

**ANTIBACTERIAL EFFICACY OF EXTRACT OF GYMNEMA SYLVESTRE
AGAINST ENTEROCOCCUS FAECALIS – AN IN VITRO STUDY****Keerthika .R.**

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Abstract**Introduction**

Increasing emergence of resistance to the currently available antibiotics has necessitated continued search for new antimicrobial compounds. *Gymnema sylvestre* is a woody vine common in tropical areas of Asia, Africa and Australia and it is believed that this plant possesses antiviral, antibacterial, anti-allergic, anti-oxidants and hypoglycemic properties. Thus this study aims to evaluate the antibacterial efficacy of *Gymnema sylvestre* against *Enterococcus faecalis* biofilm.

Materials and method

Ethanollic extracts prepared from *Gymnema sylvestre* leaves were screened for in vitro antimicrobial activity against *Enterococcus faecalis* using disk diffusion method. For statistical analysis of data, multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by the LSD test for post hoc analysis. Statistical significance was accepted at a level of $P < 0.05$. Data were analyzed using SPSS (version 11).

Results

A dose dependent antibacterial effect of extracts of *Gymnema sylvestre* leaf was noted with maximum antibacterial effect at the concentration of 50 µg/ml.

Conclusion

Ethanollic extract of *Gymnema sylvestre* demonstrated antimicrobial activity against *Enterococcus faecalis* recommending further in vivo and toxicology studies to prove its use in the clinical situation.

Keywords: *Gymnema sylvestre* ,Antimicrobial activity ,*Enterococcus faecalis* ,Disk diffusion method.

Introduction:

Gymnema sylvestre is a perennial woody vine native to tropical Asia, China, Africa and Australia. It has been widely used in ayurvedic medicine for treating various illnesses. (Kapoor, 2017) This exhibits a broad range of therapeutic effects for various conditions such as diabetes,

arthritis, diuretics, anemia, hypercholesterolemia, etc... It also shows antibacterial, anti-inflammatory and anti-carcinogenic properties. (Ozturk & Hakeem, 2018)(Peter, 2012) The methanolic extract of the leaves of *Gymnema sylvestra* are used in the aqueous form as the intracanal medication. (Eloff, 1998)

The chloroform, petroleum ether, and methanolic leaf extracts of *Gymnema sylvestre* at various concentrations of 25, 50, and 100 mg/mL were tested against microbial dental infections and found to be significantly effective against the cariogenic bacteria particularly the methanolic extract which showed highest activity at minimum concentration. (Ahirwal et al., 2015)(Arora & Sood, 2017; . et al., 2019)

The antibiotic and antimicrobial activity of different extracts of *Gymnema sylvestre* was determined against a number of pathogens, namely, *S. aureus*, *E. coli*, and *B. subtilis* and the activity was observed even against gram-negative bacteria. (Shanmugavadivu & Velmurugan, 2018) *Gymnema sylvestre* leaf extracts showed good prospects as an antibiotic herbal remedy and effective herbal formulation for the treatment of microbe-related infections. The methanolic and ethanolic leaf extract of *Gymnema sylvestre* possesses considerable antibiotic and antimicrobial activity which could be used as a better intracanal medication for better and effective local drug delivery system. (. et al., 2019)(Delihias, 1997)

The bioactive constituents in *Gymnema sylvestre* known as tannins and saponins are responsible for the anti-inflammatory activity which helps to bring down the inflammation in the periapical region of the teeth. (Kanetkar et al., 2007; Ye, n.d.) In general after the endodontic treatment anti-inflammatory medication is prescribed as an oral medication but by incorporating this invention as an intra-canal medication local delivery of anti-inflammatory mediators can be achieved thus by eliminating the need for systemic administration. (Karojari et al., 2022; "Polypharmacy of Alternative and Herbal Medications," 2002)

Enterococcus faecalis is an anaerobic gram-positive coccus that usually starts in the human mouth cavity, gastrointestinal system, and vaginal cavity because it has shown good adaptation to conditions with high nutritional density, low oxygen levels, and a complicated ecology. (Pinheiro, Gomes, Ferraz, Sousa, et al., 2003) *Enterococcus faecalis* was discovered more frequently in cases of unsuccessful endodontic therapy than in cases of original infections, according to several investigations. *Enterococcus faecalis* is the most commonly identified bacteria in cases of post-endodontic therapy pain and infection, with prevalence rates as high as 90%. *Enterococcus faecalis* was more likely to be related with asymptomatic cases than symptomatic ones among all instances of primary endodontic infection. (Gomes et al., 2008; Sousa et al., 2003)(Rôças et al., 2004)

