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Antimicrobial study of some *Ganoderma* species against gram-positive bacterial isolates of diabetic foot ulcer

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Abstract
Ganoderma (Ganodermataceae) is known for its therapeutic applications. Its species *such as G. applanatum*, *G. boninense*, *G. lucidum*, *G. resinaceum* and *G. tsugae*, are known for their specific biologically active and macromolecules such as polysaccharides, triterpenoids, steroids, phenolic compounds, lipids and alkaloids revealing its vast biomolecular diversity. The antimicrobial studies of these species was carried out against some Gram positive bacterial isolates of diabetic foot ulcer. In present study, the antibacterial and inhibitory effects of various *Ganoderma* extracts in petroleum ether, chloroform, acetone, ethanol, methanol and aqueous were used against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Enterococcus faecium* and *Streptococcus pneumoniae* by agar well diffusion method. All the extracts exhibited various degree of inhibition against all tested bacteria. *G. applanatum* and *G. tsugae* shown the best results against Gram positive test bacteria and therefore, represent a good model for the development of new drug formulations for diabetic patients.

Keywords: *G. applanatum*, *G. boninense*, *G. lucidum*, *G. resinaceum*, *G. tsugae*, various extracts, agar well diffusion, Gram positive bacteria

1. Introduction
Ganoderma, commonly represent a group of medicinal mushrooms revered for their extensive history in traditional medicine and their rich array of bioactive compounds (Martínez-Montemayor *et al*., 2019; He *et al*., 2022) [18, 11] including polysaccharides (Zhang *et al*., 2019) [29], triterpenes (Such as ganoderic acids) (Yangchum *et al*., 2022) [27], peptides, alkaloids, flavonoids, lipids, steroids, glycosides, saponins, anthraquinone, anthocyanins, tannins and phenolic compounds (Sindhu *et al*., 2021) [22]. *Ganoderma* has so many therapeutic properties such as antioxidant, immunity booster, anti-inflammatory (Wen *et al*., 2022) [24], viral infections (Cor Andrejc *et al*., 2022) [9], antidiabetes, anticancerous properties (Cao *et al*., 2022; Wu *et al*., 2022) [5, 25], pneumatoprotective including asthma and bronchitis (Wang *et al*., 2020) [23], high blood pressure and high cholesterol, kidney disease, altitude sickness, chronic fatigue syndrome (CFS), trouble sleeping (Insomnia), stomach ulcers, poisoning, herpes pain, reducing stress and antifatigue. For millennia this mushroom have been esteemed in various cultures, particularly in traditional Chinese medicine (El-Sheikha *et al*., 2022) [10] and other Asian healing practices, due to their purported health-promoting properties and has gained attention in diabetes management due to its potential metallo protein actions. Diabetes mellitus (DM) remains a major concern for humanity, despite significant progress being made in its treatment. Diabetes leads to various complications and one of the most challenging is the development of diabetic foot ulcers. A diabetic foot ulcer is an open sore or wound that commonly occurs on the feet of individuals with diabetes. If left untreated, DFUs can lead to severe complications, including infection and gangrene which ends with amputation in extreme cases.

The present study encompasses the potential of *Ganoderma* species (Angulo-Sanchez *et al*., 2022) [3, 1] as *G. applanatum (*Peng *et al*., 2019) [20], *G. boninense* (Ma *et al*., 2014; Abdullah *et al*., 2020) [17], *G. lucidum*, *G. resinaceum* (Al-Fatimi *et al*., 2005) [2] *and G. tsugae* to combat bacterial infections in diabetic foot ulcers. The study aims to evaluate antibacterial activity in some Gram-positive bacterial strain's (*Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Enterococcus faecium* and *Streptococcus pneumoniae*) of DFU through *in vitro* assays (Shi *et al*., 2021) [21].

The findings will assist to contextualized by exploring the clinical implications of *Ganoderma* species in managing diabetic foot ulcers, considering patient safety, possible drug interactions and the feasibility of incorporating *Ganoderma*-based treatments into existing therapeutic regimens.

2. Materials and Methods

2.1 Procurement of Fungal Material

The fully grown fruiting bodies of five different species of Genus *Ganoderma* (*G. applanatum*, *G. boninense*, *G. lucidum*, *G. resinaceum* and *G. tsugae*), were obtained from ICAR- Directorate of Mushroom Research, Solan (HP). Botteled specimen with Accession Numbers were prepared according to International Rules of Botanical Nomenclature (IRBN) and respective specimen were assigned taxonomic affiliations and deposited in the Department of Biotechnology, B. N. University, Udaipur (Rajasthan) (Table: 1).

Table 1: Specimen Accession Number of studied *Ganoderma* species

S. No.	*Ganoderma* Species	Specimen Accession Number
1.	*Ganoderma applanatum*	BOT/2019-20/C/MC/01
2.	*Ganoderma boninense*	BOT/2019-20/C/MC/02
3.	*Ganoderma lucidum*	BOT/2019-20/C/MC/03
4.	*Ganoderma resinaceum*	BOT/2019-20/C/MC/04
5.	*Ganoderma tsugae*	BOT/2019-20/C/MC/05

2.2 Extract preparation

The fine powder of dried *Ganoderma* fruiting bodies was used for preparing the extracts. Petroleum ether, chloroform, acetone, ethanol, methanol and aqueous were used as solvents to obtain the pharmacologically active compounds from the mushroom. For every 10 gram of powder, 150 ml of solvent was used and was subjected to Soxhlet apparatus for extraction process. For antibacterial assay, the residues left after process, were dissolved in Dimethyl Sulfoxide (DMSO) to obtained stock solutions and were stored at 4 ºC in air tight containers. After procurement of fruiting bodies were ground to make fine powder and kept refrigerated at 4 ºC in an airtight container for further practical use.

2.3 Procurement of bacteria

Some bacterial isolates of Diabetic Foot Ulcer pathogenic strains used in present study were obtained from National Centre for Cell Science (NCCS), Pune in freeze dried form. All these bacterial isolates were preserved in 10% glycerol and stored at -20 °C. Bacteria were grown in Muller-Hinton agar for 24 hours and standardized with sterile saline to turbidity equivalent to 0.5 McFarland scale approximately1-2×10^8 CFU/ml (CLSI, 2009) and stored at 4 ºC. The antibacterial activity was determined using agar well diffusion method.

2.4 Culture media and inoculum preparation

Trypticase Soy Yeast Extract (TSYE) medium and nutrient broth medium was prepared for revival of bacteria and Nutrient agar and Muller Hinton agar medium was prepared for antibacterial assay. Agar well diffusion method was used for antibacterial testing.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of a specific extract was determined by using a broth micro-dilution bioassay in 96-well micro titre polystyrene plates. The method was modified from Yakob *et al*., 2012 [26], and involved addition of 100 µl of extracts to each well of the plates, followed by serial dilutions and bacterial inoculum. The plates were then incubated at 37 °C for 24-48 hours for bacterial growth and inhibition were observed consequently. The MIC of each extract was recorded as the lowest concentration inhibiting the growth of the bacteria.

3. Results

3.1 Evaluation of antimicrobial efficacy of *Ganoderma* species

3.1.1 Zone of Inhibition (mm) of Gram-positive bacteria

Table 2: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram positive bacteria in petroleum ether

Test species	Zone of inhibition (mm) in Petroleum ether				
	SA	EF	BS	Efm	SP
G. applanatum	5.60±0.26**	5.75±0.25**	5.90±0.20**	5.20±0.36**	3.30±0.30**
G. boninense	4.55±0.20**	4.45±0.40**	3.20±0.51*	3.90±0.45**	3.90±0.40**
G. lucidum	3.60±0.17**	4.00±0.36**	4.60±0.40**	4.85±0.35**	5.10±0.32**
G. resinaceum	3.60±0.42**	5.00±0.15**	4.10±0.31**	3.15±0.46**	4.50±0.26**
G. tsugae	5.10±0.26**	5.75±0.21**	5.15±0.30**	4.40±0.30**	5.75±0.40**
Control	6.50±0.50**	6.30±0.55*	6.45±0.45**	6.10±0.51*	6.60±0.55*

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*; Control: Tetracycline
Mean values ± SD (n=3); $p\geq0.05$ (NS), *$p<0.1$ (S), **$p\leq0.01$ (HS)

Table 3: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram positive bacteria in chloroform

Test species	Zone of inhibition (mm) in Chloroform				
	SA	EF	BS	Efm	SP
G. applanatum	4.10±0.46**	5.40±0.53*	3.80±0.30**	5.50±0.42**	4.50±0.35**
G. boninense	5.35±0.26**	3.50±0.31**	3.80±0.45**	3.00±0.35**	3.80±0.35**
G. lucidum	6.00±0.55*	3.00±0.36**	4.85±0.47**	4.25±0.26**	5.90±0.31**
G. resinaceum	3.40±0.35**	4.80±0.20**	4.35±0.45**	3.60±0.46**	5.00±0.40**
G. tsugae	4.65±0.26**	4.20±0.30**	5.85±0.35**	4.90±0.35**	5.90±0.30**
Control	6.50±0.51*	6.15±0.40**	6.30±0.45**	6.40±0.36**	6.25±0.70*

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*; Control: Tetracycline
Mean values ± SD (n=3); $p\geq0.05$ (NS), *$p<0.1$ (S), **$p\leq0.01$ (HS)

