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Research Article

Antimicrobial Studies of some *Ganoderma* Species against some Gram-Negative Bacterial Isolates of Diabetic Foot Ulcer

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Abstract

Despite the diversity within the Ganodermataceae family for its therapeutic applications, this study primarily focuses on five test species of *Ganoderma* i.e. *G. applanatum, G. boninense, G. lucidum, G. resinaceum* and *G. tsugae*. These species are known to have various biologically active macromolecules including polysaccharides, triterpenoids, steroids, phenolic compounds, lipids and alkaloids which are isolated from the fruiting bodies, mycelia and spores. These molecules have various health benefits, including anti-tumoral, anti-inflammatory, anti-allergic, anti-viral, anti-bacterial, antidiabetic and antioxidant properties. In the present study, inhibitory and antibacterial properties of several *Ganoderma* extracts in petroleum ether, chloroform, acetone, ethanol, methanol and aqueous against, were tested against Gram negative bacterial isolates of DFU. like *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium* and *Proteus vulgaris* by agar well diffusion method. For every examined bacterium, every extract showed a different level of inhibition. Comparative study reveals that *G. lucidum* and *G. tsugae* had the highest performance against Gram negative test bacteria revealing its application for creating innovative drug formulations for people with diabetes.

Keywords: *G. applanatum, G. boninense, G. lucidum, G. resinaceum, G. tsugae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Proteus vulgaris.*

Introduction

Ganoderma , commonly used to describe a class of medicinal mushrooms valued for their long history in traditional medicine and their rich array of bioactive compounds (Martinez-Montemayor, *et al.*, 2019; Bhambri, *et al.*, 2022; He, *et al.*, 2022) including polysaccharides (Zhang, *et al.*, 2019), triterpenes (such as ganoderic acids) (Yangchum, *et al.*, 2022; Ahmad, *et al.*, 2021; Kolniak-Ostek, *et al.*, 2022), peptides, alkaloids, flavonoids, lipids, steroids, glycosides, saponins, anthraquinone, anthocyanins, tannins and phenolic compounds. The benefits of *Ganoderma* include immunity stimulator, prevention of viral infections, diabetes, anticancer properties (Cao, *et al.*, 2022; Wu, *et al.*, 2022; Algehani, *et al.*, 2021), pneumatoprotective including asthma and bronchitis (Wang, *et al.*, 2020), high blood pressure and high cholesterol, hepatoprotective (Zhang, *et al.*, 2022), kidney disease, altitude sickness, chronic fatigue syndrome (CFS), trouble sleeping (insomnia), stomach ulcers, poisoning, herpes pain, reducing stress and preventing fatigue. These mushrooms have long been greatly valued for their alleged health-promoting qualities (Wu, *et al.*, 2019) in many cultures, especially traditional Chinese medicine (El-Sheikha, *et al.*, 2022) and other Asian healing practices. Because of these mushrooms' possible therapeutic benefits, they have also drawn attention in the treatment of diabetes. Even though there has been tremendous progress in the treatment of

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diabetes mellitus (DM), the condition still poses a serious threat to mankind. Diabetes may cause a number of problems, but developing diabetic foot ulcers is one of the worst. An open sore or wound that frequently develops on the feet of people with diabetes is known as a diabetic foot ulcer. If DFUs are not treated, they can cause serious side effects like infection and gangrene, which in severe situations can lead to amputation.

The potential of natural materials found in *Ganoderma* species as in *G. applanatum* (Peng, *et al.,* 2019; Shi, *et al.,* 2021), *G. boninense* (Ma, *et al.,* 2014), *G. lucidum* (Rashid, *et al.,* 2021), *G. resinaceum* (Al-Fatimi, *et al.,* 2005; Chen, *et al.,* 2017) and *G. tsugae* to treat bacterial infections in diabetic foot ulcers is investigated in current study (Angulo-Sanchez, *et al.,* 2022). Their ability to combat Gram-negative bacteria from diabetic foot ulcers, however, is dependent on a number of variables. These consist of the type of bacteria, the amount and make-up of the active ingredients, the techniques used for extraction and the synergistic effects.

2. Materials and Methods

2.1 Procurement of Fungal Material

The completely developed fruiting bodies of five distinct species of Genus *Ganoderma* - *G. applanatum, G. boninense, G. lucidum, G. resinaceum,* and *G. tsugae*, were acquired from the ICAR- Directorate of Mushroom Research, Solan (HP). The specimens' accession numbers were created in accordance with the International Rules of Botanical Nomenclature (IRBN) and deposited in the Department of Biotechnology at B. N. University, Udaipur (Rajasthan) (Table: 2.1).

Table 2.1: 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 Fungal Samples: Preparation and Storage

The fruiting bodies were collected, cleaned and sun dried to provide test samples for assessing each *Ganoderma* species' antibacterial activity. They were pounded into a fine powder and stored in an airtight jar at 4°C to be used for further practises. When necessary, remove the sample from refrigerator and store it at room temperature before the antimicrobial activity assay.

2.3 Extract preparation

The extracts were made using finely ground dried *Ganoderma* species fruiting bodies. The solvents that were utilised to extract the pharmacologically active chemicals from the mushroom were petroleum ether, chloroform, acetone, ethanol, methanol and water. A soxhlet device was utilised to extract 10g of powder from 150 ml of solvent. After being processed, the leftover residues were dissolved in dimethyl sulfoxide (DMSO) to create stock solutions for the antibacterial assay. These solutions were then sealed in airtight containers and kept at 4°C.

2.4 Procurement of bacteria

Clinical bacterial isolates were obtained in freeze-dried form from the National Centre for Cell Science (NCCS), Pune. Until they were needed, all of these bacterial isolates were kept at -20°C in 10% glycerol. Muller-Hinton agar was used to cultivate the bacteria for 24-48 hours. After that, they were standardised using sterile saline to a turbidity of 0.5 McFarland scale, or around 1-2×10^8 CFU/ml (CLSI, 2009) and then they were kept at 4°C. The agar well diffusion method was employed to determine the antibacterial activity.

2.5 Culture media and inoculum preparation

Nutrient agar and Muller Hinton agar media were made for the antibacterial test, while Trypticase Soy Yeast Extract (TSYE) medium

and nutrient broth medium were prepared for the revival of bacteria. Additionally, the IMViC test was used to identify the purity of bacterial cultures. The antimicrobial test was conducted using the Agar well diffusion technique.

2.6 Determination of Minimum Inhibitory Concentration (MIC)

In 96-well micro titre polystyrene plates, a broth micro-dilution bioassay was used to assess the minimum inhibitory concentration (MIC) of a particular extract. Based on the modified procedure of Yakob, *et al.* (2012) and included dilutions in steps, adding a bacterial inoculum and addition of 100 µl of extracts to each well of the plates. For the next 24 to 48 hours, the plates were incubated at 37°C. There was evidence of both bacterial proliferation and suppression. Each extract's minimum inhibitory concentration (MIC) was determined by measuring the lowest concentration at which the bacteria could grow.