Enterococcus faecalis was the most frequently isolated bacteria from the root canal systems (45.8%) in previously treated cases. (Pinheiro et al., 2004) *Enterococcus faecalis* is a biological

indicator that has been extensively studied in the field of endodontics . Several laboratory investigations looked at *Enterococcus faecalis* susceptibility to endodontic therapy and found that it was very resistant to antimicrobial medications. Furthermore, *Enterococcus faecalis* may thrive in a variety of challenging conditions, including low nutrition availability and high alkaline pH of up to 11.5. *Enterococcus faecalis* ability to proliferate as a biofilm on root canal walls and as a mono-infection in treated canals without synergistic support from other bacteria makes it a root canal pathogen with great resistance to antimicrobial treatments.(Siqueira & Rôças, 2004)(Barbosa-Ribeiro et al., 2016; Pinheiro, Gomes, Ferraz, Teixeira, et al., 2003)

Development of alternative drug discovery has led to new pharmaceutical compounds from natural products . The Bioactive components from these natural products have shown to provide better therapeutic effects. Previously our team has a rich experience in working on various research projects across multiple disciplines .(Azeem & Sureshababu, 2018; Felicita, 2017; Felicita et al., 2012; A. R. Jain, 2017; Krishnan & Lakshmi, 2013; Kumar et al., 2006; Mp, 2017; Patturaja, 2016; Rao & Kumar, 2018; Sekar et al., 2019; Sivamurthy & Sundari, 2016) Now the growing trend in this area motivated us to pursue this project. Thus this study aims to evaluate the antibacterial efficacy of *Gymnema sylvestre* against *Enterococcus faecalis* biofilm.

METHODOLOGY

Extract preparation

For preparing extract, 500 gm of the powdered *Gymnema sylvestre* sample is soaked with 70% ethanol (in ratio ethanol:plant (6:1)) for 72 hrs and filtered using Whatman No. 1 paper. The filtrate was placed into the thimble of the Soxhlet extraction apparatus chamber. The sample was extracted for 12 hours at 4 cycles per hour.

After extraction, the solvent was removed by the means of a vacuum evaporator, yielding the extracted compound. The crude extracts were weighed and stored at 0°C-4°C before analysis.

Bacterial strains and media

Enterococcus faecalis strain ATCC 29212 was maintained in stock culture at -80°C in Trypticase Soy Broth containing 25% glycerol. The 10 µl of the strains were inoculated into brain-heart infusion (BHI) broth and incubated in an aerobic atmosphere at 37°C. After 24 hours, the turbidity of the culture medium was assessed using a spectrophotometer. Microorganisms were grown on Blood agar plates, and checked for purity. The bacterial cell suspensions were adjusted to a density containing approximately 1×10^8 CFU/ml. and diluted with media to contain 10^5 CFU/ml.

Antimicrobial susceptibility testing



A 100 µl of bacterial suspension was spread on each Mueller Hinton Agar plate. The 20 µl of various different concentrations (10, 25 and 50 µg/ml) of ethanolic extract of *Gymnema Sylvestre* were impregnated on sterilized 6 mm blank discs. Distilled water loaded discs were used as negative controls respectively. The 0.2% Chlorhexidine used as positive controls for all strains. All impregnated discs were ensured to be fully dried in a 45°C incubator for 18 to 24 prior to the application of bacteria. The discs which had been impregnated with irrigants using sterile forceps were applied on the inoculated Mueller Hinton agar once it had completely dried. The discs were pressed gently to ensure uniform contact with the agar surface. Within 15 min of application, plates were kept in an incubator for 48 h. After incubation was complete, the diameter of the inhibition zone around the treated discs were measured for the antibacterial activity assessment. If present, their diameters were measured to the nearest whole millimeter with a ruler. All tests were carried out three times to ensure the reliability, and the average of the three replicates of irrigants, and negative control were calculated.

Statistical analysis

For statistical analysis of data, multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by the LSD test for post hoc analysis. Statistical significance was accepted at a level of $P < 0.05$. Data were analyzed using SPSS (version 11).

