669 ARG672 ARG673 lYS676 ASN708 GLU712 ARG713 ILE715 PRO716 SER717 ASP720 lEU722 lYS723 PRO724 ARG727 lYS728 lEU829 GLU837 PRO838 GLU839 TRP840 PHE1003 ASP1004 HIS1005 VAL1006 GLY1007 CYS1008
Paradols	topoisomerase IIα	-6.8	Chain A: ASP541 GLN542 ASP543 GLN544 SER547 lYS550 ILE577 TYR590 SER591 lEU592 PRO593 MET669 ARG672 ARG673 lYS676 GLU682 ASP683 TYR684 lEU685 TYR686 GLU702 lEU705 ASN708 GLU712 ARG713 ILE715 PRO716 SER717 ASP720 lEU722 lYS723 PRO724 ARG727 lYS728 lEU829 ASP831 GLU837 PRO838 GLU839 TRP840 PHE1003 ASP1004 HIS1005 VAL1006 GLY1007

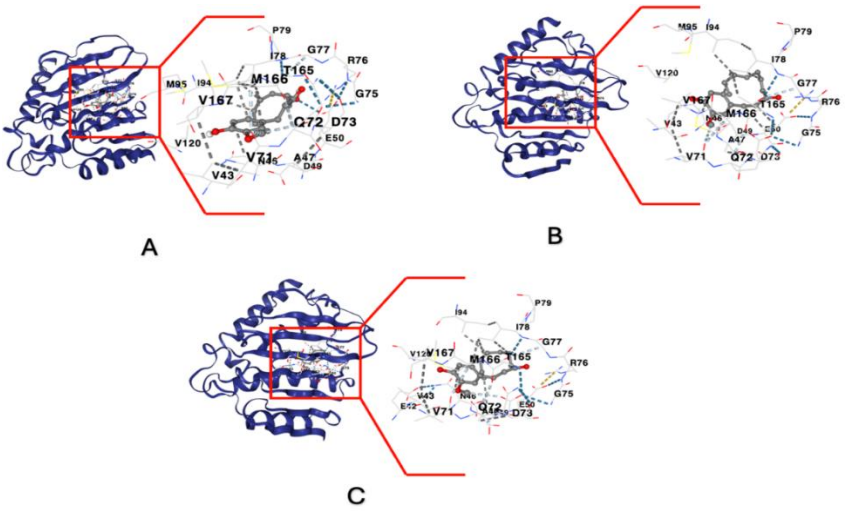


Fig. 1: Docking visualization DNA gyrase B with, A: Gingerols; B: shogaols; C: paradols

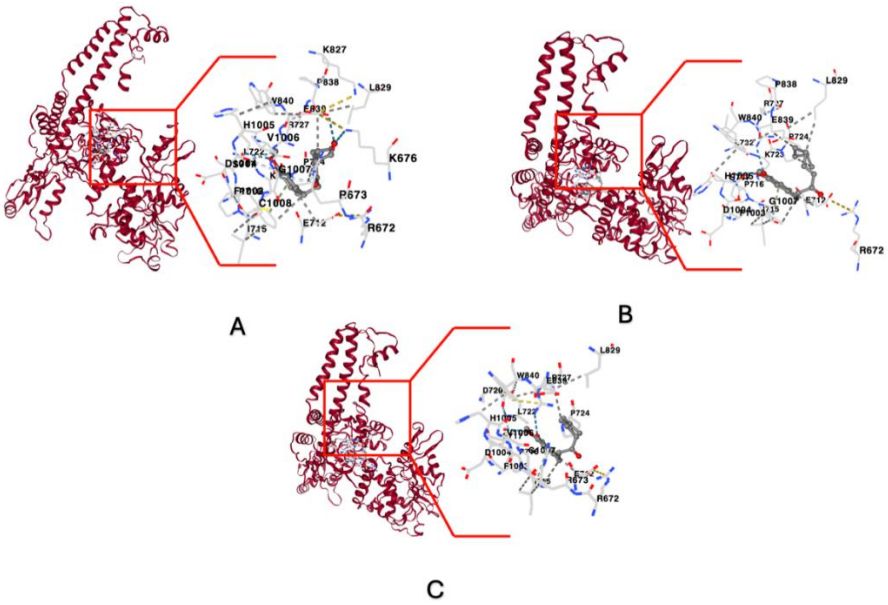


Fig. 2: Docking visualization human topoisomerase IIα with, A: Gingerols; B: shogaols; C: paradols

