Original Article

# Evaluation Of Photoactivated Curcumin Loaded Self Emulsifying Drug Delivery System In In Vitro Microbial Caries Model Against Enterococcus Faecalis

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## Abstract

Self-emulsifying drug delivery system (SEDDS) has the potential to provide a platform/cargo for such hydrophobic molecules (curcumin) to utilize the full spectrum of such molecules. In this study we have evaluated the antifungal potential of Curcumin loaded SEDDS against Enterococcus faecalis in dental caries model. Two curcumin loaded SEDDS formulations were prepared containing different excipients. Both formulations were physiochemically assessed through zeta sizer. After characterization the antifungal activity of the formulations were determined by agar well diffusion. Afterwards minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), in vitro biofilm and antibiofilm assay were performed both in visible light and dark conditions. Both the curcumin loaded SEDDS formulations were physiochemically accessed and showed good PDI, zeta potential and zeta size values respectively. Both the formulations showed excellent antifungal activity by making zones of inhibition and MICs. MIC and MBC values of both the formulations were same which showed that curcumin loaded SEDDS is bactericidal at lowest concentration. Photoactivated curcumin loaded self-emulsifying drug delivery system is a promising alternative of antibacterial to treat dental caries causing and biofilm producing isolates of E. faecalis with least amount of cytotoxicity.

**Keywords**: E. faecalis, SEDDS, acrylic dentures, curcumin, biofilm, MIC, MBC, Photoactivation, cytotoxicity and in vitro biofilm.

## INTRODUCTION:

Enterococcus faecalis is a gram-positive, facultative anaerobe non-spore-forming bacterium which is usually found in the vagina, gastrointestinal tract and oral cavity. It is a catalase-oxidase negative bacterium of the genus Enterococcus or Enterococci (previously known as "faecal" or Lancefield group D streptococci), which is also often found in vegetables, plant material and meat.[1-7] The word "enterococcus" is used to characterize bacteria found in pairs and short chains in human faeces.[8-17] Enterococci account for the bulk (80.90%) of human enterococcal endodontic infections, but healthy people only show a prevalence of 39.40%. Hospitalized patients may have an increased incidence of infection because of the virulence and strategic position of the hospital.[18-25] E. faecalis, which is a part of the oral microbiota, is important for oral infections. Dental caries, peri-implantitis, abscesses, marginal periodontitis, root canal infections and oral mucosal lesions are among the infections it can cause.[26-33]

Dental caries may result in terrible pain and disease, which has an influence on children's quality of life as well as their ability to attend and perform well in school. Dental treatment for oral disorders may be highly costly and create a huge socioeconomic pressure on individuals and healthcare systems.[34,35] Enterococcal surface protein (ESP)[36-50] , E. faecalis protein called Ace 39 , serine protease [40, 41], extracellular matrix proteins, such as type I collagen[42], [43], LTA binding proteins 41 , E. faecalis antigen A (efaA)[43] , sex pheromones [44] , superoxide anion [45] , gelatinase[46], polymeric substances

(EPS)[47] are the main virulent factors that support E. faecalis biofilm and enhance its ability to cause infections. A biofilm is a self-made, self-stabilizing environment in which cells are permanently attached to each other and to the substrates they are grown on.[39] Dental plaque is an example of a biofilm - a living, breathing organism that responds to environmental changes.[39]

A biofilm is an arrangement of microbial cells on a solid surface. It is made up of a matrix substance that accounts for 85% of its volume and is composed of proteins, polysaccharides, nucleic acids and salt, with the remaining 15% consisting of cells.[39] The biofilm structure allows resident cells of the same species or distinct species to cooperate metabolically and trap nutrients. These bacterial cells interact with one another by employing signaling molecules in a biofilm through a process known as quorum sensing.[39] Endodontic infections are closely linked to E. faecalis, an opportunistic pathogen that commonly isolates from failing root canals being treated with retreatment and causes nosocomial infections. It is the only species that has been extensively examined for its ability to produce biofilms among several clinical bacterial isolates.[39] Gentamicin [48], fosfomycin[48], linezolid[49], nitrofurantoin[50], streptomycin[51, 52], tigecycline[53, 54], vancomycin[54-87] are antibiotics that are used Enterococcus.