3. Results

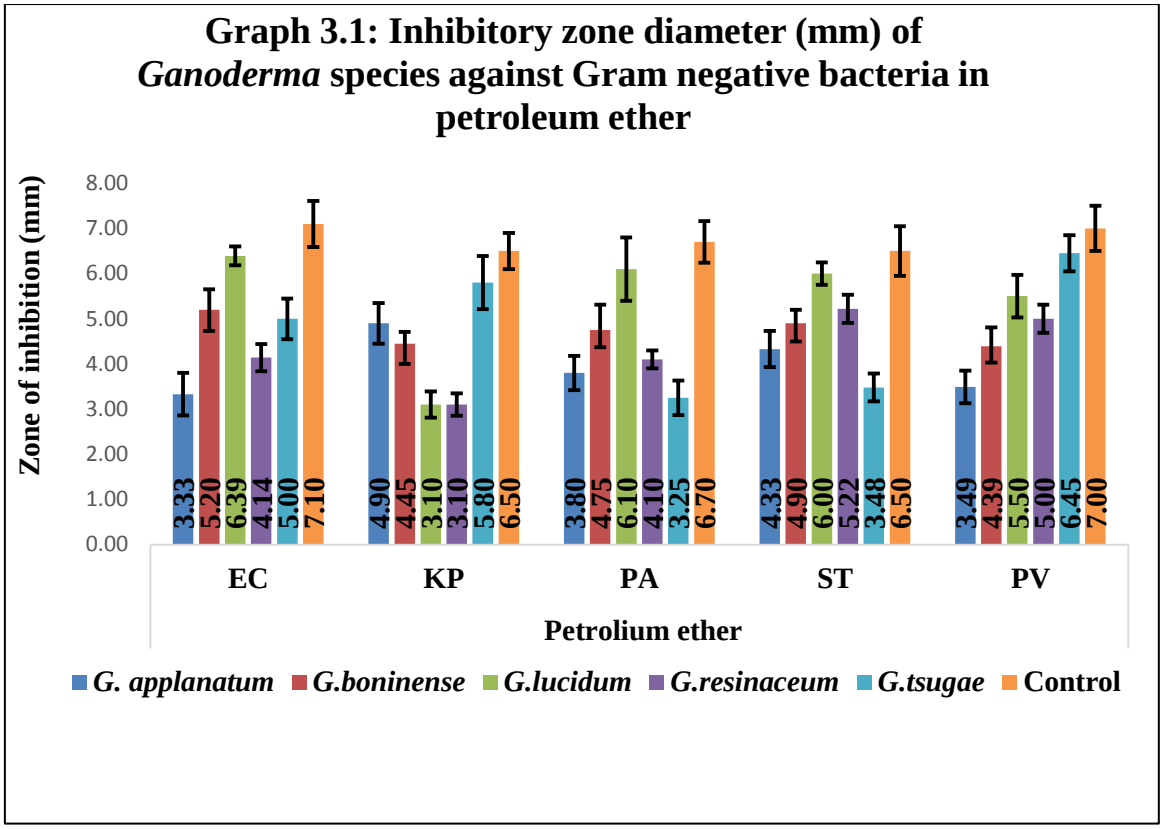
3.1 Evaluation of antimicrobial efficacy of *Ganoderma* species

3.1.1 Zone of inhibition (ZOI) of Gram-negative bacteria

Table 3.1: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram negative bacteria in petroleum ether

Test species	Zone of inhibition (mm) in Petroleum ether				
	EC	KP	PA	ST	PV
G. applanatum	3.33±0.47**	4.90±0.45**	3.80±0.38**	4.33±0.40**	3.49±0.36**
G. boninense	5.20±0.45**	4.45±0.26**	4.75±0.56*	4.90±0.30**	4.39±0.42**
G. lucidum	6.39±0.21**	3.10±0.29**	6.10±0.70*	6.00±0.25**	5.50±0.47**
G. resinaceum	4.14±0.30**	3.10±0.25**	4.10±0.20**	5.22±0.31**	5.00±0.31**
G. tsugae	5.00±0.45**	5.80±0.59*	3.25±0.38**	3.48±0.31**	6.45±0.40**
Control	7.10±0.51*	6.50±0.40 **	6.70±0.46**	6.50±0.55*	7.00±0.50**

EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*;
ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*; Control: Tetracycline
Mean values ± SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)



EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris; C: Tetracycline*

Table 3.2: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram negative bacteria in chloroform

Test species	Zone of inhibition (mm) in Chloroform				
	EC	KP	PA	ST	PV
G. applanatum	6.50±0.30**	3.10±0.31**	3.90±0.26**	4.25±0.26**	6.00±0.45**
G. boninense	3.80±0.56*	4.60±0.26**	5.30±0.45**	3.60±0.31**	3.75±0.26**
G. lucidum	5.60±0.47**	6.30±0.35**	6.00±0.50**	5.90±0.46**	4.60±0.61*
G. resinaceum	4.38±0.26**	3.80±0.32**	4.75±0.21**	2.60±0.35**	3.00±0.35**
G. tsugae	6.50±0.42**	5.45±0.26**	6.60±0.50**	5.90±0.40**	5.20±0.49**
Control	7.10±0.45**	6.90±0.30**	7.00±0.61*	6.50±0.50**	6.75±0.51*

EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*; Control: Tetracycline
Mean values ± SD (n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)

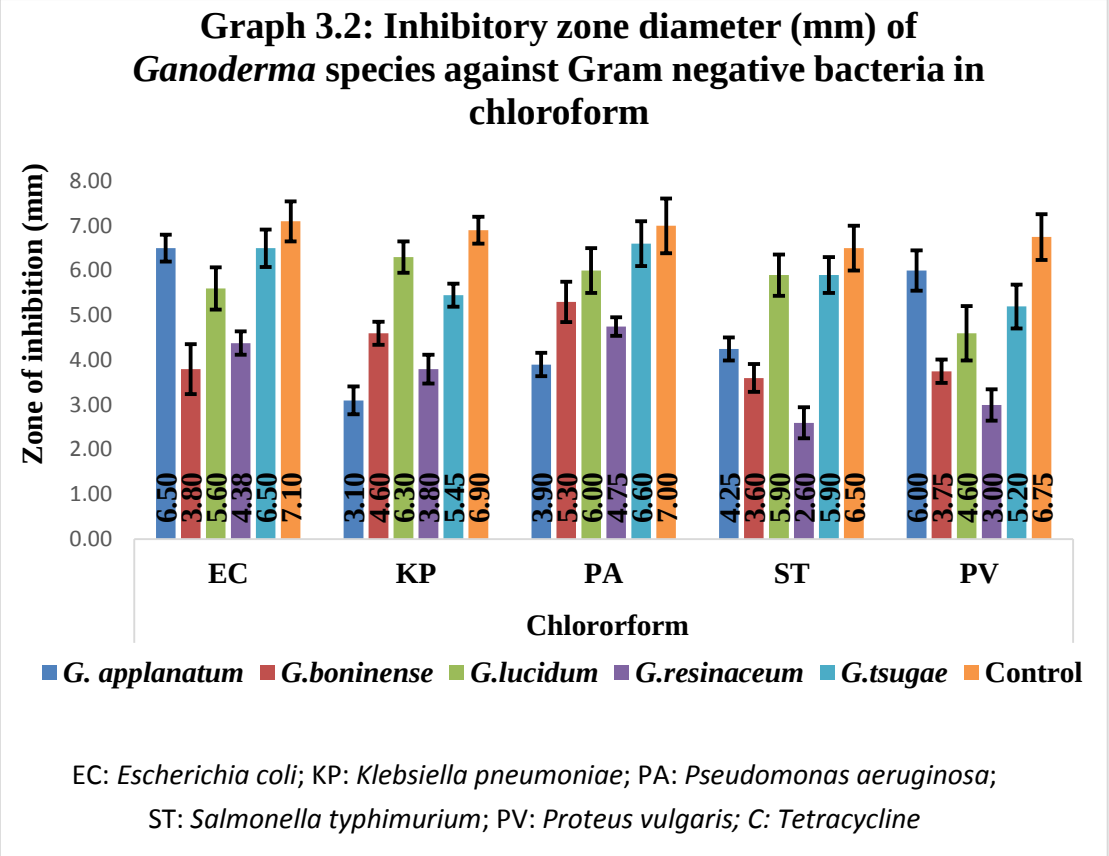


Table 3.3: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram negative bacteria in acetone