The molecular docking results indicate that both gingerols and shogaols show strong binding affinities towards DNA gyrase B and human topoisomerase IIα, suggesting their potential as effective antibacterial agents. The binding energies observed are comparable to those reported in similar studies. For instance, a study found that ginger compounds exhibited similar binding affinities against bacterial targets, reinforcing the potential of *Zingiber officinale Rosc.* Extract in antimicrobial applications [21]. Comparatively, shogaols exhibited the highest binding affinity among the three compounds, suggesting that the structural differences between these compounds may influence their interaction with the target proteins. These results are consistent with previous findings, who also reported higher efficacy of shogaols in antimicrobial activity compared to other ginger constituents [22]. Overall, this study provides valuable insights into the molecular interactions and potential therapeutic applications of *Zingiber officinale Rosc.* Extract, particularly in combating *P. gingivalis* infections. Further *in vitro* and *in vivo* studies are warranted to validate these findings and explore the clinical efficacy of these compounds.

Insilico toxicity prediction

The in silico toxicity predictions supported the potential safety of ginger extract compounds. Ensuring the safety of *Zingiber officinale* extracts or their main compounds is crucial. The results of the in silico toxicity prediction are presented in tables 5 and 6.

Table 5: Prediction of toxicity (pKCSM) *Zingiber officinale* main compound

Property	Model name	Predicted value			Unit
		Gingerol	Shogaols	Paradols	
Toxicity	AMES toxicity	No	No	No	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	0.635	0.759	0.819	Numeric (log mg/kg/d)
Toxicity	hERG I inhibitor	No	No	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	No	Yes	Yes	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	1.958	2.081	2.108	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	1.631	2.159	2.18	Numeric (log mg/kg_bw/d)
Toxicity	Hepatotoxicity	No	No	No	Categorical (Yes/No)
Toxicity	Skin Sensitisation	No	Yes	Yes	Categorical (Yes/No)
Toxicity	*T. Pyriformis* toxicity	1.487	2.475	2.462	Numeric (log ug/l)
Toxicity	Minnow toxicity	0.966	0.15	0.022	Numeric (log

Table 6: Toxicity class of zingiber officinale main compound (protox online)

No.	Parameters	Gingerol	Shogaols	Paradols
1.	Predicted LD 50	250 mg/kg	687 mg/kg	2580 mg/kg
2.	Predicted toxicity class	Class 3	Class 4	Class 5
3.	Average similarity	100%	100%	87.52 %
4.	Prediction accuracy	100%	100%	70.97%

The *in silico* toxicity prediction models for the main compounds of *Zingiber officinale* (gingerol, shogaols, and paradols) suggest a generally favorable safety profile, with the absence of AMES toxicity and hepatotoxicity indicating a low likelihood of mutagenic or liver-damaging effects, consistent with other studies on similar natural compounds. The prediction results show that gingerol does not inhibit hERG I or II, while shogaols and paradols inhibit hERG II, a critical factor since hERG inhibition can lead to cardiotoxicity. The non-inhibition of hERG I by all three compounds is a positive indicator of their safety concerning potential cardiac effects. Predicted oral rat acute toxicity (LD50) values for gingerol, shogaols, and paradols are 1.958, 2.081, and 2.108 mol/kg, respectively, suggesting moderate acute toxicity. Chronic toxicity (LOAEL) values further support moderate toxicity, with gingerol showing the lowest

and paradols the highest values, aligning with other studies on natural compounds. The absence of hepatotoxicity across all three compounds indicates a lower risk of liver damage, consistent with the traditional use of ginger in herbal medicine. However, the skin sensitization potential of shogaols and paradols necessitates caution for topical applications, supported by studies on skin irritants among natural compounds. Differences in *T. pyriformis* and minnow toxicity values reflect variations in environmental toxicity, with paradols showing the lowest toxicity towards minnows, beneficial for environmental safety. Overall, the in silico toxicity prediction indicates that *Zingiber officinale* compounds exhibit a generally safe profile with specific considerations for hERG inhibition and skin sensitization, aligning with existing literature on the safety of natural compounds and providing a basis for further i*n vivo* and clinical studies to confirm these predictions.