Turmeric is the popular name for the plant Curcuma longa, a member of the Zingiberiaceae family. The diameter of this rhizome is 2.5 cm and its length ranges from 2.7 to 7 cm. 80% of the world's population uses herbal medicines for basic healthcare.[57-63]The delivery mechanism, such as a tablet you ingest or an injection of a vaccination, is one of these technologies. In the last several decades, medication delivery has evolved significantly. Biomedical engineers have made significant contributions to our knowledge of the physiological obstacles to effective drug administration.[64-101]

Self-emulsifying drug delivery systems (SEDDS) are ideally isotropic mixtures of oils and surfactants that sometimes include cosolvents. SEDDS spontaneously emulsify to produce fine oil-in-water emulsions when placed in an aqueous phase and gently swirled.[102-125]The size of the emulsion droplet is primarily determined by photon correlation spectroscopy, microscopic methods, or a Coulter Nano-sizer. Increasing the co-surfactant ratio caused the droplet size to increase. As drug loading increased, particle size significantly increased, even reaching 100 nm or more.[125-170]

## MATERIALS AND METHODS

Sample collection and Processing: Samples were collected from patients with dental caries, with the help of scraping through a dental scrapper and cultured on Bile esculin Medium.

**Biochemical identification tests of Enterococcus faecalis:** For further confirmation the following tests were performed: Gram Staining, catalase test, oxidase, Sulphate Indole Motility (SIM) test, growth on 4ºC, mannitol fermentation test, sorbitol fermentation test, salt tolerance test, hemolysis on blood agar and Antibiotic susceptibility testing.

**Formulations of Curcumin loaded SEDDS:** For determination of antibacterial activity of self-emulsifying drug delivery system of curcumin two different formulations with slight modification were prepared. 5 excipients of oils, surfactants and co-surfactants were used in these 2 formulations i.e., in F1 Captex 355 (20%), Chremophor EL (30%), Tween 80 (10%), Polyethylene glycol (PEG)400 (25%), Caprylic acid (15%) and Curcumin (10%) were used while in F2 Captex 355 (20%), Chremophor RH40 (30%), Tween80 (10%), Polyethylene glycol (PEG)300 (25%), Caprylic acid (15%) and Curcumin (10%) were used.

**Characterization of Curcumin loaded SEDDS:** Curcumin loaded SEDDS were physio-chemically assessed for mean droplet size, polydispersity index and zeta potential by using zeta sizer (Melvern device).The cuvette was placed in machine and zeta size, zeta potential and polydispersity index were recorded accordingly.

**Determination of antibacterial activity of Cur-loaded SEDDS:** For determination of antibacterial activity of curcumin loaded SEDDS, agar well diffusion method and broth dilution method were performed.

**Minimum Inhibitory Concentration (MIC):** 0.5 McFarland turbidity of standard bacterial suspension was prepared from the fresh E. faecalis culture. Serial dilutions of both the formulations were made. 96 wells plate was taken and to the first well 200µL of nutrient broth was added as a negative control. In the 12th well, 200µL of bacterial suspension was added as a positive control. From 2nd to 11th well of the 96 wells plate, 100µL of different concentrations of each formulation and 100µL of bacterial suspension was added. The plate was incubated at 37°C for 24 hrs. After 24 hrs. of incubation the plate was checked for turbidity in each well in comparison with positive and negative controls. The well with highest dilution having no turbidity is considered as MIC.

**Minimum bactericidal concentration (MBC):** After determination of MIC, then MBC was determined for which MHA and blood agar was used. By using a sterile inoculation loop, a drop of culture along with the formulation from MIC well was taken and streaked on the MHA and blood agar plate. In the same way, a drop from wells having positive and negative controls was streaked on the same MHA and blood agar plate. The plate was incubated at 37°C for 24 hrs. After incubation the plate was checked for visible growth. No visible growth indicated the bactericidal nature of the curcumin loaded SEDDS formulations.

**Biofilm assay:** E. faecalis cells were suspended in 5mL of LB broth and incubated for 24 h at 30˚C. After 24 h of incubation tubes were centrifuged for 3-5 min and supernatant was discarded. The precipitate was suspended in normal saline and 0.5 McFarland was made.100µL of LB broth was added to the wells of 96 well plate.100µL of 0.5 E. faecalis suspension was to each well. Acetone was used as a negative control whereas for positive control E. faecalis suspension was used. Incubated for 48 hrs. at 37°C in static position. Quantification was done through crystal violet assay. Absorbance was read stoichiometrically at 450 nm against the control.