Table 4: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram positive bacteria in acetone

Test species	Zone of inhibition (mm) in Acetone				
	SA	EF	BS	Efm	SP
G. applanatum	6.00±0.47**	5.50±0.25**	6.25±0.40**	5.30±0.30**	3.25±0.35**
G. boninense	4.20±0.35**	3.70±0.36**	3.80±0.25**	3.10±0.40**	4.00±0.40**
G. lucidum	4.75±0.50**	6.20±0.42**	4.50±0.46**	4.80±0.25**	5.90±0.35**
G. resinaceum	3.55±0.20**	4.90±0.25**	3.80±0.45**	4.20±0.42**	4.65±0.46**
G. tsugae	5.60±0.50**	4.24±0.27**	5.80±0.25**	5.90±0.45**	5.25±0.36**
Control	6.50±0.55*	6.75±0.61*	6.70±0.46**	6.25±0.51*	6.45±0.50**

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*; Control: Tetracycline
Mean values ± SD (n=3); $p \geq 0.05$ (NS), *$p < 0.1$ (S), **$p \leq 0.01$ (HS)

Table 5: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram positive bacteria in ethanol

Test species	Zone of inhibition (mm) in Ethanol				
	SA	EF	BS	Efm	SP
G. applanatum	6.20±0.60*	6.10±0.21**	6.50±0.56*	5.00±0.35**	5.70±0.35**
G. boninense	3.85±0.31**	4.10±0.60*	4.50±0.35**	3.90±0.45**	3.75±0.60*
G. lucidum	5.90±0.31**	6.50±0.26**	3.90±0.40**	4.60±0.46**	5.70±0.36**
G. resinaceum	4.40±0.49**	4.10±0.30**	5.10±0.25**	3.00±0.25**	4.40±0.45**
G. tsugae	5.20±0.45**	4.70±0.30**	5.80±0.50**	5.80±0.45**	5.15±0.40**
Control	6.75±0.50**	7.00±0.66*	7.00±0.45**	6.30±0.36**	6.45±0.30 **

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*; Control: Tetracycline
Mean values ± SD (n=3); $p \geq 0.05$ (NS), *$p < 0.1$ (S), **$p \leq 0.01$ (HS)

Table 6: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram positive bacteria in methanol

Test species	Zone of inhibition (mm) in Methanol				
	SA	EF	BS	Efm	SP
G. applanatum	6.15±0.40**	6.50±0.45**	5.15±0.30**	5.00±0.25**	6.25±0.50**
G. boninense	4.80±0.35**	5.30±0.21**	5.85±0.31**	6.00±0.50**	5.65±0.25**
G. lucidum	5.55±0.35**	6.50±0.50**	7.10±0.55*	4.00±0.45**	3.50±0.45**
G. resinaceum	4.15±0.36**	4.20±0.40**	4.40±0.40**	4.70±0.40**	3.50±0.40**
G. tsugae	6.80±0.44**	5.95±0.38**	6.50±0.46**	5.45±0.45**	5.00±0.45**
Control	7.50±0.51*	7.25±0.46**	7.60±0.70*	7.00±0.55*	6.75±0.61*

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*; Control: Tetracycline
Mean values ± SD (n=3); $p \geq 0.05$ (NS), *$p < 0.1$ (S), **$p \leq 0.01$ (HS)

Table 7: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram positive bacteria in aqueous

Test species	Zone of inhibition (mm) in Aqueous				
	SA	EF	BS	Efm	SP
G. applanatum	6.90±0.20**	5.35±0.35**	4.30 ±0.20**	5.90±0.36**	5.15 ±0.35**
G. boninense	4.10±0.35**	4.75±0.46**	5.15±0.30**	4.40±0.26**	3.15 ±0.25**
G. lucidum	6.90±0.46**	3.30±0.36**	6.50±0.51*	3.85±0.35**	5.80 ±0.35**
G. resinaceum	5.40±0.26**	4.10±0.32**	5.90±0.36**	3.10±0.35**	4.45±0.45**
G. tsugae	4.70±0.25**	6.00±0.35**	3.60±0.35**	5.20±0.25**	3.80±0.50**
Control	7.50±0.33**	6.75±0.55*	7.10±0.50**	6.80±0.45**	6.50±0.55*

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*; Control: Tetracycline
Mean values ± SD (n=3); $p \geq 0.05$ (NS), *$p < 0.1$ (S), **$p \leq 0.01$ (HS)

Table 8: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in petroleum ether

Test species	Minimum inhibitory concentration (µg/ml) in Petroleum ether				
	SA	EF	BS	Efm	SP
G. applanatum	4.53±0.70*	4.52±0.36**	4.54±0.10**	4.55±0.30**	4.56±0.51*
G. boninense	4.57±0.30**	4.60±0.25**	4.58±0.61*	4.56±0.50**	4.59±0.45**
G. lucidum	4.62±0.40**	4.59±0.60*	4.62±0.55*	4.59±0.52*	4.61±0.55*
G. resinaceum	4.63±0.50**	4.64±0.40**	4.62±0.45**	4.65±0.70*	4.61±0.41**
G. tsugae	4.62±0.33**	4.63±0.25**	4.66±0.33**	4.64±0.30**	4.65±0.10**

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*;
Mean values ± SD (n=3); $p \geq 0.05$ (NS), *$p < 0.1$ (S), **$p \leq 0.01$ (HS)

Table 9: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in chloroform

Test species	Minimum inhibitory concentration (µg/ml) in Chloroform				
	SA	EF	BS	Efm	SP
G. applanatum	4.52±0.51*	4.55±0.33**	4.56±0.10**	4.53±0.45**	4.54±0.50**
G. boninense	4.56±0.55*	4.59±0.66*	4.58±0.36**	4.57±0.45**	4.55±0.41**
G. lucidum	4.61±0.45**	4.63±0.50**	4.63±0.51*	4.59±0.41**	4.62±0.40**
G. resinaceum	4.62±0.40**	4.62±0.10**	4.61±0.35**	4.63±0.46**	4.65±0.61*
G. tsugae	4.64±0.25**	4.60±0.36**	4.62±0.20**	4.66±0.33**	4.64±0.45**

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*;
Mean values ± SD (n=3); $p \geq 0.05$ (NS), *$p < 0.1$ (S), **$p \leq 0.01$ (HS)

Table 10: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in acetone

Test species	Minimum inhibitory concentration (µg/ml) in Acetone				
	SA	EF	BS	Efm	SP
G. applanatum	4.57±0.33**	4.55±0.30**	4.56±0.35**	4.54±0.51*	4.59±0.10**
G. boninense	4.56±0.51*	4.58±0.33**	4.61±0.40**	4.57±0.60*	4.59±0.15**
G. lucidum	4.63±0.36**	4.61±0.51*	4.60±0.55*	4.62±0.51*	4.63±0.25**
G. resinaceum	4.63±0.50**	4.65±0.45**	4.64±0.46**	4.62±0.55*	4.61±0.45**
G. tsugae	4.65±0.66*	4.62±0.25**	4.66±0.20**	4.66±0.33**	4.67±0.51*

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*;
Mean values ± SD (n=3); $p \geq 0.05$ (NS), *$P < 0.1$ (S), **$p \leq 0.01$ (HS)

Table 11: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in ethanol

Test species	Minimum inhibitory concentration (µg/ml) in Ethanol				
	SA	EF	BS	Efm	SP
G. applanatum	4.55±1.00*	4.53±0.66**	4.59±0.10**	4.52±0.60*	4.58±0.50**
G. boninense	4.59±0.33**	4.57±0.50**	4.60±0.50**	4.58±0.33**	4.61±0.60*
G. lucidum	4.58±0.50**	4.65±0.10**	4.63±0.50**	4.60±1.00*	4.65±0.33**
G. resinaceum	4.59±0.50**	4.56±0.60*	4.55±0.10**	4.60±0.10*	4.59±0.60*
G. tsugae	4.62±0.50**	4.65±0.33**	4.64±0.33**	4.63±0.33**	4.61±0.33**

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*;
Mean values ± SD (n=3); p≥0.05 (NS), *$p < 0.1$ (S), **$p \leq 0.01$ (HS)