Comparatively, long-term administration of *Zingiber officinale* has been found to potentially lead to liver and kidney damage in rats [23]. Despite this, the plant has been shown to be less toxic and rich in phytochemicals, with the local variety having a higher toxic effect [24]. *Zingiber officinale* has also been found to mitigate lead acetate-induced toxicity in rats, reversing hematological and biochemical alterations [25, 26]. Furthermore, the plant's standardized extract has demonstrated no evidence of toxicity in preclinical tests and has shown potential anti-cholangiocarcinoma activity [27]. Lastly, the ethyl acetate fraction of *Zingiber officinale* extract has been found to attenuate lead-induced brain damage in rats [26].

Observation of MIC and MBC of ginger extract on *P. gingivalis* ATCC®33277™

The determination of MIC and MBC is carried out to find the smallest concentration of the extract that can inhibit or kill the bacteria. After 24 h of incubation at 37 ℃ the MIC and MBC values for *P. gingivalis* ATCC®33277™ were observed by assessing the turbidity level in each tube (table 7 and 8).

Table 7: Turbidity examination level of ginger extract against *P. gingivalis*

Group test	Replication			
	I	II	III	IV
60%	+	+	+	+
40%	+	+	+	+
20%	+	+	+	+
10%	+	+	+	+
Chlorhexidine 0.2%	-	-	-	-
DMSO	+	+	+	+

Description: += cloudy;-= clear (not cloudy)

Table 8: The results of calculating the number of *P. gingivalis* ATCC®33277™ colonies from culturing the test solution

Group	Number of bacterial colonies (CFU/ml)				
	Replication				Mean±SD
	I	II	III	IV	
60%	0	0	0	0	0
40%	0	0	0	0	0
20%	17	39	33	27	29±9.3
10%	166	182	141	196	171±23.5
Chlorhexidine 0,2%	0	0	0	0	0
DMSO	≥300	≥300	≥300	≥300	≥300

All values are mean±SD values (Number of experiment, n= 4)

The results of the MIC and MBC tests of ginger extract on *P. gingivalis* ATCC®33277™ reveal significant antibacterial activity. The MIC value was observed at a concentration of 20%, where the bacterial growth was still present but substantially reduced, indicated by a slight cloudiness. In contrast, at concentrations of 40% and 60%, the solution remained clear, suggesting complete inhibition of bacterial growth.

The MBC results further corroborate these findings. No bacterial colonies were observed at 40% and 60% concentrations, indicating that these concentrations are bactericidal. However, at 20%, although the bacterial count was reduced, colonies were still present (mean 29±9.3 CFU/ml), indicating that this concentration is inhibitory but not completely bactericidal. At 10%, the colony count was significantly higher (mean 171±23.5 CFU/ml), showing insufficient bactericidal activity at this concentration. Chlorhexidine 0.2%, used as a positive control, showed no bacterial growth, confirming its effective antibacterial properties. DMSO, the negative control, showed extensive bacterial growth, validating the results obtained for the ginger extract.

Comparatively, the antibacterial activity of ginger extract against *P. gingivalis* aligns with findings from previous studies. For instance, Park*et al.*, in 2008 demonstrated that ginger extract exhibited strong inhibitory effects against various oral pathogens, with MIC values ranging between 10% and 40% [28]. Similarly, other research found that ginger extract was effective against *Streptococcus mutans*, another significant oral pathogen, with MIC values consistent with the present study [29].

The variability in MIC and MBC values across different studies can be attributed to factors such as the ginger variety used, extraction methods, and differences in bacterial strains tested. Nonetheless, the present study highlights the potential of ginger extract as a natural antibacterial agent against *P. gingivalis*, suggesting its possible application in oral health products. Further research is warranted to explore its clinical efficacy and safety in human subjects.

Inhibition zone of ginger extract on the growth of *P. gingivalis* ATCC®33277™ using the diffusion method

Using 5% Brucella Agar Sheep Blood as the substrate, the diffusion technique was employed to determine the inhibitory zone. A suspension of *P gingivalis* ATCC®33277™ bacteria was uniformly spread over Brucella Agar Sheep Blood media using sterile tips. After incubation, the inhibition zones were analyzed. A blank disc containing ginger extract was adhered to the 5% Brucella Agar Sheep Blood surface, incubated for 24 h, and then observed. Measurements were taken four times for each material across all groups simultaneously. The inhibitory process of the ginger extract was demonstrated by the establishment of a clear zone around the disc area on the solid medium. The diameter of the inhibition zone was measured using a slide caliper (table 3).