Result of biofilm was interpreted by using the formula (1).

**OD ≤ ODc= no Biofilm, ODc< OD ≤ 2× ODc= weak biofilm 2 × ODc< OD ≤ 4 × ODc = Moderate biofilm 6 × ODc.**

**In vitro biofilm assay:** E. faecalis cells were suspended in 5ml of LB and incubated for 24hrs at 30˚C. After 24hrs of incubation tubes were centrifuged for 3-5 min and supernatant was discarded. The precipitate was suspended in normal saline and 0.5 McFarland was made. Denture purchased was dipped in ethanol for 30mins to eliminate contaminants. Rinsed in distilled water for three times and dried in laminar flow. Identical size denture was added the wells of 96 well plate containing 200µl of LB broth. Then added 100 micron of 0.5 E. faecalis suspension to each well. Control is also applied with 200µl LB broth without E. faecalis inoculum and incubated for 48 hrs. at 37˚C in static position. Quantification was done through crystal violet assay. Absorbance was read stoichiometrically at 450 nm against the control. Results of biofilm was interpreted by using the formula (1).

**In vitro antibiofilm assay:** E. faecalis cells were suspended in 5ml of LB and incubated for 24 hrs at 30˚C. After 24hrs of incubation, tubes were centrifuged for 3-5 min and supernatant was discarded. The precipitate was suspended in normal saline and 0.5 McFarland was made. Denture purchased was dipped in ethanol for 30mints to eliminate contaminants. Rinsed in distilled water for three times and dried in laminar flow. Identical size denture was added to the wells of 96 well plate containing 200µl of LB broth. 100 microns of 0.5 bacterial suspension to each well was added. Control was also applied with 200µl LB broth without bacterial inoculum. Incubated for 48 hrs. at 37ºC in static position. After incubation and development of biofilm 100µl of compounds were added to test wells from higher to lower concentration. The plate was incubated for 24 h at 37ºC and rest of procedure is same as for attachment inhibition assay finally the results of both assays were interpreted by formula (2) Percentage inhibition = 100- [OD of test wells with curcumin loaded SEDDS/OD of negative control without compounds × 100].

**In vitro antibiofilm assay after Photoactivation:** To check whether there is any effect of light and dark on the activity of both formulations, both F1 and F2 were exposed to dark and light conditions. In case of photoactivation, after incubation and development of biofilm 100µL of compounds were added to test wells from higher to lower concentration**.** The plate was exposed to light for 15 minutes. And in case of dark reaction the compounds were added

in dark and covered with aluminum foil and incubated for 24 h at 28ºC. The rest of procedure is same as for attachment inhibition assay. The results of both assays were interpreted by using the above-mentioned formula.

**Cytotoxicity assay:** The cytotoxicity of formulations was determined by using sheep blood. 3cc sheep blood was diluted with 27mL phosphate buffer. Diluted sheep blood of 500µL was taken as a constant concentration. Both the formulations in different concentrations (20mg, 10mg and 5mg) were added into each Eppendorf tube. Triton X was used as a positive control whereas sheep blood without formulations was used as a negative control. The tubes were incubated for 2 hours at 37oC. Tubes were centrifuged at 1200 rpm for 15 minutes after incubation. After centrifugation supernatants were analyzed by spectrophotometer. Calculation was carried out by using the below formula:

**% Hemolysis = sample – negative control x 100/ positive control – negative control**

## RESULTS

### Confirmation and identification of Enterococci:

For confirmation, E. faecalis was cultured on Bile Esculin and incubated at 37°C for 24hrs. After incubation Brown point colonies appeared. The bacterial cells were stained according to the standard protocol of gram staining. After staining, the bacterial cells were observed under the microscope. All these characteristics matched with that of the E. faecalis.