Table 12: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in methanol

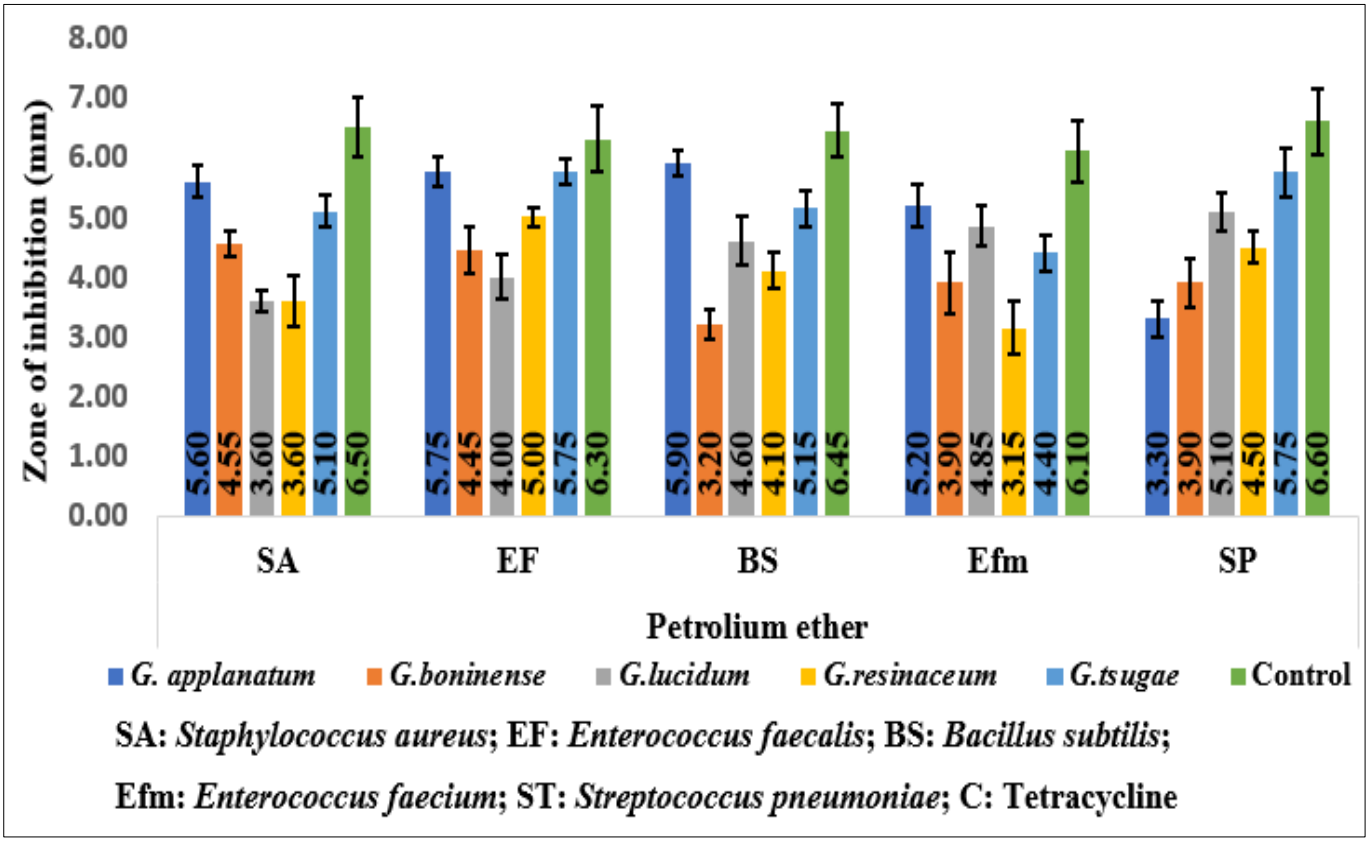
Test species	Minimum inhibitory concentration (µg/ml) in Methanol				
	SA	EF	BS	Efm	SP
G. applanatum	4.59±0.35**	4.57±0.55*	4.54±0.45**	4.56±0.30**	4.55±0.50**
G. boninense	4.58±0.66*	4.57±0.20**	4.59±0.33**	4.55±0.36**	4.61±0.25**
G. lucidum	4.63±0.20**	4.60±0.51*	4.62±0.25**	4.63±0.45**	4.61±0.35**
G. resinaceum	4.60±0.25**	4.61±0.35**	4.65±0.30**	4.63±0.33**	4.61±0.43**
G. tsugae	4.63±0.33**	4.67±0.46**	4.64±0.10**	4.65±0.51*	4.62±0.41**

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*;
Mean values ± SD (n=3); $p \geq 0.05$ (NS), *$p < 0.1$ (S), **$p \leq 0.01$ (HS)

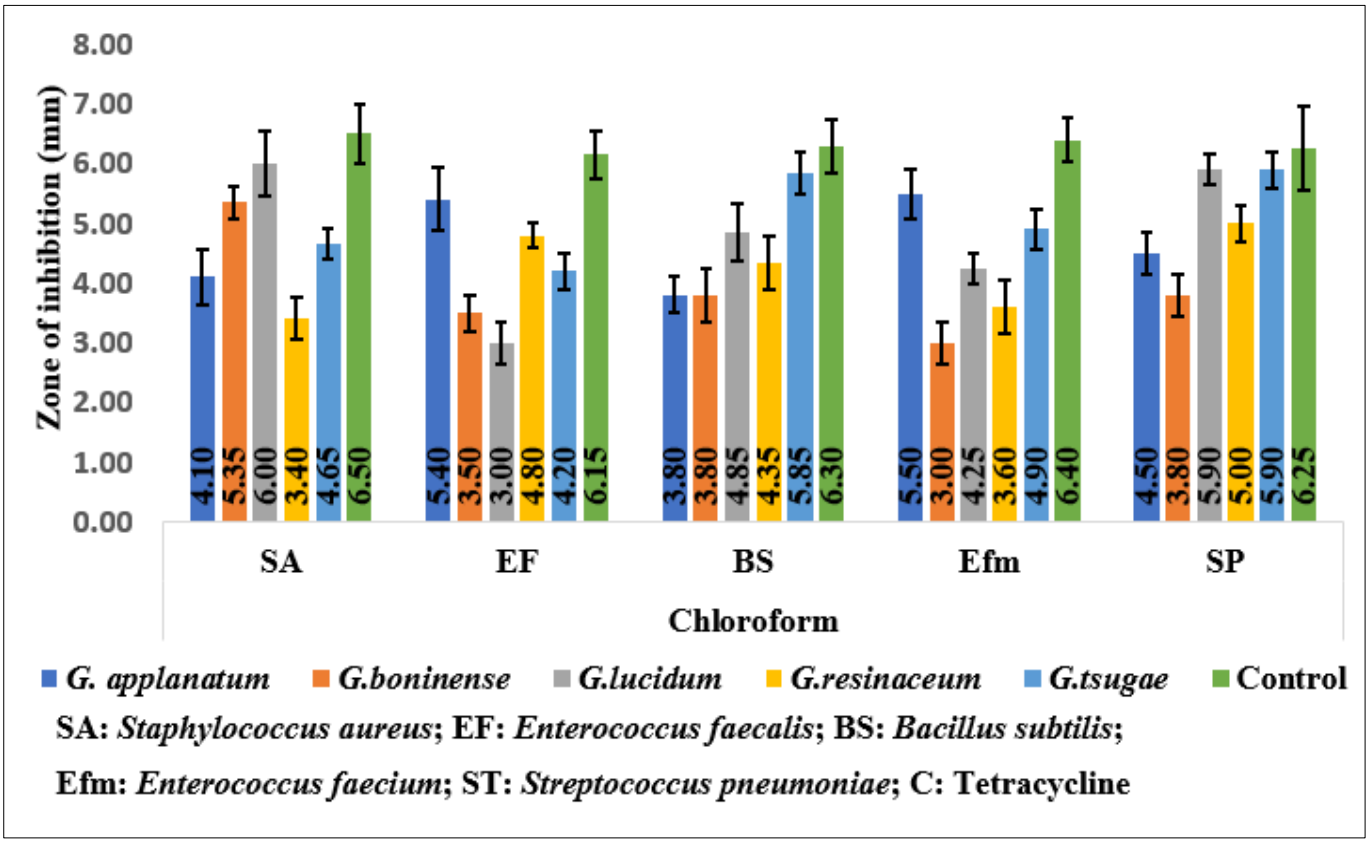
Table 13: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in aqueous

Test species	Minimum inhibitory concentration (µg/ml) in Aqueous				
	SA	EF	BS	Efm	SP
G. applanatum	4.59 ±0.20**	4.56±0.45**	4.58±0.51*	4.60±0.50**	4.55±0.41**
G. boninense	4.58±0.30**	4.61±0.45**	4.59±0.33**	4.55±1.00*	4.57±0.35**
G. lucidum	4.63±0.55*	4.59±0.33**	4.63±0.50**	4.60 ±0.25**	4.62±0.70*
G. resinaceum	4.60±0.40**	4.61±0.66*	4.63±0.25**	4.64±0.33**	4.65±0.10**
G. tsugae	4.67±0.50**	4.67±0.25**	4.61±0.46**	4.63 ±0.51*	4.62 ±0.43**