Test species	Zone of inhibition (mm) in Acetone				
	EC	KP	PA	ST	PV
G. applanatum	3.90±0.31**	6.50±0.26**	3.65±0.26**	5.65±0.35**	6.45±0.46**
G. boninense	4.85±0.31**	3.60±0.40**	5.00±0.55*	4.90±0.40**	3.70±0.32**
G. lucidum	5.65±0.40**	5.85±0.53*	5.75±0.31**	6.45±0.35**	6.45±0.30**
G. resinaceum	3.90±0.36**	4.25±0.40**	4.35±0.55*	4.10±0.35**	4.45±0.25**
G. tsugae	6.30±0.42**	4.90±0.31**	6.25±0.55*	3.25±0.46**	5.15±0.40**
Control	7.00±0.66*	7.10±0.50**	6.80±0.45**	6.75±0.55*	6.90±0.36**
EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*; Control: Tetracycline Mean values ± SD (n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)					

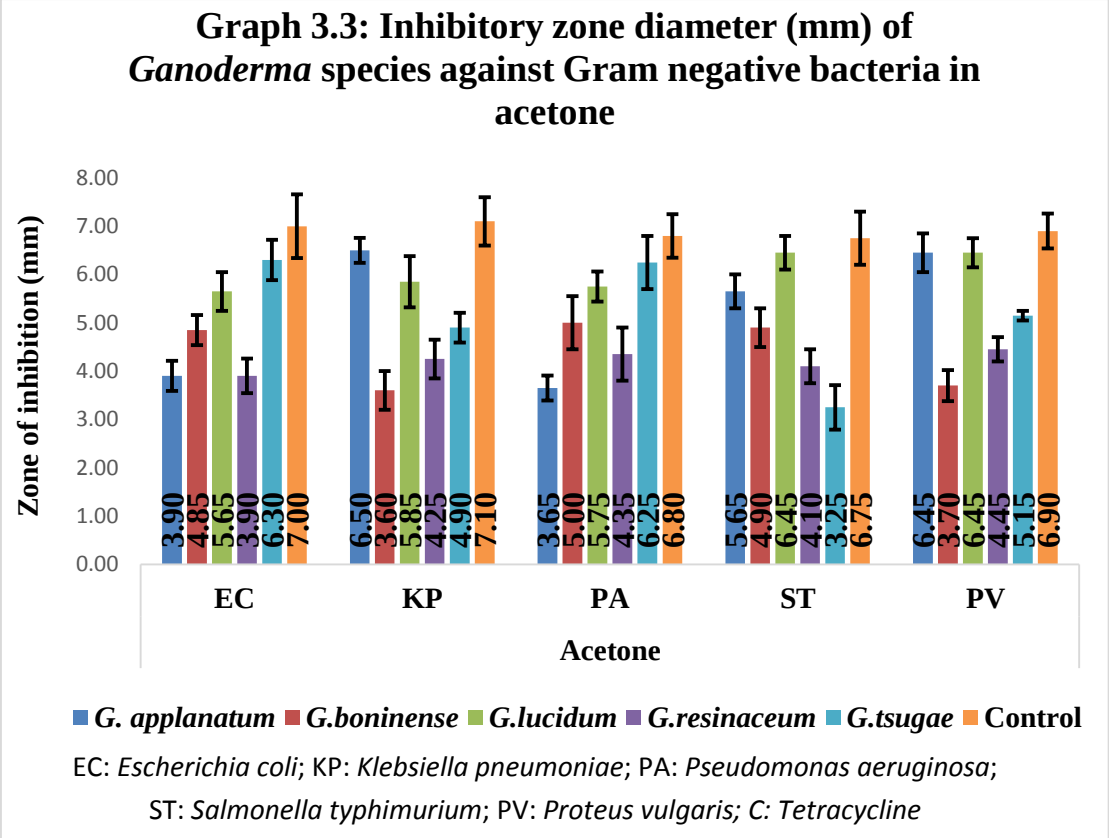
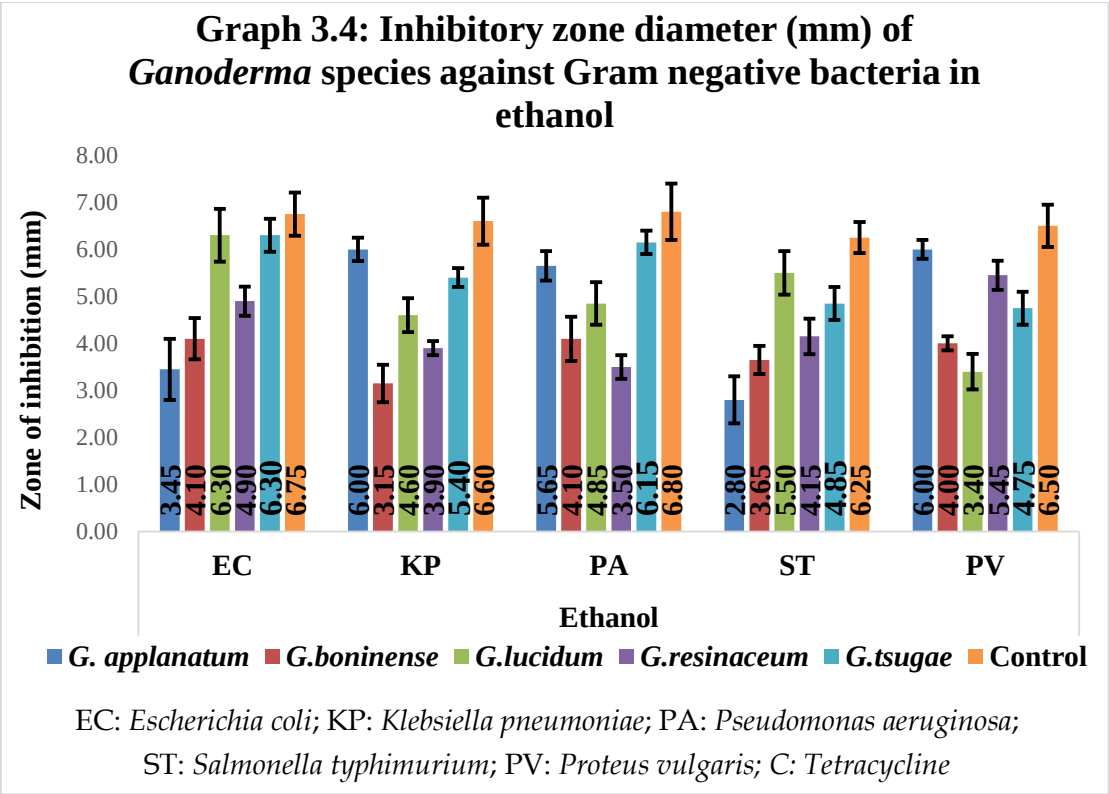


Table 3.4: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram negative bacteria in ethanol

Test species	Zone of inhibition (mm) in Ethanol				
	EC	KP	PA	ST	PV
G. applanatum	3.45±0.65*	6.00±0.25**	5.65±0.31**	2.80±0.50**	6.00±0.20**
G. boninense	4.10±0.44**	3.15±0.40**	4.10±0.47**	3.65±0.30**	4.00±0.15**
G. lucidum	6.30±0.56*	4.60±0.36**	4.85±0.45**	5.50±0.46**	3.40±0.38**
G. resinaceum	4.90±0.31**	3.90±0.15**	3.50±0.25**	4.15±0.38**	5.45±0.31**
G. tsugae	6.30±0.35**	5.40±0.20**	6.15±0.25**	4.85±0.35**	4.75±0.35**
Control	6.75±0.46**	6.60±0.50**	6.80±0.60*	6.25±0.33**	6.50±0.45**
EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*; Control: Tetracycline Mean values ± SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)					



EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris; C: Tetracycline*

Table 3.5: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram negative bacteria in methanol