**Table 1: Confirmation and identification of E. faecalis**

| S.no | Biochemical tests | Results |
|---|---|---|
| 1 | Catalase test | Negative |
| 2 | Oxidase test | Negative |
| 3 | Sulphate Indole Motility (SIM) | Negative |
| 4 | Growth on 4°C | Negative |
| 5 | Mannitol fermentation test | Positive |
| 6 | Sorbitol fermentation test | Positive |
| 7 | Salt tolerance test | Positive |
| 8 | Hemolysis on blood agar | Alpha hemolysis |

### Antibiotic susceptibility testing:

**Table 2: Zones of inhibition shown by different antibiotics**

| Antibiotics (concentration in µg) | Zones of inhibition |
|---|---|
| Ciprofloxacin (5) | Nil |
| Vancomycin (30) | Nil |
| Erythromycin (15) | Nil |
| Ampicillin (15) | Nil |
| Linezolid (15) | Nil |
| Fosfomycin (30) | 9mm |
| Gentamicin (20) | 8mm |

### Preparation of Curcumin loaded SEDDS formulations:

**Table 3: Composition of F1 and F2**

| **Components (F1)** | **Percentages** |
|---|---|
| Captex 355 | 20% |
| Chremophor EL | 30% |
| Tween 80 | 10% |
| Polyethylene glycol (PEG)400 | 25% |
| Caprylic acid | 15% |
| Curcumin | 10% |
| **Components (F2)** | **Percentage** |
| Captex 355 | 20% |
| Chremophor RH40 | 25% |
| Tween 80 | 10% |
| Polyethylene glycol (PEG)300 | 25% |
| Caprylic acid | 20% |
| Curcumin | 10% |

## Characterization of Curcumin loaded SEDDS formulations:

**Table 4: Characterization results of Cur-loaded SEDDS formulations**

| **Formulations** | **Mean droplet size(nm)** | **Polydispersity index (PDI)** | **Zeta potential(mv)** |
|---|---|---|---|
| F1 | 30.52nm | 0.148 | -6.70mv |
| F2 | 72.06nm | 0.159 | -5.62mv |

## Antibacterial activity of curcumin loaded SEDDS by using agar well diffusion method:

**Table 5: Zones of inhibition of F1 and F2**

| **Cur-loaded SEDDS formulations** | **Zone of inhibition(mm) ± SD** |
|---|---|
| F1 | 12 ±1.5 |
| F2 | 14 ± 1.0 |
| Gentamicin (Positive control) | 8 ± 0.1 |
| Solo Curcumin | 3.2 ± 0.1 mm |
| F1 without Cur | Nil |
| F2 without Cur | Nil |
| DMSO (Negative control) | Nil |

## MIC and MBC determination:

**Table 6: MIC and MBC values of F1 and F2**

| **Curcumin loaded SEDDS formulations** | **MIC value(mg/mL) ±SD** |
|---|---|
| F1 | 0.3± 2.5mg/mL (300 µg/µL) |
| F2 | 0.15± 1.1mg/mL (150 µg/µL) |
| **Curcumin loaded SEDDS formulations** | **MBC value(mg/mL) ±SD** |
| F1 | 0.3± 2.5 mg/mL (300 µg/µL) |
| F2 | 0. 15± 1.1mg/mL (150 µg /µL) |

**Biofilm assay:** Biofilm assay was performed in order to assess the biofilm forming isolates of E. faecalis. Out of 8 samples, 5 were biofilm formers and rest of the 3 were non biofilm producers as shown in table 7.

**Table 7: Biofilm producing isolates**

| Biofilm producing isolates codes | Category | Optical density |
|---|---|---|
| E. FBF1 | Strong | 6 × ODc <OD |
| E. FBF2 | Moderate | 2 × ODc < OD ≤ 4 × ODc |
| E. FBF3 | Weak | ODc < OD ≤ 2× ODc |
| E. FBF4 | Moderate | 2 × ODc < OD ≤ 4 × ODc |
| E. FBF5 | Weak | ODc < OD ≤ 2× ODc |

**In vitro Biofilm assay:** Biofilm assay was performed for which acrylic dental denture was used and E. faecalis was allowed to make biofilm. After incubation the results were noted. Among 5 biofilm producing isolates, one was strong biofilm former, 2 moderate and 2 were weak biofilm producing species.

**In vitro Antibiofilm assay:** The best activity was shown by both the formulations against weak biofilm producer as compared to moderate and high biofilm producing samples. The percentages of inhibition are listed in the table 8-9.