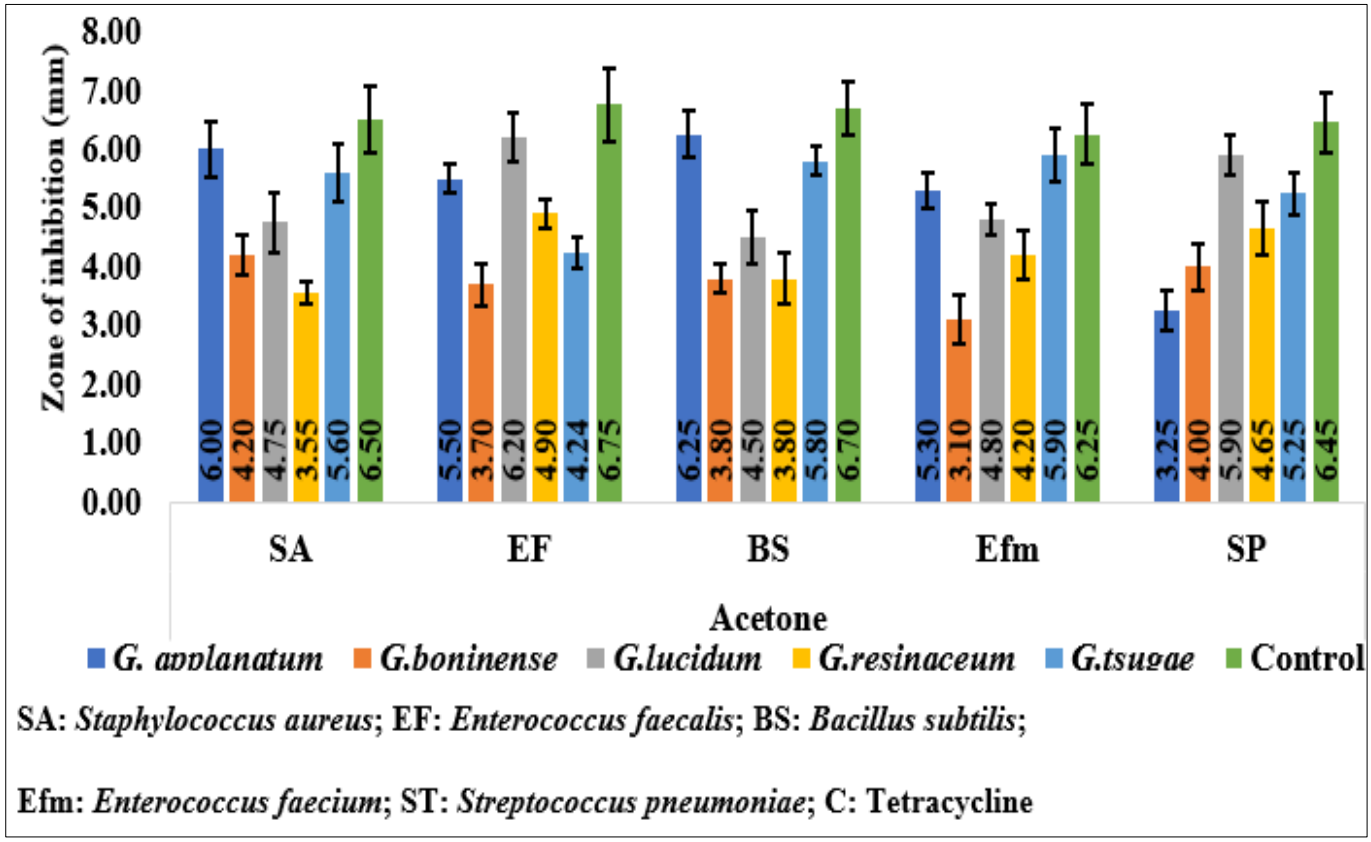
SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; BS: *Bacillus subtilis*;
Efm: *Enterococcus faecium*; ST: *Streptococcus pneumoniae*
011Mean values ± SD(n=3); $p \geq 0.05$ (NS), *$p < 0.1$ (S), **$p \leq 0.01$ (HS)



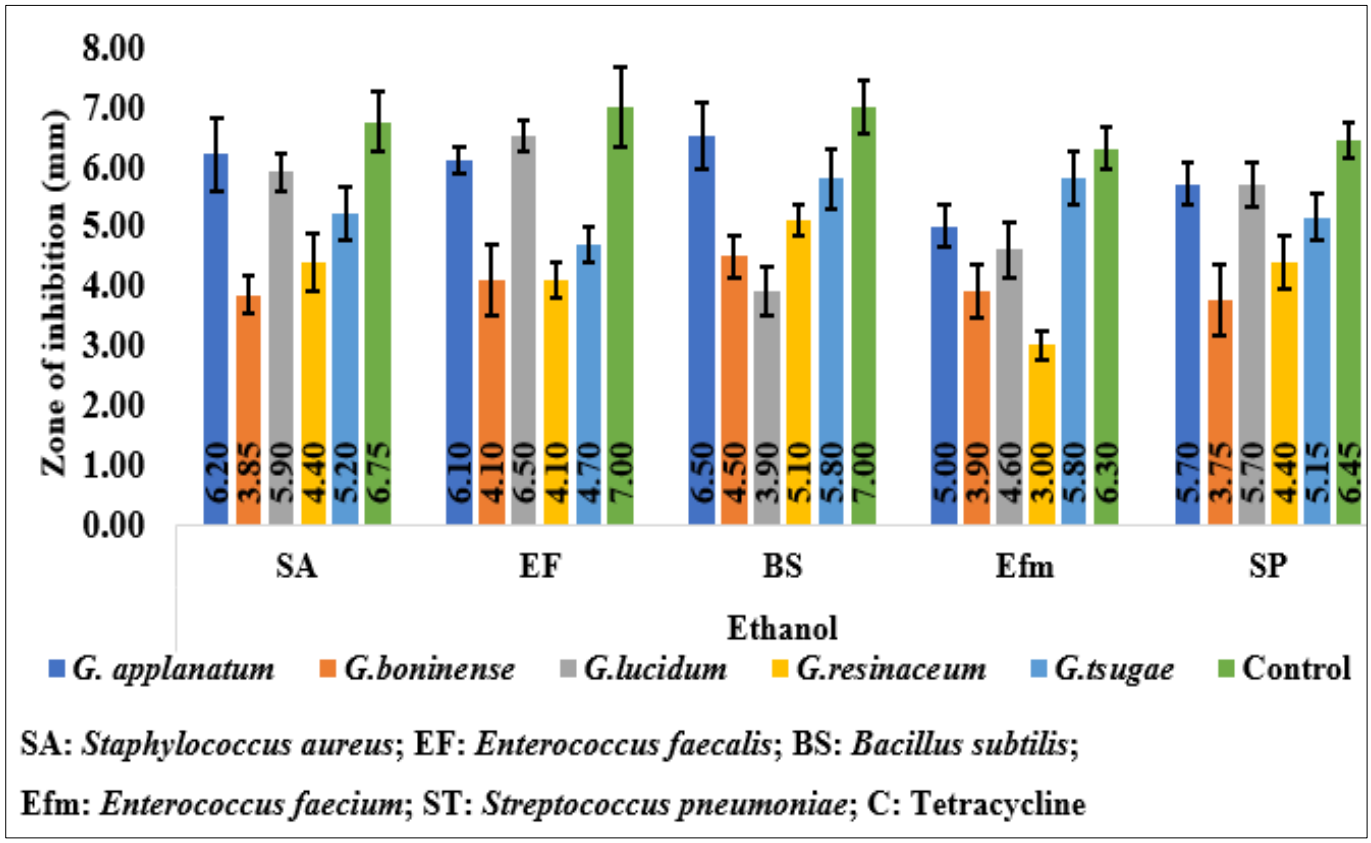
Graph 1: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram positive bacteria in petroleum ether



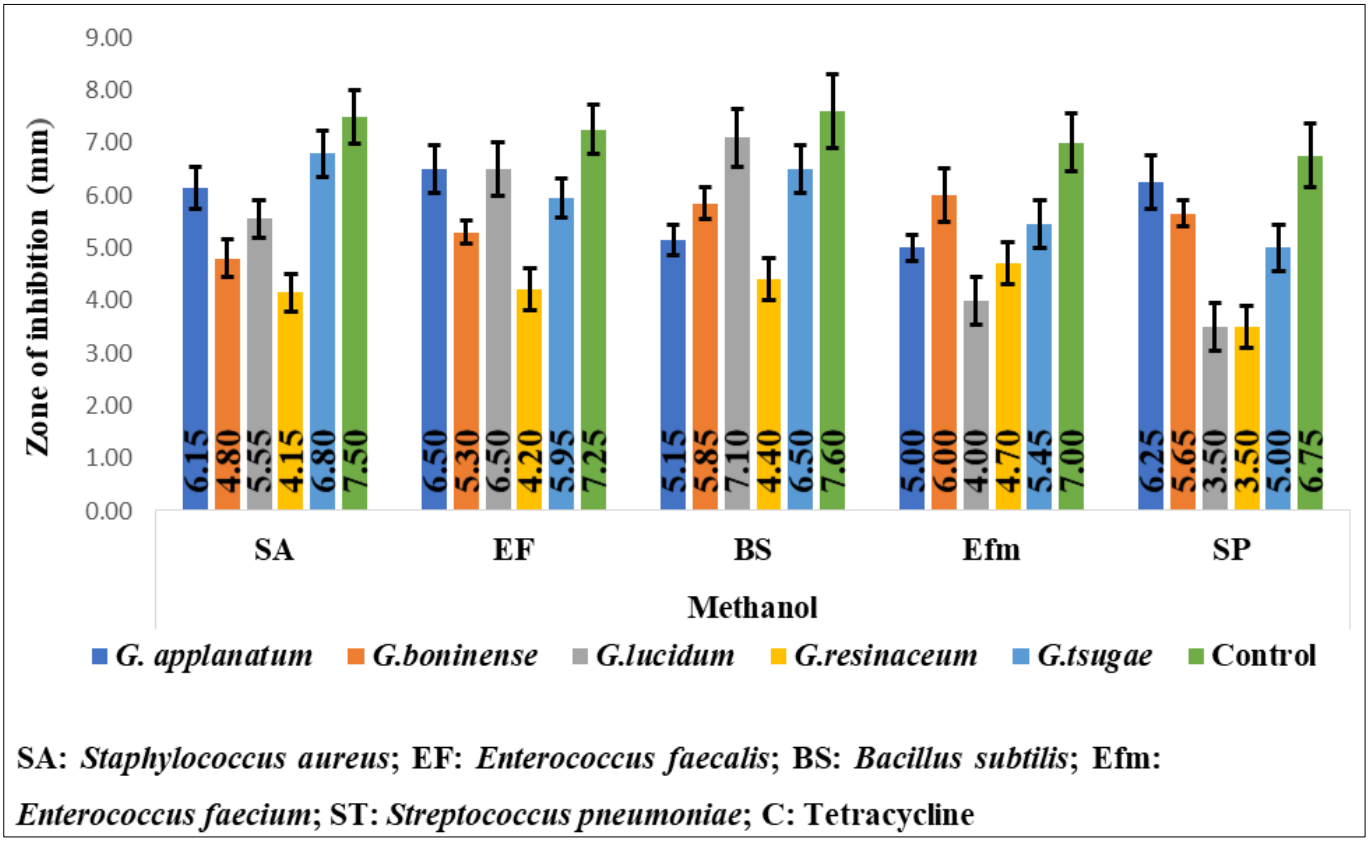
Graph 2: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram positive bacteria in chloroform