Test species	Zone of inhibition (mm) in Methanol				
	EC	KP	PA	ST	PV
G. applanatum	5.80±0.35**	6.50±0.26**	6.40±0.26**	3.90±0.46**	3.50±0.25**
G. boninense	5.00±0.40**	3.85±0.31**	5.75±0.25**	3.90±0.31**	4.25±0.40**
G. lucidum	6.35±0.15**	5.10±0.46**	7.15±0.47**	6.15±0.40**	5.90±0.36**
G. resinaceum	4.30±0.44**	4.40±0.32**	4.65±0.25**	5.25±0.21**	5.15±0.15**
G. tsugae	7.00±0.32**	5.70±0.35**	7.15±0.20**	6.80±0.31**	6.50±0.35**
Control	7.60±0.50**	7.00±0.45**	7.60±0.51*	7.25±0.60*	6.90±0.40**
EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*; Control: Tetracycline Mean values ± SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)					

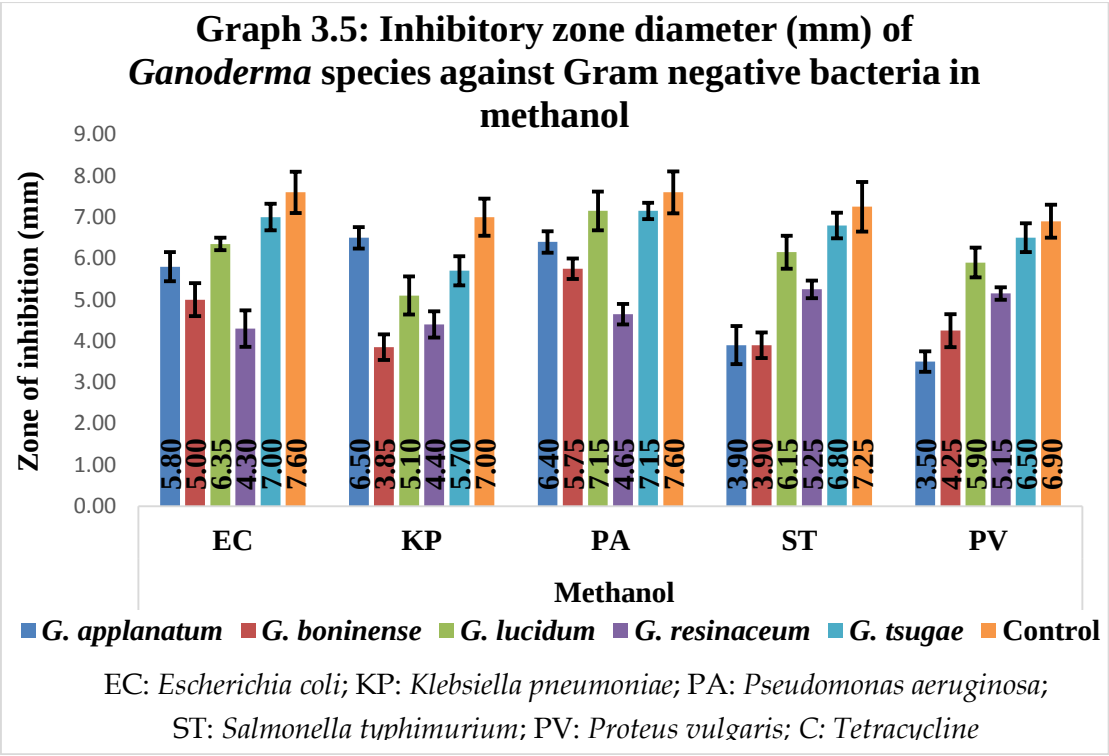
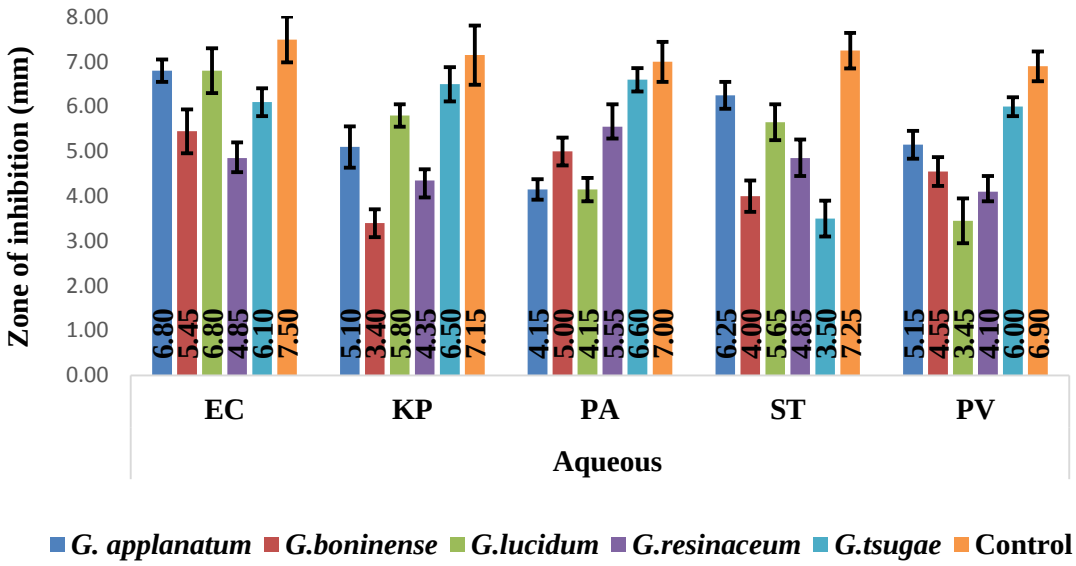


Table 3.6: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram negative bacteria in aqueous

Test species	Zone of inhibition (mm) in Aqueous				
	EC	KP	PA	ST	PV
G. applanatum	6.80 ±0.25**	5.10 ±0.46**	4.15±0.23**	6.25±0.30**	5.15 ±0.31**
G. boninense	5.45 ±0.49**	3.40±0.31**	5.00±0.31**	4.00± 0.35**	4.55 ±0.32**
G. lucidum	6.80 ±0.50**	5.80±0.25**	4.15± 0.26**	5.65±0.40**	3.45 ±0.50**
G. resinaceum	4.85±0.35**	4.35 ±0.25**	5.55±0.50**	4.85±0.42**	4.10 ±0.35**
G. tsugae	6.10 ±0.31**	6.50±0.38**	6.60±0.26**	3.50±0.40**	6.00±0.21**
Control	7.50±0.51*	7.15±0.66*	7.00±0.45**	7.25±0.40**	6.90±0.33**

EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*;
ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*; Control: Tetracycline
Mean values±SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)

Graph 3.6: Inhibitory zone diameter (mm) of *Ganoderma* species against Gram negative bacteria in aqueous