**Table 8: Percentage inhibition of E. faecalis by different concentrations of F1 in dark, light and non photo activated conditions.**

| F1in different conditions | IC50 values(mg/ml) | Percentage inhibition± SD |
|---|---|---|
| Non photoactivated | 0.6 | 71.2 ± 0.96 |
| Photoactivated | 0.1 | 77.7 ± 0.47 |
| Dark | 0.6 | 64.4±0.20 |

**Table 9: Percentage inhibition of E. faecalis by different concentrations of F2 in dark, light and non photo activated conditions.**

| F2 in different conditions | IC50 values(mg/ml) | Percentage inhibition± SD |
|---|---|---|
| Non photoactivated | 0.6 | 72.2 ± 0.96 |
| Photoactivated | 0.1 | 78.7 ± 0.47 |
| Dark | 0.6 | 64.4±0.20 |

## Cytotoxicity assay of F1 and F2 in Dark and Light

Cytotoxicity of Cur-loaded SEDDS i.e., F1 and F2 as shown in table 3.11 was determined by the formula given below:

**Percent hemolysis = Sample OD -- Negative control OD x 100**

**Positive control OD -- negative control OD**

**Positive control** (Triton x 100) = 2.02 OD

**Negative control** (Sheep blood) = 0.01 OD

**Figure 1-2: Percent hemolysis of F1 and F2 in different concentrations**

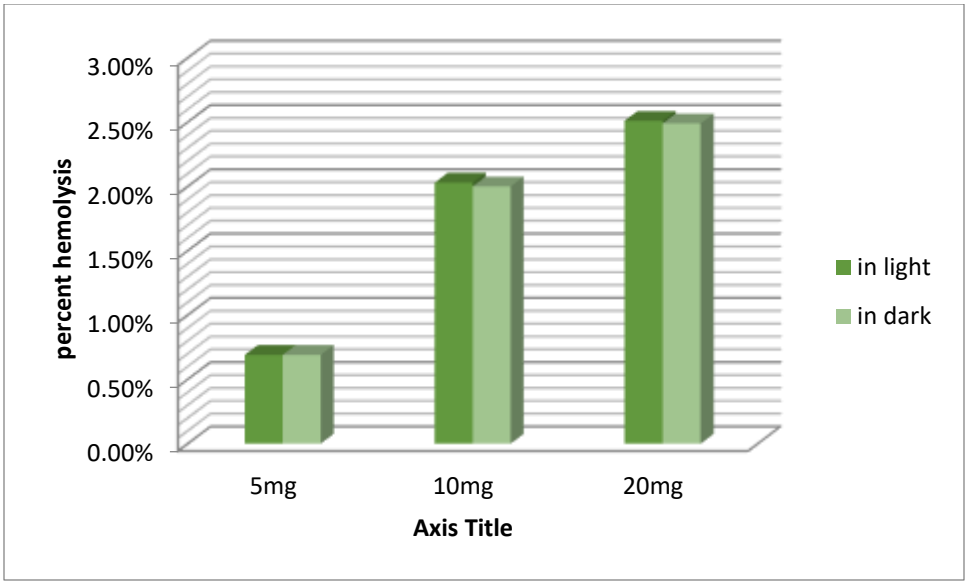


**Fig 1: Cytotoxicity percentages of F1 in dark and light**

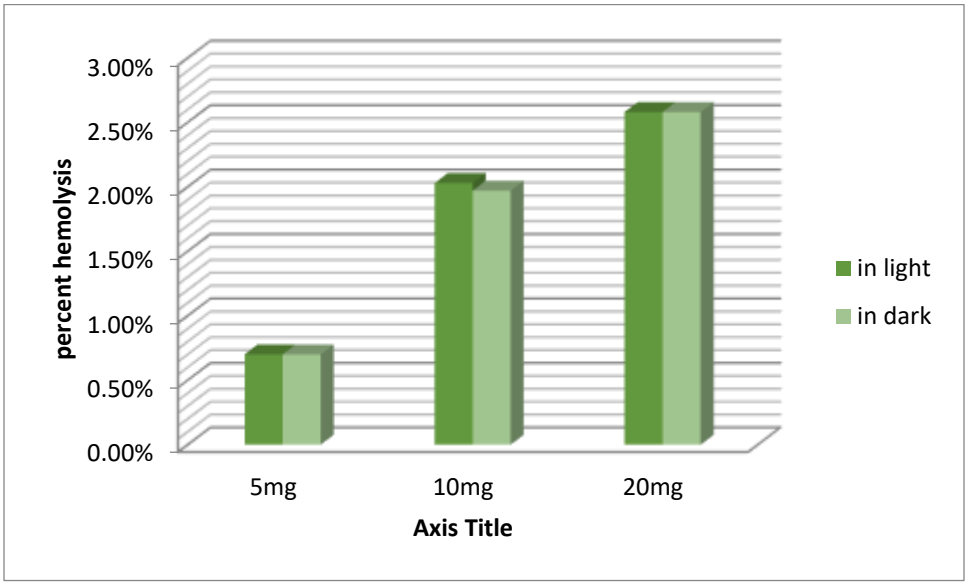


**Fig 2: Cytotoxicity percentages of F2 in dark and light**

## DISCUSSION

E. faecalis is non-spore-forming gram-positive bacterium which usually resides in gastrointestinal tract and oral cavity. It is a catalase-oxidase negative bacterium of the genus Enterococcus.[2, 3] Enterococci contribute to infections with various bacteria in the necrotic root canal. It is commonly encountered in botched root-filled dental operations and may remain as a single organism in the root canal.[3]

In this study E. faecalis was isolated from dental caries specimens. The Enterococcus family was first identified by proceeding its growth on bile esculin, whether the culture gives shinny grey colonies with breakdown of esculin to form iron ions, which resulted in affirmative manner. This identification test was correlated with the results of Collins et. el., (1993) in phylotypic analysis of Enterococci.[3] Gram staining, which is an essential protocol to confirm whether the bacterium has a thick or thin peptidoglycan layer making its cell membrane, in this current study the bacterium