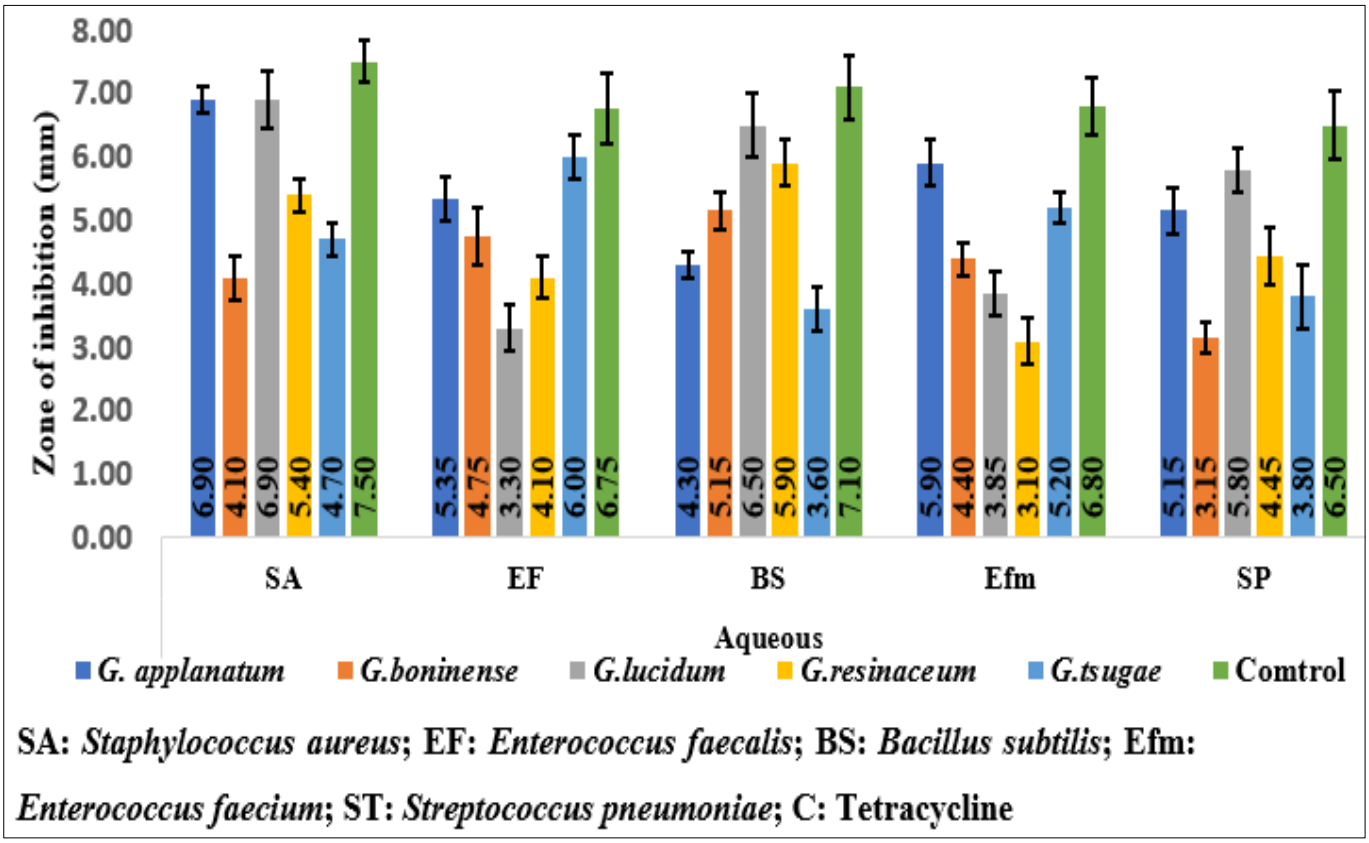
Graph 3: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram positive bacteria in acetone



Graph 4: Inhibitory zone diameter (mm) of Ganoderma species against Gram positive bacteria in ethanol

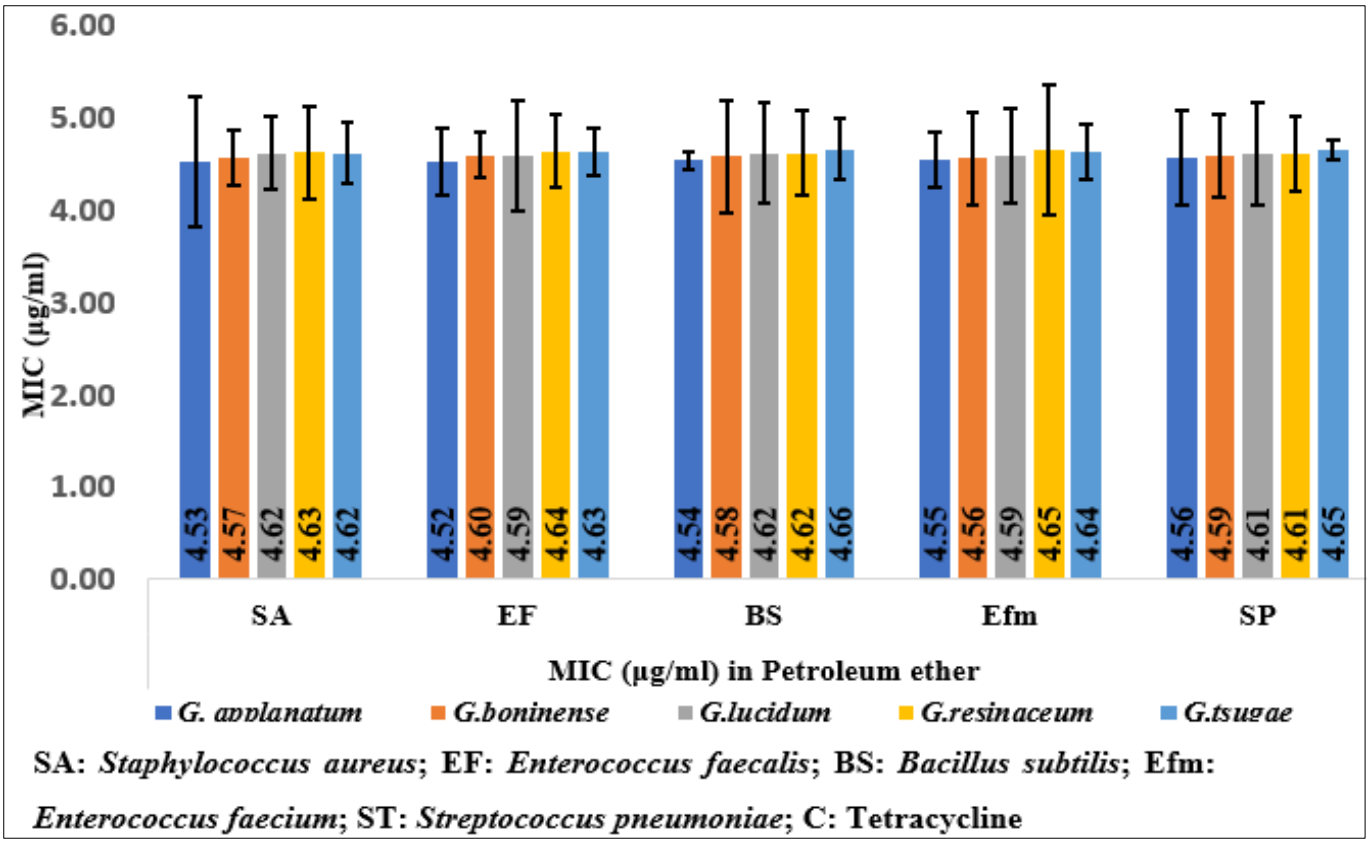


Graph 5: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram positive bacteria in methanol