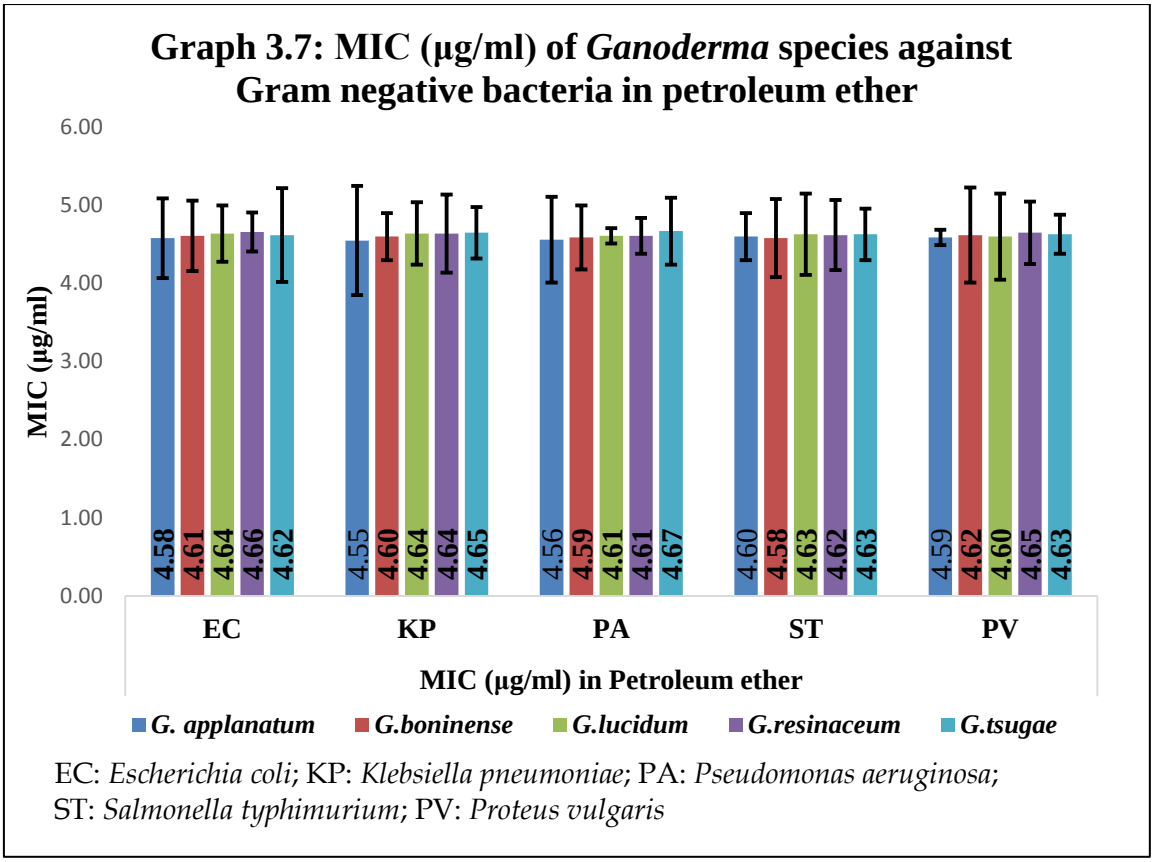
EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris; C:* Tetracycline

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

Table 3.7: MIC (µg/ml) of *Ganoderma* species against Gram negative bacteria in petroleum ether

Test species	Minimum inhibitory concentration (µg/ml) in Petroleum ether				
	EC	KP	PA	ST	PV
G. applanatum	4.58±0.51*	4.55±0.70*	4.56±0.55*	4.60±0.30**	4.59±0.10**
G. boninense	4.61±0.45**	4.60±0.30**	4.59±0.41**	4.58±0.50**	4.62±0.61*
G. lucidum	4.64±0.36**	4.64±0.40**	4.61±0.10**	4.63±0.52*	4.60±0.55*
G. resinaceum	4.66±0.25**	4.64±0.50**	4.61±0.23**	4.62±0.45**	4.65±0.40**
G. tsugae	4.62±0.60*	4.65±0.33**	4.67±0.43**	4.63±0.33**	4.63±0.25**

EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*;
Mean values±SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)



EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*

Table 3.8: MIC (µg/ml) of *Ganoderma* species against Gram negative bacteria in chloroform

Test species	Minimum inhibitory concentration (µg/ml) in Chloroform				
	EC	KP	PA	ST	PV
G. applanatum	4.59±0.51*	4.58±0.50**	4.57±0.33**	4.60±0.10**	4.56±0.61*
G. boninense	4.58±0.35**	4.62±0.41**	4.59±0.66*	4.61±0.36**	4.60±0.45**
G. lucidum	4.60±0.20**	4.59±0.40**	4.64±0.50**	4.63±0.45**	4.64±0.51*
G. resinaceum	4.66±0.46**	4.61 ±0.46**	4.63±0.40**	4.65±0.45**	4.62±0.55*
G. tsugae	4.64±0.33**	4.63±0.25**	4.66±0.25**	4.61±0.41**	4.67±0.45**

EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*;
Mean values±SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)

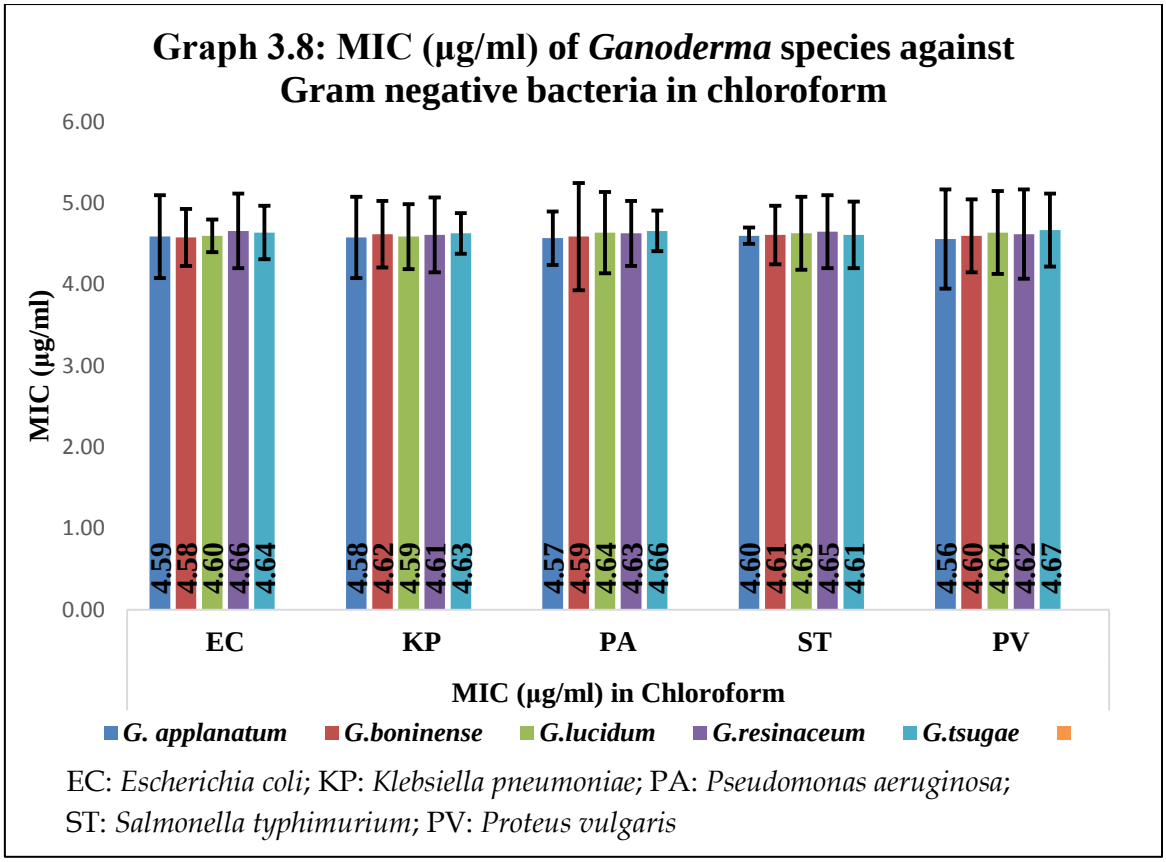


Table 3.9: MIC (µg/ml) of *Ganoderma* species against Gram negative bacteria in acetone

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

EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*;
ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*;
Mean values±SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)