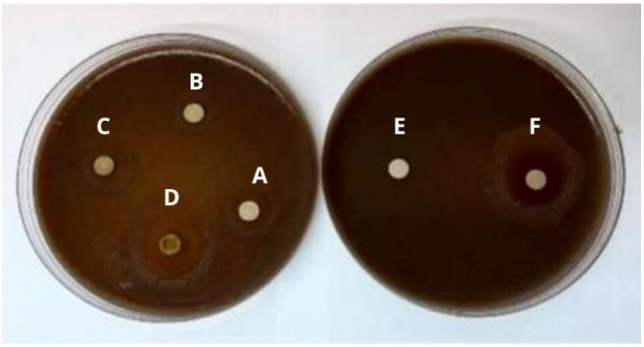


Fig. 3: Inhibition zone of *Porphyromonas gingivalis* ATCC®33277™. A: Dose 60%, B: Dose 40%; C: Dose 20%; D: Dose 10%; E: DMSO; F: Chlorhexidine 0.2%

Table 9: Average inhibition zones of several concentrations of ginger extract against *Porphyromonas gingivalis* ATCC®33277™

Group	Diameter of inhibition zone (mm)				
	Repetition				Mean±SD
	I	II	III	IV	
60%	23.5	22.5	22.5	23	22.87±0.47
40%	18.5	19	18.5	18	18.5±0.40
20%	14	14.5	15	14.5	14.5±0.40
10%	11,25	11,5	11	11,5	11.31±0.23
Chlorhexidine 0.2%	29,5	30	31	29.5	30±0.70
DMSO	0	0	0	0	0

All values are mean±SD values (Number of experiment, n= 4)

Results showed that the mean diameter of the ginger extract's inhibitory zone on *P. gingivalis* ATCC®33277™ growth was 22.87 mm, 18.5 mm, 14.5 mm, and 11 mm for concentrations of 60%, 40%, 20%, and 10% respectively. Ginger extract demonstrated inhibition of *P. gingivalis* ATCC®33277™ at all tested concentrations.

The results of the inhibition zone tests indicate that ginger extract possesses significant antibacterial activity against *P. gingivalis* ATCC®33277™. The diameter of the inhibition zones was proportional to the concentration of the ginger extract, with the largest zone observed at 60% concentration (22.87 mm) and the smallest at 10% concentration (11.31 mm). This suggests a dose-dependent inhibitory effect. The performance of ginger extract was compared with chlorhexidine 0.2%, a standard antimicrobial agent, which exhibited a larger inhibition zone (30 mm), indicating superior antibacterial activity. However, the inhibition zones produced by ginger extract are substantial, suggesting its potential as a natural antibacterial agent.

Comparatively, previous studies have reported similar findings regarding the antibacterial properties of ginger. For instance, Ginger extract has been found to be effective against a range of oral pathogens, including *P. gingivalis*. Studies have demonstrated its inhibitory and bactericidal power against *P. gingivalis* and *Actinobacillus Actinomycetemcomitans* [30], as well as its antifungal and antimicrobial properties against Candida species and some bacterial pathogens [5]. Furthermore, it has been found to be an effective antimicrobial herb against several Gram-positive bacteria [31]. Ginger extract has also been found to have a therapeutic effect against *Cryptosporidium parvum* in experimentally infected mice [32].

The variability in inhibition zone diameters across different studies can be attributed to factors such as the type of ginger used, extraction methods, and differences in bacterial strains tested. Nonetheless, the current study underscores the potential of ginger extract as an effective natural antibacterial agent against *P. gingivalis*, highlighting its possible application in the development of oral health products. Further research is warranted to explore its clinical efficacy and safety in human subjects.

CONCLUSION

The study demonstrated that gingerol, shogaols, and paradols from *Zingiber officinale* possess significant antibacterial properties, inhibiting key bacterial enzymes and exhibiting effective inhibition zones at varying concentrations. Their toxicity profiles and predicted molecular interactions suggest potential therapeutic applications, though their toxicity class warrants careful consideration for dosage in practical use. Finally, *Zingiber officinale* rhizome extract showed growth inhibition activity of *P. gingivalis* ATCC®33277™.

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AUTHORS CONTRIBUTIONS

Minasari conceived and designed the study, supervised the project, wrote the manuscript, and conducted the computational analysis and in silico toxicity prediction. Filia Dana Tyasingsih performed the *in vitro* antimicrobial efficacy experiments, analyzed data and contributed to result interpretation. Rini Oktavia Nasution assisted with computational analysis, in silico toxicity prediction, and manuscript writing and editing. Fidelia Nava Shakira helped with the *in vitro* experiments, data collection and analysis, and manuscript preparation.

CONFLICT OF INTERESTS

Declared none

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