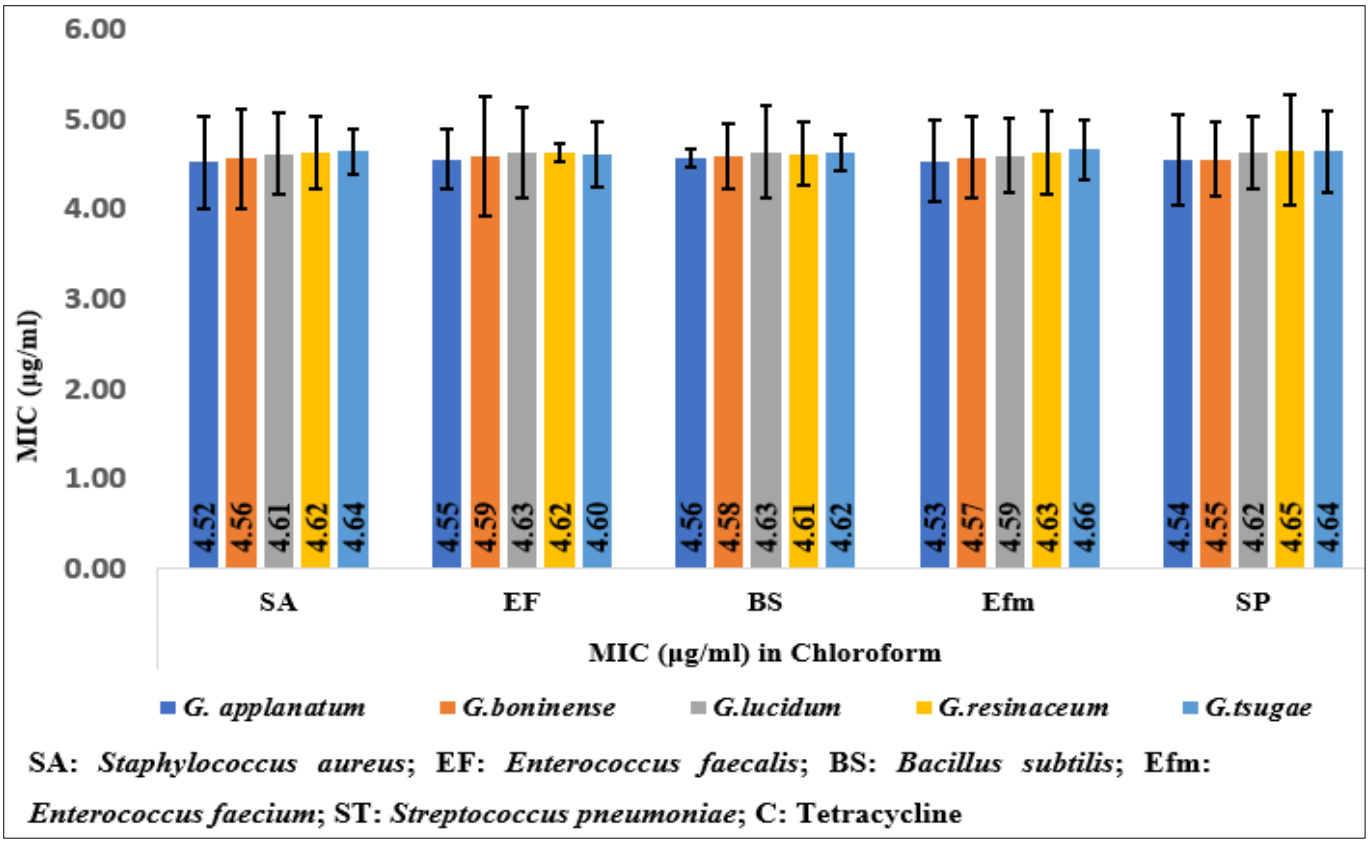


Graph 6: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram positive bacteria in aqueous

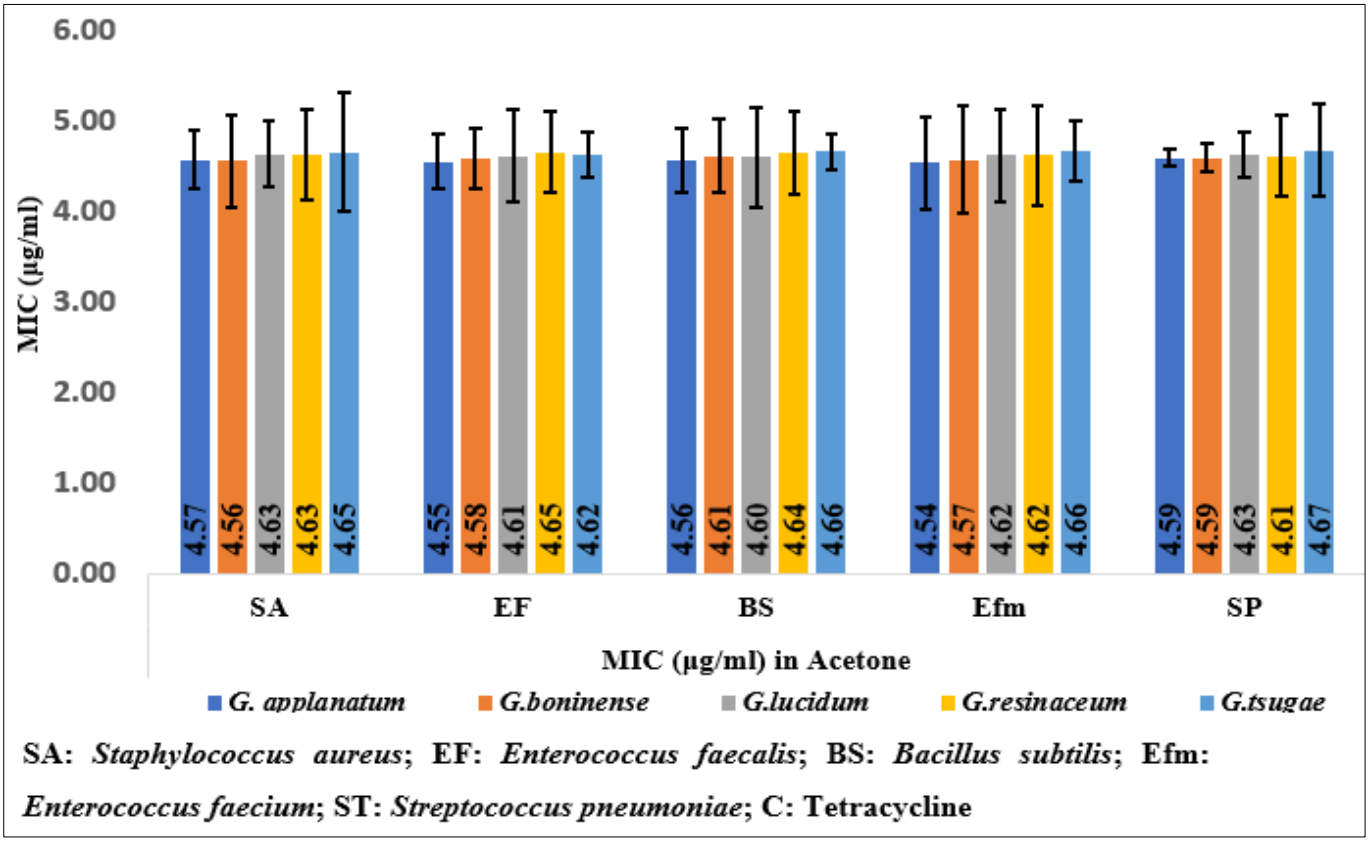
3.1.2 Minimum Inhibitory Concentration of *Ganoderma* species extract against Gram positive bacteria



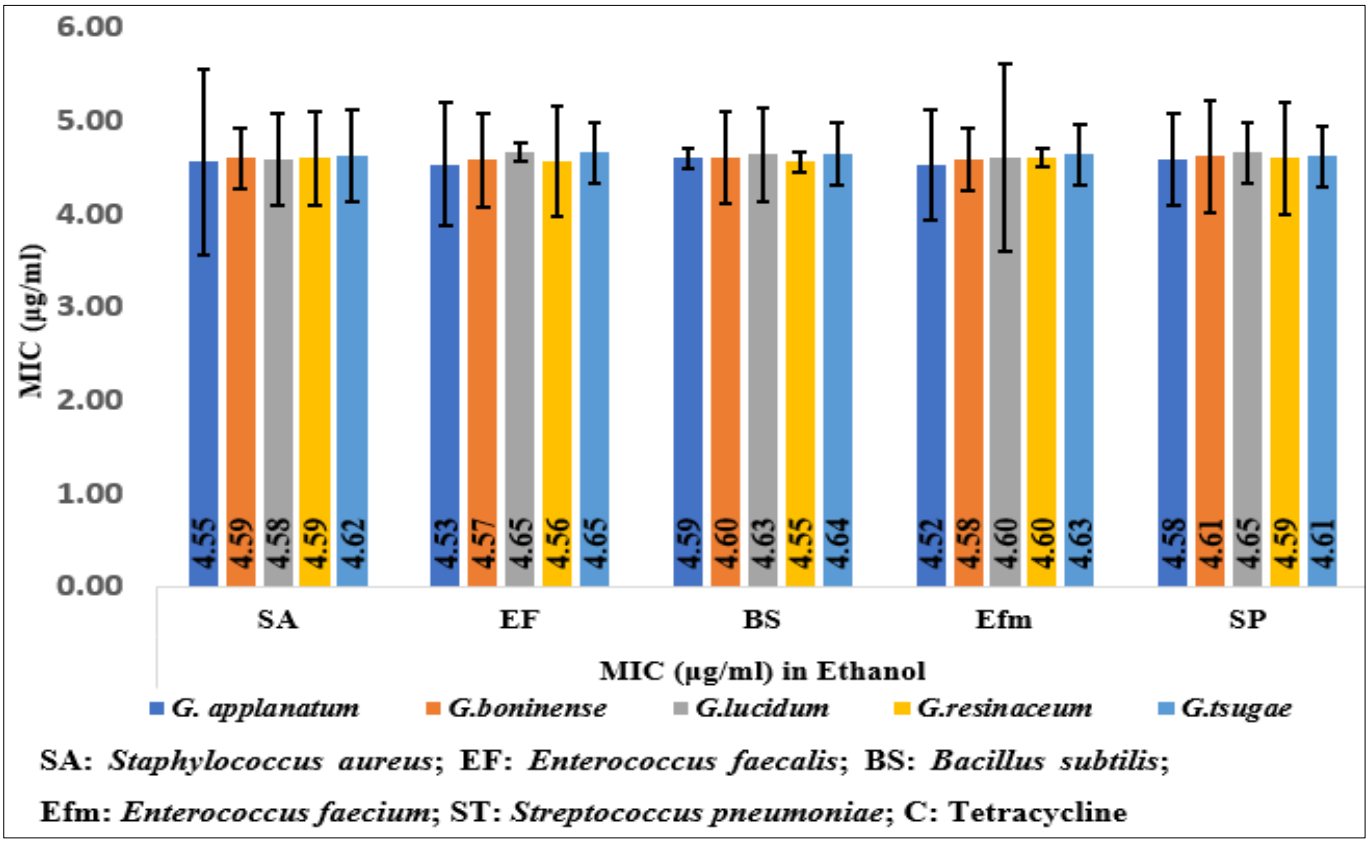
Graph 7: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in petroleum ether