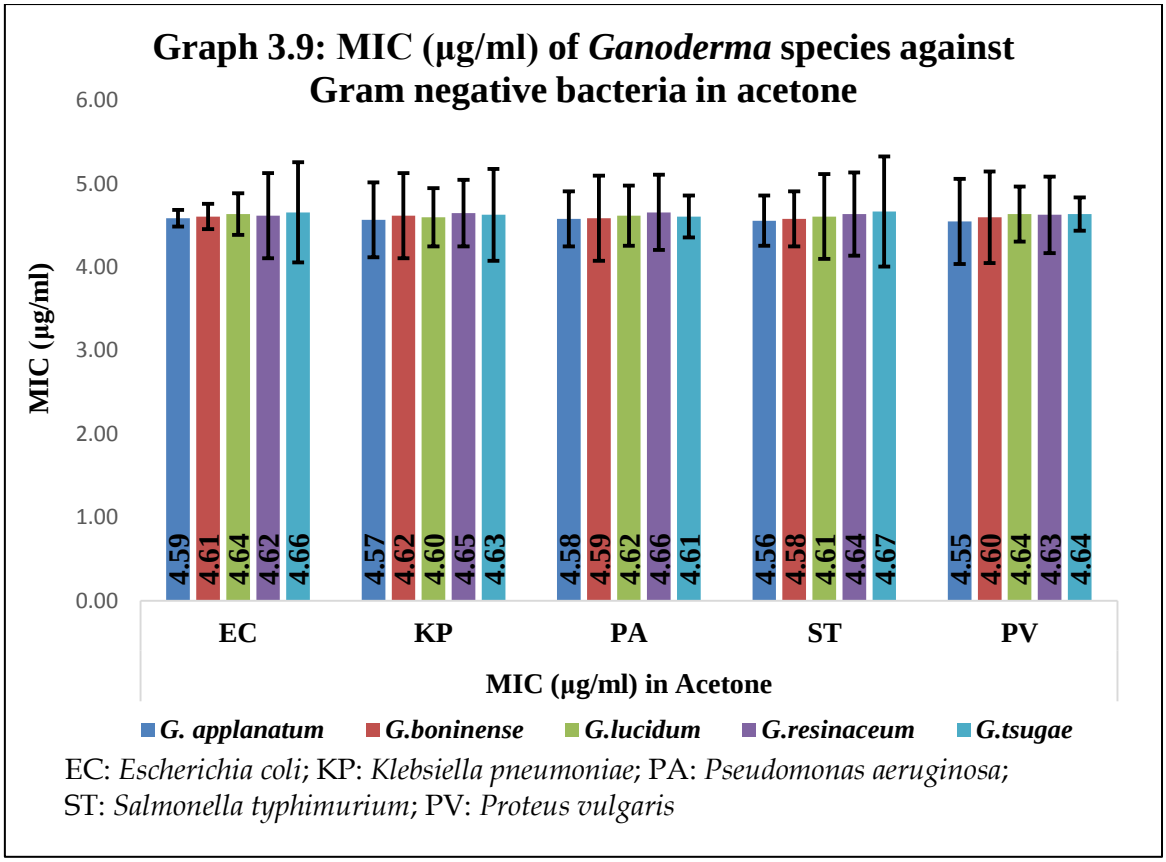


Table 3.10: MIC (µg/ml) of *Ganoderma* species against Gram negative bacteria in ethanol

Test species	Minimum inhibitory concentration (µg/ml) in Ethanol				
	EC	KP	PA	ST	PV
G. applanatum	4.59±0.50**	4.60±0.30**	4.58±0.35**	4.57±0.55*	4.56±0.45**
G. boninense	4.62±0.25**	4.61±0.36**	4.59±0.66*	4.58±0.20**	4.60±0.33**
G. lucidum	4.64±0.35**	4.62±0.45**	4.63±0.20**	4.64±0.51*	4.63±0.25**
G. resinaceum	4.61±0.43**	4.63±0.33**	4.64±0.25**	4.65±0.35**	4.66±0.30**
G. tsugae	4.65±0.41**	4.66±0.51*	4.67±0.33**	4.62±0.46**	4.63±0.10**
EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*; Mean values±SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)					

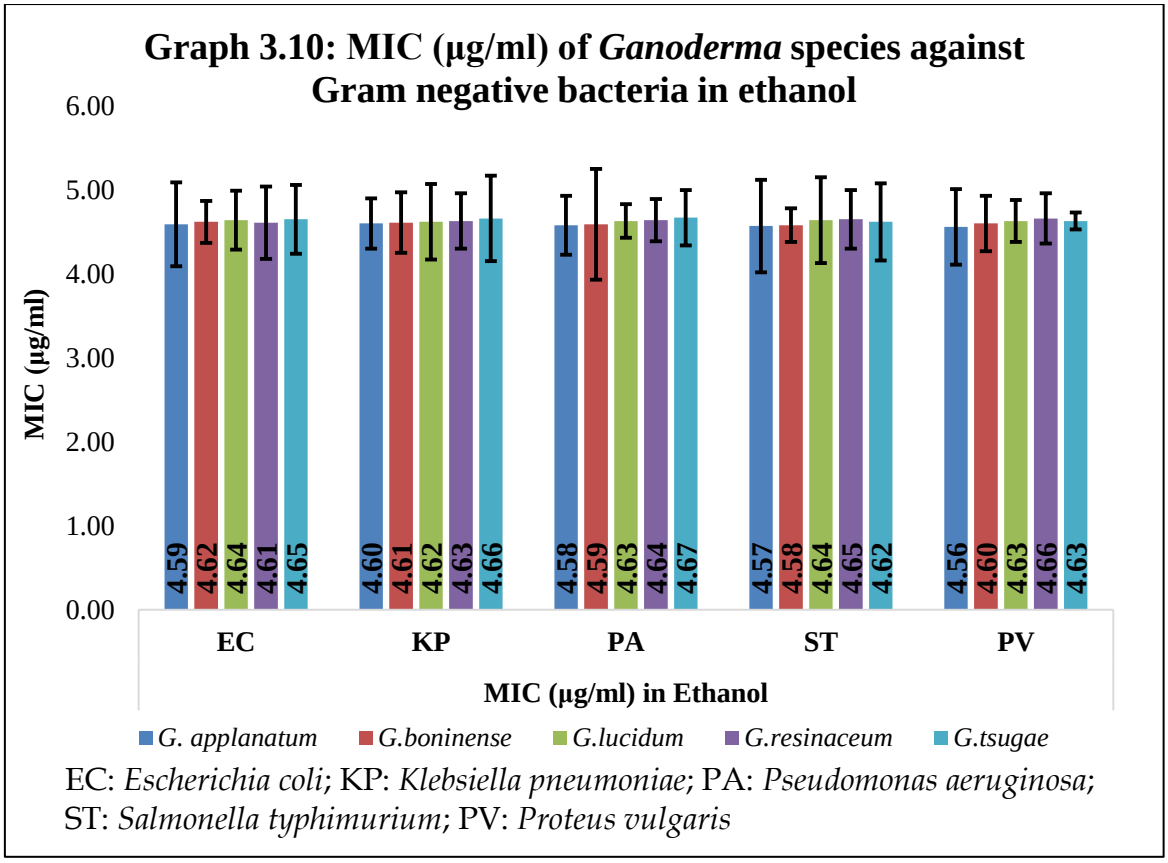


Table 3.11: MIC (µg/ml) of *Ganoderma* species against Gram negative bacteria in methanol

Test species	Minimum inhibitory concentration (µg/ml) in Methanol				
	EC	KP	PA	ST	PV
G. applanatum	4.57±0.66*	4.56±0.10**	4.60±0.50**	4.55±0.50**	4.58±0.50**
G. boninense	4.58±0.50**	4.61±0.33**	4.60±0.60*	4.59±0.50**	4.62±0.50**
G. lucidum	4.64±0.60*	4.64±0.10**	4.60±0.33**	4.63±0.10**	4.64±0.60*
G. resinaceum	4.64±0.33**	4.61±0.60*	4.62±1.00*	4.66±0.33**	4.65±0.33**
G. tsugae	4.67±0.10**	4.65±0.33**	4.64±0.33**	4.63±0.50**	4.62±1.00*

EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*;
ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*;
Mean values±SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)