showed a gram-positive nature, which according to Govindarajan et. al., (2022) was gram positive as well.[11] Catalase test was negative in the current study to confirm the enterococcal genre supported by identification study conducted by Hederstedt et.al., (2022).[12] Oxidase negative negative result shown by by Enterococcus also was confirm taking the report of Zang et.al., (2015) as a standard showing similar identification results.[52] Sulphate reduction, motility and conversion of tryptophan amino acid into indole was examined to confirm the E. faecalis after the confirmation of Enterococcus. In the current there was no sulphate reduction, motility and indole ring formation noticed as well which was justified by study of Collins et. al., (1997).[3] Mannitol and Sorbitol fermentation tests were conducted to confirm the E. faecalis strain more specifically along with a 4$^{o}$C growth test, which resulted in affirmative manner for mannitol and sorbitol fermentation but there was no growth of E. faecalis inoculums at 4$^{o}$C, where E. faecium showed a growth at same temperature. A similar study was reported in the Manero and Blanch (1999) and Collins et. al., (1993).[3,12]

Antibiotic susceptibility test was performed by using Kirby Bauer method and it was found that E. faecalis was highly resistant to ciprofloxacin, vancomycin, erythromycin, ampicillin and linezolid showing no zones of inhibition which is also shown in research studies of Thomas et.al., (2018) and Rkyadi's et.al., (2013) where E. faecalis was susceptible to Gentamicin and Fosfomycin with zones of 8mm and 9mm respectively. [157-158]

The cur-loaded formulations i.e., F1 and F2 were characterized for their droplet size, polydispersity index and zeta potential which was consisted of 1% (10 mg) curcumin and excipients as discussed above. F1 exhibited PDI, zeta potential and zeta sizer values of 0.148, -6.70mv and 30.52nm respectively, while F2 showed PDI, zeta potential and zeta sizer values of 0.159, -5.62 mV and 72.0nm respectively which was also shown in the study conducted by Momin et.al., (2019) where Cur-loaded SEDDS showed zeta size, zeta potential and PDI values of 34.6nm, -4.5 and 0.18 respectively.[165]

To address the antibacterial activity of Cur-loaded SEDDS were used. By utilizing drug delivery systems, these barriers can be addressed in order to improve bioavailability of curcumin. A study conducted by Hegg et al., (2011) used curcumin loaded alginate foams as a delivery system for curcumin against E. faecalis.[159] Similarly another study conducted by Zheng et.al., (2020) used colloidal delivery system for curcumin to enhance its antimicrobial properties.[160] In the current study where the formulations i.e. F1and F2 consisting of 10mg curcumin were subjected to examine its further activity with addition of different excipients i.e. Captex 355, Chremophor EL, Chremophor EL, Tween 80, Polyethylene glycol (PEG)400, Caprylic acid, where in the study of Shajari et. al., (2022) and Zheng et.al., (2020) only PEGs and Tween 80 were loaded in the formulations.[160,91]