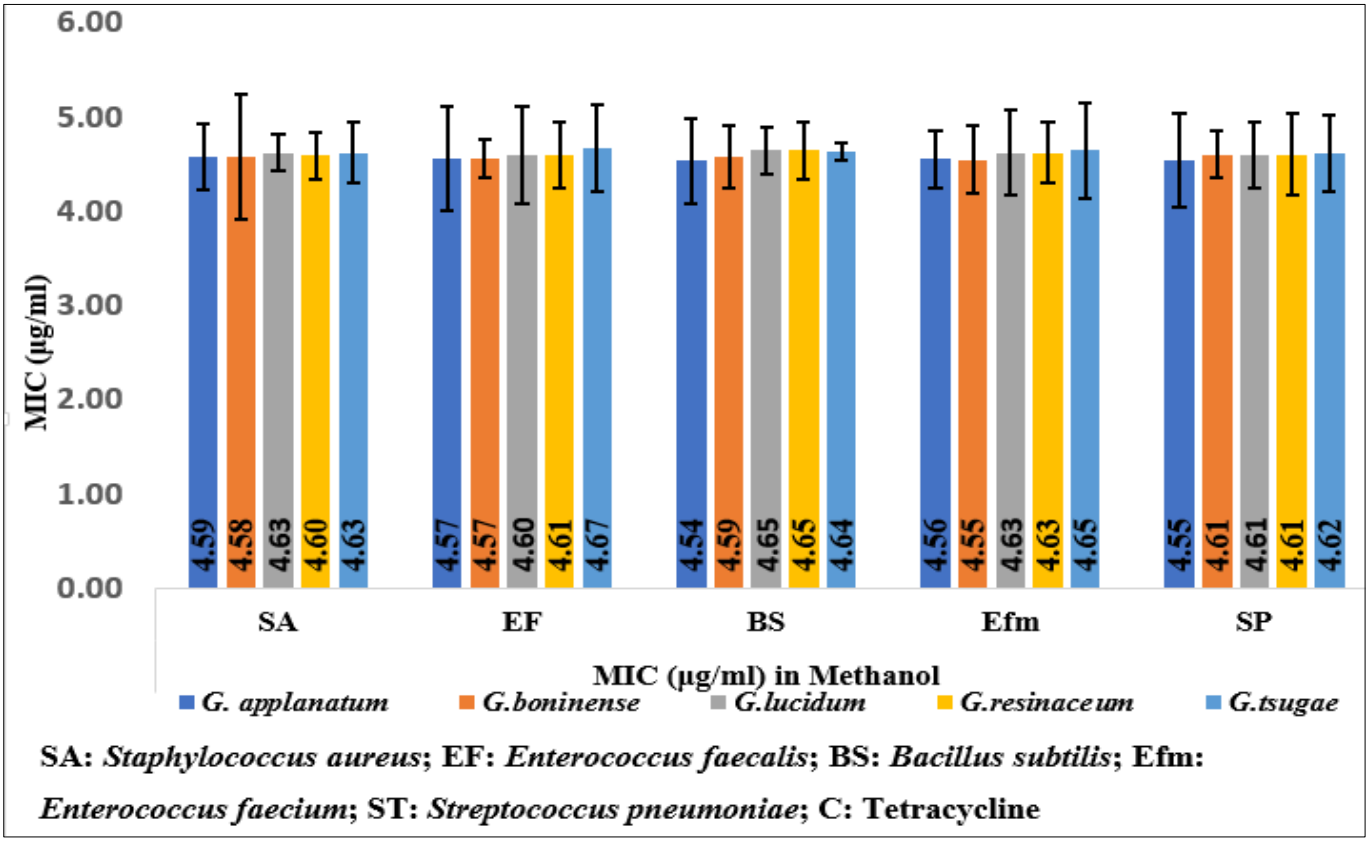
Graph 8: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in chloroform



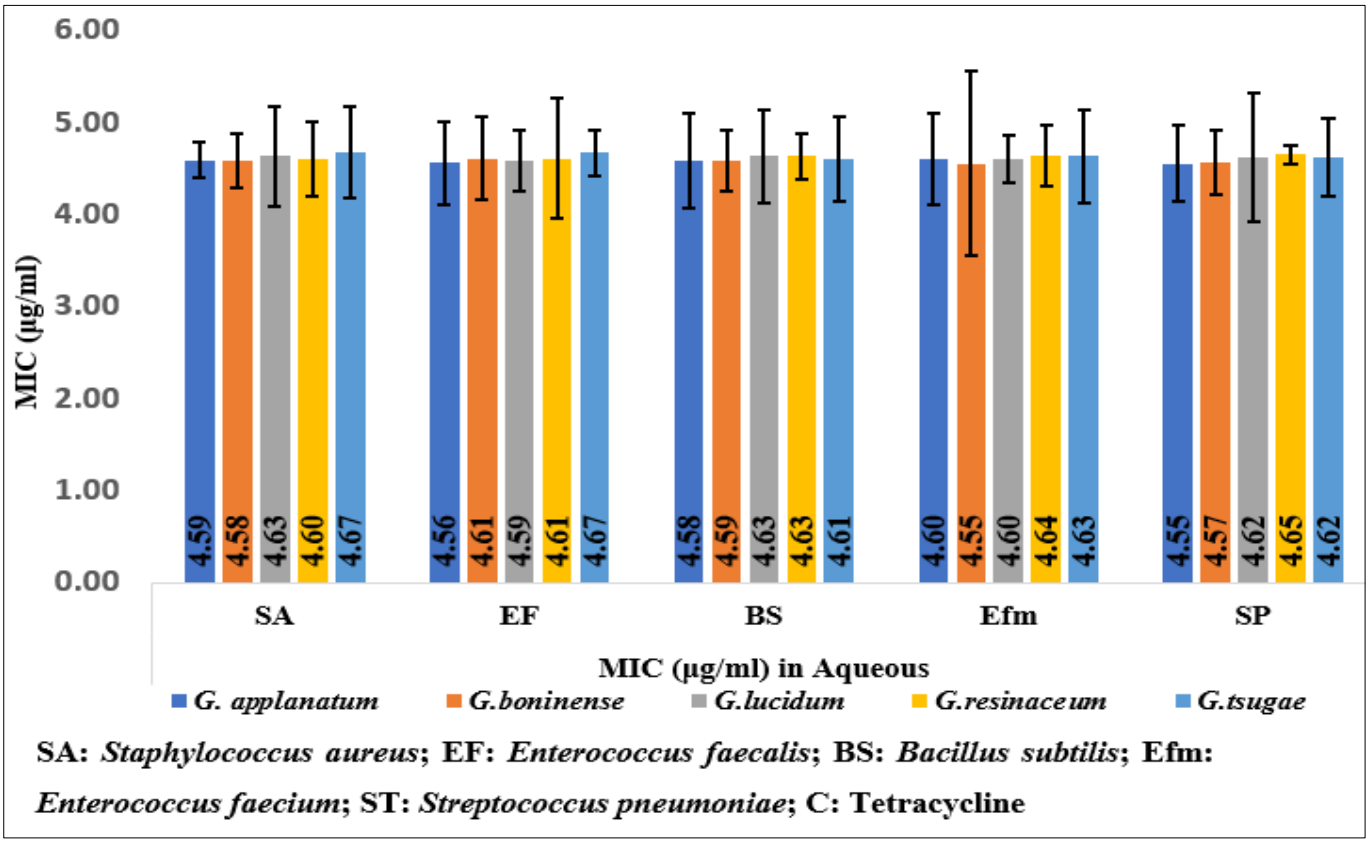
Graph 9: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in acetone



Graph 10: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in ethanol



Graph 11: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in methanol



Graph 12: MIC (µg/ml) of *Ganoderma* species against Gram positive bacteria in aqueous

4. Discussion

This present study encompasses the antibacterial potential of the various extract of fruiting bodies of all tested *Ganoderma* species as a result of its concomitant and/or predominant antibacterial, anti-inflammatory and regenerative properties which entirely depends on their bioactive compounds such as polysaccharides, triterpenes, peptides, proteins, steroids, tannins, alkaloids, phenolic compounds and flavonoids etc. Genus *Ganoderma* is traditionally used to heal wounds and ensure smooth tissue regeneration. The petroleum ether extract of *G. applanatum* exhibits maximum zone of inhibition against *B. subtilis* (5.90 mm). *G. tsugae* showed

moderate inhibition against *E. faecalis* and *S. pneumoniae* (5.75mm for both) and *G. resinaceum* showed minimum inhibition against *E. faecium* (4.40mm) (Table 2).