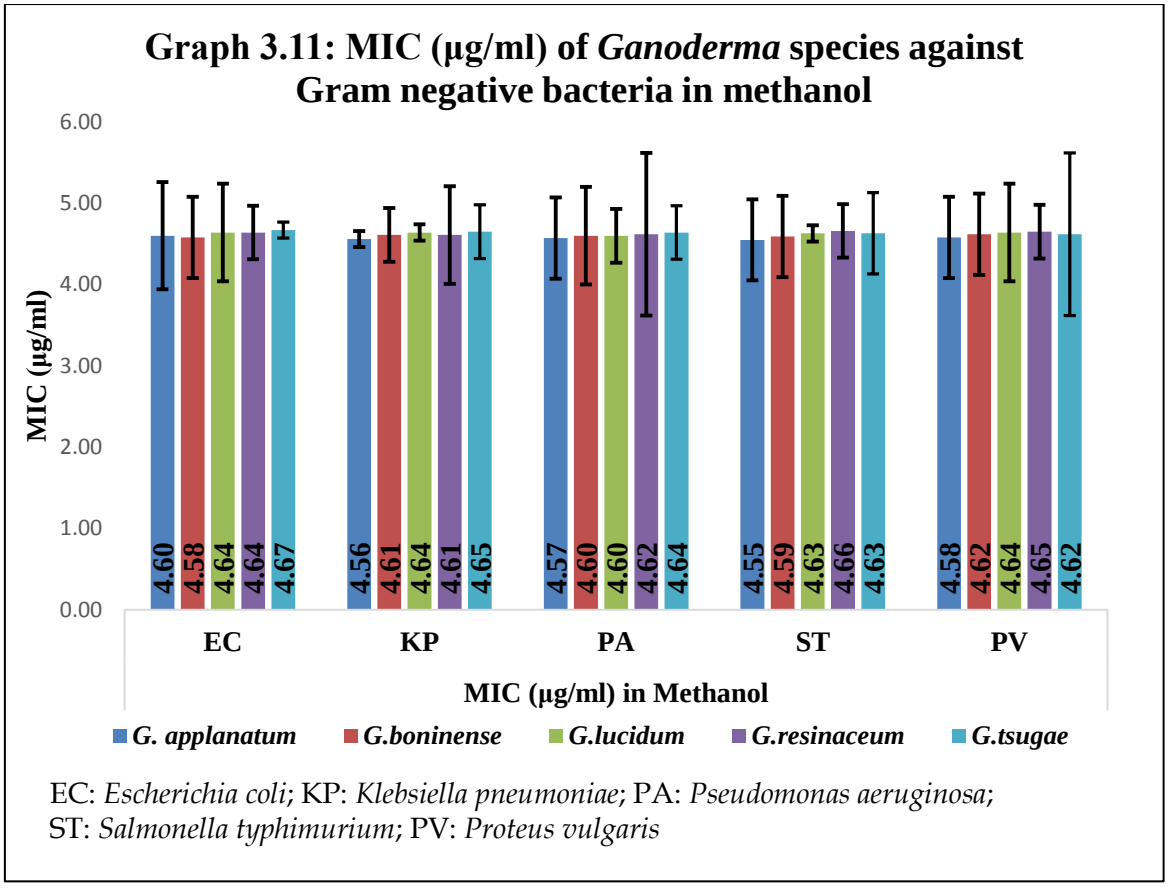
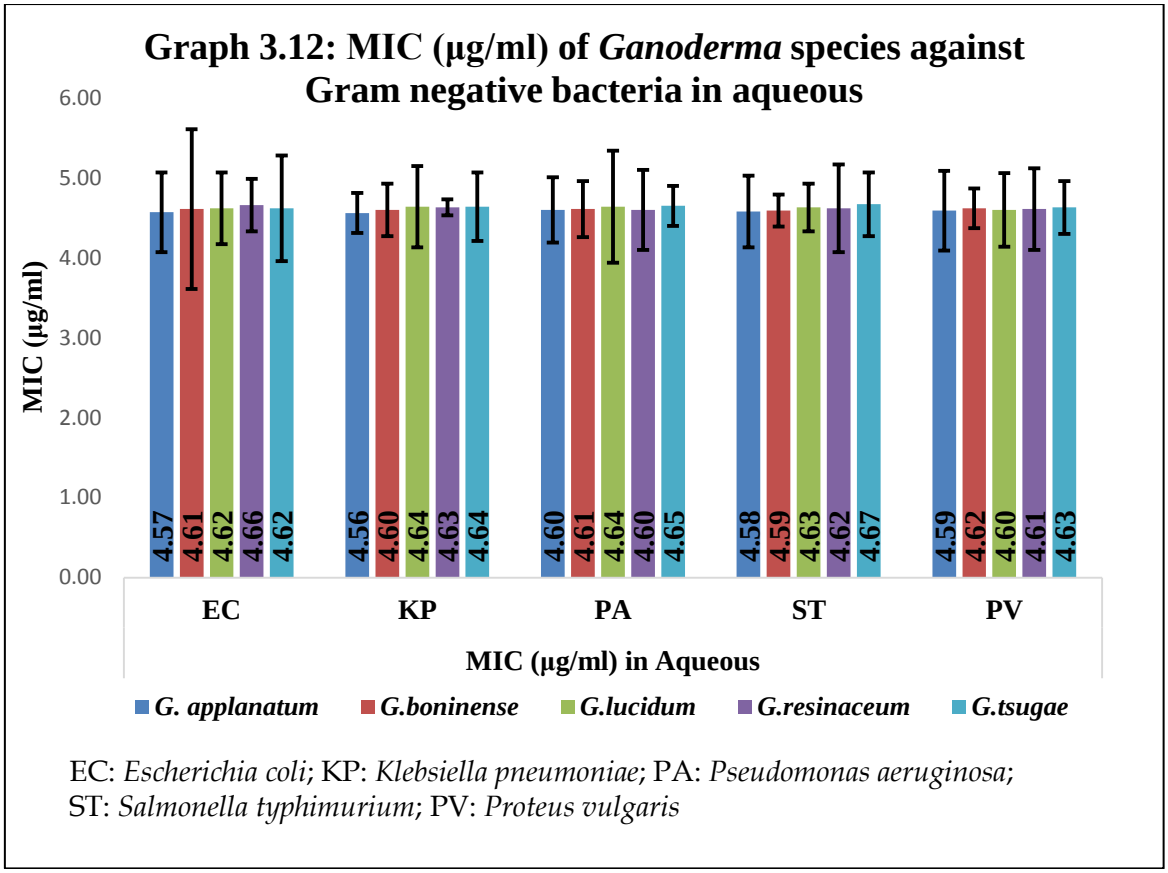


Table 3.12: MIC (µg/ml) of *Ganoderma* species against Gram negative bacteria in aqueous

Test species	Minimum inhibitory concentration (µg/ml) in Aqueous				
	EC	KP	PA	ST	PV
G. applanatum	4.57±0.50**	4.56 ±0.25**	4.60±0.41**	4.58±0.45**	4.59±0.50**
G. boninense	4.61±1.00*	4.60±0.33**	4.61±0.35**	4.59±0.20**	4.62±0.25**
G. lucidum	4.62±0.45**	4.64±0.51*	4.64±0.70*	4.63±0.30**	4.60±0.46**
G. resinaceum	4.66±0.33**	4.63±0.10**	4.60±0.50**	4.62±0.55*	4.61±0.51*
G. tsugae	4.62±0.66*	4.64 ±0.43**	4.65±0.25**	4.67±0.40**	4.63±0.33**
EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*; Mean values±SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)					



EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; ST: *Salmonella typhimurium*; PV: *Proteus vulgaris*

Diabetic Foot Ulcers (DFUs) is a serious complication of diabetes that can lead to infections due to the compromised immune system and reduced blood flow to the extremities. Several microorganisms are commonly found in DFUs and these infections are often polymicrobial, i.e. they involve multiple types of bacteria. Some of the most prevalent gram positive and negative bacteria found in DFUs include *Staphylococcus aureus*, *Streptococcus* species, *Enterococcus* species, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus* species, *Proteus* species, *Salmonella* species and anaerobic bacteria (Du, *et al*., 2022; Sultana, *et al*., 2023).