Results

A dose dependent antibacterial effect of extracts of *Gymnema sylvestre* leaf was noted with maximum antibacterial effect at the concentration of 50 µg/ml.(Table 1)(fig 1)

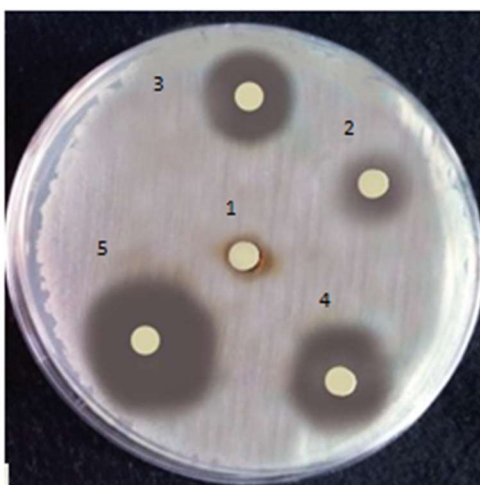
Table 1: Antibacterial activity of GSE

Test compounds	Zone of Inhibition (mm)
	<i>E. Faecalis</i>
GSE (10 µg/ml)	5.5 ± 0.56 ^{***c}
GSE (25 µg/ml)	9.9 ± 0.89 ^{***b}

GSE (50 µg/ml)	12.1 ± 1.1 ^{***a}
CHX (0.2%)	16.3 ± 1.5 ^{***}
Negative control	NI

NI means no inhibition zone. Each value is expressed as mean ± SD (n = 3). *p<0.05; **p<0.01 and ***p<0.001 statistically significant as compared with negative control). ^ap<0.05; ^bp<0.01 and ^cp<0.001 statistically significant as compared with CHX(0.2%).

Figure 1:



1- Negative control; 2- GSE(10 µg/ml); 3- GSE(25 µg/ml); 4- GSE(50 µg/ml); 5- CHX (0.2%)

Discussion

Antibacterial activity of Ethanol extract was evaluated against *E.faecalis* by disc diffusion method. It was observed that there was a dose dependent antibacterial effect of extracts of *Gymnema sylvestre* leaf. The dose dependent antimicrobial activity was also noted by other authors (Vlietinck et al., 1995)

A study by Selvaraj et al has also shown there was 100% antibacterial efficacy of ethanolic extract of *Gymnema sylvestre*, he has also compared the efficacy of various extracts of *Gymnema sylvestre*. They also observed that this plant was effective against several gram positive and negative bacteria such as *S. aureus*, *E. Coli*, *K. pneumoniae* and *P. aeruginosa*. (et

al., 2019)

In the studies by David et al & Tahir et al , methanolic extract of the leaves of this plant was reported to show antimicrobial activity against *E. coli*, *B. cereus*, *C. albicans*, and *C. kefyr*. The aqueous extract showed moderately anti-microbial activity against *S. aureus*, *C. krusei*, *C. perfringens* type-A and *C. kefyr* where the hexane extract showed activity against *S. aureus*, *B. cereus*, *S. enterica*, *H. paragallinarum* and *C. perfringens* type-A. (Bhandari et al., 2012; Tahir et al., 2016)

Pasha et al and Paul et al have shown that both the aqueous and ethanol extract are active against pathogenic *Salmonella* species (*Salmonella typhi*, *S. Typhimurium*, and *S. paratyphi*). Ethanolic, chloroform, and ethyl acetate extracts were reported to be active against *P. vulgaris*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus* . (Bhalerao et al., 2022; Patel, 2017)

G. sylvestris leaf extracts showed good prospects as an antibiotic herbal remedy was effective as herbal formulation for the treatment of microbe's related infections.. Presence of gymnemic acid has showed significant antimicrobial effect against the microbes. (Sudhanshu & Sudhanshu, 2012) The ethanolic and methanolic extract of *Gymnema sylvestris* has proven to be effective against broad range of organisms. (Arora & Sood, 2017) (David & Sudarsanam, 2013) (Satdive et al., 2003)

The bioactive constituents in *G. sylvestris* known as tannins and saponins are responsible for the anti-inflammatory activity of the plant The flavone act as anti-inflammatory agent which can significantly affect bacteria. (Chodiseti et al., 2013; Khanna & Kannabiran, 2008)

Our institution is passionate about high quality evidence based research and has excelled in various fields. (R. K. Jain et al., 2014; Johnson et al., 2019; Keerthana & Thenmozhi, 2016; Lakshmi et al., 2015; Neelakantan et al., 2011) We hope this study adds to this rich legacy.

Limitation(s)

Only herbs of local origin were obtainable. The applied methods of preservation of herbal materials before being used in this study could be questionable. For sensitivity tests, both disc and wells should have been used.

Conclusion

A dose dependent antibacterial effect of extracts of *Gymnema sylvestris* leaf was noted with maximum antibacterial effect at the concentration of 50 µg/ml. The antibacterial activity of *Gymnema sylvestris* leaf extract against *E. faecalis* recommends further *in vivo* and toxicology studies to prove its use in the clinical situation. The present study supports the traditional use of the natural products in the treatment of several diseases.

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