In order to determine the antibacterial activity of Cur-loaded SEDDS F1 and F2, agar well-diffusion method was used which resulted in 12mm and 14mm of zones of inhibition, respectively. Furthermore, through broth microdilution method, where F1 showed MIC of 0.3 mg/mL and F2 showed MIC of 0.15 mg/mL. As per result of this study MBC value was same as MIC, which showed the bactericidal nature of Cur-loaded SEDDS at 0.3 mg/mL and 0.15 mg/mL. The MIC values of Cur-loaded SEDDS against E. faecalis reported by Neelakantan et.al., (2013) and Gulen et.al., (2016) was almost the same (0.6 mg/mL and 0.16 mg/mL).[162-163]

Dimethyl sulfoxide has an antimicrobial activity due to which, different dilutions of DMSO/distilled water were made in order to examine the concentration at which it has no activity against E. faecalis. DMSO was found to have no activity against E. faecalis. Similar study by Mechmachani et.al., (2022) has recently been conducted where DMSO doesn't have any effect on the growth of E. faecalis.[161]

To determine antibiofilm activity of F1 and F2 E. faecalis biofilm was created in 96 well flat bottom plate using LB broth and kept for 24hrs. following the protocol generated by Hamza et. al., (2020) and Gholibegloo et.al., (2022) where the protocol was used for production of Candida biofilm but in current it was for the production E. faecalis biofilm.[70,164]

Biofilm inhibition by cur-loaded SEDDS was determined against E. faecalis on the acrylic dental denture. F1 inhibited 66%, 67.5% and 71.2% biofilm formed by strong (E. FBF1), moderate (E. FBF2) and weak (E. FBF3) isolate of E.

faecalis whereas F2 inhibited 66.9%, 68.9%, and 72.6% biofilm formed by strong (E. FBF3), moderate (E. FBF2) and weak (E. FBF1) isolate of E. faecalis at highest concentration of curcumin. High rate of inhibition was observed at high concentration as compared to the low concentration of F1 and F2, which slightly showed difference in inhibition of biofilm of up to 70% where there was no strength i.e., strong, moderate and weak categories mentioned in terms of identifying the E. faecalis strains Hamza et. al., (2020) and Gholibegloo et.al., (2022).[70,164]

Finally, in this study, the cytotoxicity of F1 and F2 was determined to know how much hemolysis F1 and F2 cause to assure it can be further used as a therapeutic agent with low cytotoxicity by using photo spectrometer following photometry protocol at 570nm, where the average percentage did not exceed the toxic level which was 2.49% for F1 and 2.58% for F2. The level of toxicity of a compound was compared with the permissible level of cytoxicity of phenolic compound which should not exceed 5% mention in Gajalakshmi et. al., (2014) study.[166]

The formulations tested in this study effectively inhibited biofilm formation of E. faecalis. Both formulations exhibited promising results in inhibiting E. faecalis biofilm formation on acrylic dental dentures under non-photosensitized, photosensitized, and dark conditions. The high concentration of both formulations demonstrated a higher rate of inhibition compared to the low concentration. In non-photosensitized, photosensitized, and dark conditions, the IC50 value of F1 was 0.6mg/ml, 0.1mg/ml, and 0.6mg/ml, respectively, while the IC50 value of F2 was 1mg/ml, 0.3mg/ml, and 1mg/ml, respectively. These results indicate that light exposure enhanced the antibacterial activity of both formulations, but there was no significant decrease in activity under dark conditions. Additionally, the study evaluated the antibiofilm activity of free curcumin under both light and dark conditions, but no activity was observed at the IC50 values of F1 and F2 in all three conditions.

Best activity was shown by both the formulations against weak biofilm producer as compared to moderate and high biofilm producing samples as shown in the results section. It was found that inhibition capacity of Cur-loaded SEDDS reduced with the reduction in concentration.

## Conclusions

The present study investigated the feasibility of using an herbal compound to prevent dental caries caused by E. faecalis. The results revealed that curcumin-loaded self-emulsifying drug delivery system (SEDDS) formulations possess potential antibacterial activity against E. faecalis strains isolated from dental caries. Moreover, these formulations can effectively prevent and eliminate biofilm formation by E. faecalis under photosensitized, non-photosensitized, and dark conditions. Therefore, it can be inferred that curcumin-loaded SEDDS may serve as a viable alternative to conventional antibacterials for treating biofilm-producing strains of E. faecalis.

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