The inhibitory zone diameter (mm) of *Ganoderma* species in petroleum ether against Gram-negative bacteria revealed that *G. tsugae* exhibits maximum zone of inhibition against *P. vulgaris* (6.45mm) while *G. lucidum* showed moderate inhibition against *P. aeruginosa* and *S. typhimurium* (6.10mm and 6.00mm respectively) and *G. resinaceum* and *G. lucidum* showed minimum inhibition against *K. pneumoniae* (3.10mm) (Table 3.1). The inhibitory zone diameter (mm) of *Ganoderma* species in chloroform against Gram- negative bacteria revealed that maximum inhibition occurred by *G. tsugae* against *P. aeruginosa* (6.60mm), moderate inhibition occurred by *G. lucidum* against *K. pneumoniae* (6.30mm). Similarly, minimum inhibition occurred by *G. resinaceum* against *P. vulgaris* (3.00mm). Maximum inhibition occurred by *G. boninense* against *P. aeruginosa* (5.30mm) and minimum inhibition against *S. typhimurium* (3.60mm). This result is consistent with the earlier findings as that of Sim, *et al*., 2019, where they observed highest antibacterial activity of chloroform-extracted GBMA (*G. boninense* media agar) against *E. coli, P. aeruginosa* and *K. pneumoniae*. It is possible that the bioactive chemicals in the crude extracts were in low quantities or were not effectively active to prevent bacterial growth (Nwachukwu, *et al*., 2017). (Table 3.2).

Maximum inhibition in acetone was caused by *G. lucidum* against *S. typhimurium* and *P. vulgaris* (6.45mm), moderate inhibition by *G. tsugae* for *E. coli* (6.30mm). Similarly, minimum inhibition was shown by *G. tsugae* against *S. typhimurium* (3.25mm). Similar

study by Suansia, *et al.,* (2021) confirms the same (Table 3.2). The inhibitory zone diameter (mm) of *Ganoderma* species in ethanol showed the maximum inhibition by *G. lucidum* and *G. tsugae* against *E. coli* (6.30mm), thus supporting the result as observed by Djide, *et al.,* (2014). Moderate inhibition was shown by *G. applanatum* and *G. tsugae* against *K. pneumoniae* and *P. aeruginosa* (6mm). Similarly, lowest inhibition by *G. applanatum* against *S. typhimurium* (2.80mm). Similarly, *G. tsugae* showed maximum inhibition against *E. coli* (6.30mm) and minimum inhibition against *P. vulgaris* (4.75mm). This result is consistent with the research conducted by Li, *et al.,* 2018, they also found highest antibacterial activity against *E. coli* because triterpenoids present in *G. tsugae* extract showed strong antibacterial activity against all tested bacteria (Table 3.4).

Maximum inhibition in methanol is indicated by *G. lucidum* and *G. tsugae* against *P. aeruginosa* (7.15mm), moderate inhibition by *G. tsugae* against *S. typhimurium* (6.80mm). Similarly, lowest inhibition by *G. applanatum* against *P. vulgaris* (3.50mm). The methanolic extract of *G. lucidum* showed the strongest antibacterial activity among all extracts against the tested bacterial strains along with *G. tsugae*. This suggests that methanol may dissolve the active ingredient that prevents the development of sensitive bacteria more easily than the other solvents. The same outcomes are likewise obtained in the methanolic extract of *G. lucidum* by Shah, *et al.,* (2014) (Table 3.5).

The inhibitory zone diameter (mm) of *Ganoderma* species in aqueous phase revealed maximum inhibition by *G. applanatum* and *G. lucidum* against *E. coli* (6.80mm), moderate inhibition by *G. tsugae* against *K. pneumoniae* and *P. aeruginosa* (6.50mm and 6.60mm). Similarly, lowest inhibition by *G. tsugae* against *S. typhimurium* (3.50mm). The antibacterial activity of methanolic extract of *G. applanatum* was also studied by Elisashvili, *et al.,* (2022) reveals the highest inhibition zone against *E. coli, Pseudomonas fluorescens* and *P. aeruginosa*. On the basis of these studies, we can say that the biochemicals of the extract dissolve well in the solvent and caused maximum inhibition (Table 3.6).

The *G. tsugae* also showed the highest MIC against *P. aeruginosa* (4.67 µg/ml) whereas *G. lucidum* extract demonstrated moderate MIC against *P. vulgaris* (4.60 µg/ml) and *G. applanatum* with lowest MIC against *K. pneumoniae* (4.55 µg/ml) (Table 3.7).

The *G. tsugae* had the highest minimum inhibitory concentration (MIC) against *P. vulgaris* (4.67 µg/ml). *G. boninense* and *G. resinacium* extract demonstrated the moderate MIC against *K. pneumoniae* and *P. vulgaris* (4.62 µg/ml). While, *G. applanatum* extract exhibited the lowest MIC against *P. aeruginosa* (4.57 µg/ml) (Table 3.8).

4. Discussion

Medicinal mushrooms are considered one of the richest sources of natural bioactive constituents and various species of them inhibit the growth of a wide diversity of microorganisms including Gram negative bacteria. Gram-negative bacilli are the most common bacterial pathogens and are often resistant to medicinal products. Monitoring for antimicrobial resistance in this population is critical since resistance has been linked to increased morbidity and mortality. *Ganoderma*, is a genus of well-known medicinal mushroom and has many pharmacological and biological activities including an antimicrobial effect, although few studies have investigated the antibacterial and antifungal effects of its purified compounds (Vazirian, *et al.,* 2014). Using a progressive and discriminative extraction and isolation process of the main classes of compounds from fresh and dried fruit bodies of *Ganoderma* via the initial use of petroleum ether, chloroform, acetone, ethanol, methanol and water extraction methods (Gao, *et al.,* 2005), this research investigated the potential antimicrobial, anti-inflammatory and regenerative properties with respect to the complex wound healing potential of the primary extracts.

Research have been conducted to isolate and distinguish the antibacterial activity of *G. lucidum* culture fluids against several gram-negative plant pathogenic bacteria like *Acidovorax avenae*, *Agrobacterium*, *Erwinia carotovora*, *Pseudomonas fluorescens*, *Xanthomonas*. Nearly all of the studied bacteria were unable to grow in the freshly obtained culture fluids of *G. lucidum* (Costa*, et al.,* 2020). Investigations were conducted to evaluate the antimicrobial potential of the autochthonous *Ganoderma* species (*G. resinaceum*, *G. pfeifferi*, *G. lucidum* and *G. applanatum*). CHCl3 extract of *G. resinaceum* had the strongest antibacterial activity against *P. aeruginosa*, EtOH extracts of *G. pfeifferi* and *G. resinaceum* were shown to have the strongest antibacterial activity against *A. niger*, whereas *G. pfeifferi* exhibited the maximum antibacterial activity against both *E. coli* and *S. aureus* (Thapa*, et al.,* 2022).

Study have been done to distinguish the antibacterial activity of methanol and water extract of *G. lucidum* and *G. applanatum* against *Bacillus subtilis*, *Staphylococcus epidermidis*, *Micrococcus* and *Pseudomonas aeruginosa* and both *Ganoderma* species exhibited strong antibiotic activity against all bacterial strains tested (Elisashvili*, et al.,* 2022; Sedefoglu*, et al.,* 2022).

Conclusion

The antimicrobial investigations related to *Ganoderma* species and Gram-negative bacterial isolates linked to diabetic foot ulcers (DFUs) have demonstrated encouraging outcomes. According to this study, all tested *Ganoderma* species have compounds or extracts that exhibit good antibacterial properties against all tested Gram-negative bacteria, including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium* and *Proteus vulgaris*, which are frequently linked to diabetic foot infections. Because *Ganoderma* is a valuable fungus with a wide range of therapeutic benefits that can be used as complementary and alternative methods to prevent and treat a wide range of diseases, including diabetes, these findings suggest the potential for *Ganoderma*-based treatments to combat infections in DFUs, potentially offering an alternative or adjunct to conventional antibiotics. while *Ganoderma* shows promise in supporting diabetic management but 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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