The study reveals that chloroform extract of *Ganoderma* species, specifically *G. lucidum* and *G. tsugae*, exhibited maximum inhibition against *S. aureus* (6mm), moderate inhibition occurred by *G. lucidum* and *G. tsugae* against *S. pneumoniae* (5.90mm for both). Akin to study by Chen *et al*., (2019) [39] also showed that *G. lucidum*'s chloroform extract is comparatively more effective against S. *aureus* with lowest against *E. faecalis*, while *G. boninense*'s extract counters lowest against *E. faeceum*. Study found lower polysaccharide content in *G. resinaceum*, but higher triterpenoid content in *G. lucidum*. In present study, chloroform extract of *G. boninense* also showed maximum inhibition against *S. aureus* (5.35mm) and minimum inhibition against *E. faecium* (3mm). This result is aligned with the previous research conducted by Abdullah *et al.* (2020) [17] and Sim *et al*., (2019) [32], where highest antibacterial activity of chloroform-extracted GBMA (*G. boninense* media agar) against *S. aureus* and *Streptococcus* was revealed. *G. boninense*'s secondary metabolites in its fruiting body contributed to its antibacterial properties. The antibacterial activity of chloroform extract of *G. lucidum* was also studied by Keypour *et al*., (2008) [15] and inhibited the development of *B. subtilis* and *S. aureus*. Similarly, minimum inhibition occurred by *G. boninense* and *G. lucidum* against *E. faecium and E. faecalis* (3mm) Chan and Chong's, (2022) [6] study confirmed the antibacterial properties of *G. boninense* fruiting bodies, revealing strong susceptibility to methicillin-resistant *S. aureus* (MRSA) due to irreversible damage to cell membrane, causing cellular lysis and death (Table 3).

The maximum inhibitory zone in acetone extract of *G. applanatum* against *S. aureus* was found to be 6mm, while, moderate inhibition occurred by *G. applanatum*, *G. lucidum* and *G. tsugae* against *S. aureus*, *S. pneumoniae* and *S. pneumoniae* as 6mm and 5.90mm respectively. Similarly, minimum inhibition occurred by *G. boninense* against *E. faecium* (3.10mm) (Table 4). Likewise, ethanol and acetone extract of *G. applanatum* and *G. lucidum* demonstrated the highest antibacterial activity against *B. subtilis* and *E. faecalis*. Hu *et al*., 2023 [22] and Quereshi *et al*., 2010 [16] also demonstrated that ethanol and acetone extract of *G. lucidum* inhibits the same. Moderate inhibition is caused by *G. applanatum* against *S. aureus* and *E. faecalis* (6.20mm). Similarly, lowest inhibition occurred by *G. resinaceum* against *E. faecium* (3mm) (Table 5).

Maximum inhibition in methanol, occurred by *G. lucidum* against *B. subtilis* (7.10mm), moderate inhibition by *G. applanatum*, *G. lucidum* and *G. tsugae* against *E. faecalis*, *S. aureus* and *B. subtilis* as 6.50mm, 6.80mm and 6.50mm respectively. Similarly, lowest inhibition caused by *G. lucidum* and *G. resinaceum* against *S. pneumoniae* (3.50mm) The methanolic extract of fruiting bodies of *G. lucidum* has maximum antibacterial activity against *B. subtilis*, followed by *G. tsugae*, *G. applanatum*, *G. resinaceum* and *G. boninense*. Previously, Sande *et al*., (2019) [37] also reported similar work with hexane, ethyl acetate and methanol extracts against Methicillin-Resistant *S. aureus* (MRSA) and *Streptococcus* Spp. revealed the significant antibacterial activity against both. The antibacterial activity of methanol extract of *G. tsugae* exhibited the highest zone of inhibition against *S. aureus* and *B. subtilis* because triterpenoids present in *G. tsugae* extract (Espinosa-Garcia *et al*., 2021) [30] (Table 6). In aqueous phase, maximum inhibition was shown for *G. applanatum* and *G. lucidum* against *S. aureus* (6.90mm), moderate inhibition occurred by *G. applanatum*, *G. lucidum* and *G. tsugae* against *E. faeceum*, *S. aureus* and *E. faecalis* as 5.90mm and 6mm respectively. Aqueous extraction of *G. applanatum* and *G. lucidum* showed the highest antibacterial activity against *S. aureus* as akin to Jogaiah *et al*., (2019) [31]. The probable cause may be as distilled water is however highly polar thus more polar phytochemicals compounds can be extracted on it (Nawaz *et al*., 2020) [38]. (Table 7).

The petroleum ether extract of *G. tsugae* had the highest minimum inhibitory concentration (MIC) against *B. subtilis* (4.66 µg/ml). The extracts from *G. resinacium* and *G. tsugae* showed a moderate MIC of 4.65 µg/ml for both *E. faecium* and *S. pneumoniae*. While, *G. tsugae* extract had the lowest MIC against *E. faecalis* (4.52 µg/ml) (Table 8).

The Chloroform extract of *G. tsugae* had the highest minimum inhibitory concentration (MIC) against *E. faecium* (4.66 µg/ml). While, *G. resinacium* extract demonstrated the moderate MIC for *S. pneumoniae* (4.65 µg/ml) and *G. applanatum* extract showed the lowest MIC for *S. aureus* (4.52 µg/ml) (Table 9). The acetone extract of *G. tsugae* had the highest minimum inhibitory concentration (MIC) for *S. pneumoniae* (4.67 µg/ml) and the moderate MIC against *B. subtilis* and *E. faecium* (4.66 µg/ml). While, *G. applanatum* extract exhibited the lowest MIC for *E. faecium* (4.54 µg/ml) (Table 10). The highest minimum inhibitory concentration (MIC) showed by ethanol extract of *G. lucidum* and *G. tsugae* for *E. faecalis* and *S. pneumoniae* (4.65 µg/ml) and demonstrated the moderate MIC by *G. tsugae* for *B. subtilis* and *E. faecium* (4.64 µg/ml) (Tehranian *et al*., 2023) [35].

The highest minimum inhibitory concentration (MIC) showed by methanol extract of *G. tsugae* against *E. faecalis* (4.67 µg/ml) and demonstrated the moderate MIC by *G. resinaceum* and G. *tsugae* against *B. subtilis* and *E. faecium* (4.65 µg/ml) respectively. While, *G. applanatum* extract exhibited the lowest MIC against *B. subtilis* (4.54 µg/ml) (Table 11). The highest minimum inhibitory concentration (MIC) showed by aqueous extract of *G. tsugae* against *S. aureus* and *E. faecalis* (4.67 µg/ml) and demonstrated the moderate MIC by *G. resinaceum* against *S. pneumoniae* (4.65 µg/ml) respectively. While, *G. applanatum* extract exhibited the lowest MIC against *S. pneumoniae* (4.54 µg/ml) (Table 12).

Comparing the fruiting body extract of *Ganoderma* species to the inhibitory effect obtained by other researchers, the results showed that the methanol extract of all tested *Ganoderma* species possesses more potential as an antibacterial agent against all tested Gram-positive bacteria found in Diabetic Foot Ulcer. The extract was tested using different petroleum ether, chloroform, acetone, ethanol, methanol and aqueous extracts. From this present investigation it was proved that the methanol extract effectively controls all five bacterial strains and also demonstrated the possibility of using fruiting body extracts from *G. lucidum*, *G. tsugae*, and *G. applanatum* to treat a variety of pathogenic conditions including diabetes.

5. Conclusion

The antimicrobial studies involving *Ganoderma* species and bacterial isolates associated with Diabetic Foot Ulcers (DFUs) reveal promising results. This study has demonstrated that extracts of all tested *Ganoderma* species possess a good antibacterial property against all tested Gram-positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Enterococcus faecium*, *Streptococcus pneumoniae*, frequently implicated in diabetic foot infections. Among all studied species *G. applanatum* revealed better

results towards Gram positive DFU bacterial isolates, while *G. boninense* was less effective. In overall, summation, *Ganoderma*-based treatments to combat infections in DFUs, potentially offer an alternative or adjunct to conventional antibiotics with its role, safety and efficacy need further exploration through well-designed clinical studies before definitive conclusions can be drawn regarding its use in diabetic care.

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