

RESEARCH ARTICLE

Antimicrobial Efficacy of Silymarin and Silibinin Against Oral Microorganisms

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ABSTRACT

Objective: To evaluate the antimicrobial effect of silymarin and silibinin, a plant derived flavonoid compounds against oral microorganisms that are responsible for dental caries.

Methods: In the current investigation, we analyzed the comparative antibacterial and antifungal effect of silibinin and silymarin against clinical cariogenic oral pathogens through evaluating their minimum inhibitory and minimum bactericidal concentration. We used clinical isolates that are responsible for dental caries and these include *Candida albicans*, *Enterococcus faecalis*, *Lactobacillus acidophilus*, *Staphylococcus aureus* and *Streptococcus mutans* to evaluate the anticariogenic potential of silymarin and silibinin.

Results: In the present investigation, silymarin exhibit good antimicrobial effect against most of the oral cariogenic microorganisms tested when compared to silibinin. Interestingly, silymarin shows high sensitivity at a concentration of <5 µg/ml against *S. mutans*, *L. acidophilus* and *C. albicans*. On the other hand, silibinin also have significant antimicrobial effect against the oral pathogens.

Conclusions: Silymarin and silibinin can be used as appropriate drug candidates that control dental caries, endodontic infections. *J Microbiol Infect Dis* 2017; 7(3):139-143

Keywords: Silibinin, Silymarin, Oral infections, Oral pathogens

INTRODUCTION

The oral cavity contains over and above 600 bacterial species. Oral microorganisms are present generally in the form of a biofilm and sustain ecological equilibrium with the host body. The ecological imbalance of biofilm inexorably manifested as various oral related infectious diseases that include tooth decay, dental caries, gingivitis, thrush, apical periodontitis, periodontal (gum) diseases, pericoronitis, and craniofacial bone osteomyelitis [1]. The ecological imbalance in oral microbiota is also said to an index of systemic diseases and is implicated with several ailments including cancer, diabetes, gastrointestinal diseases, rheumatoid arthritis, cardiovascular diseases, preterm birth and others. The above diseases have strong association with oral health and

hence, oral microbiota has been considered as a potential biomarker of human diseases [2,3].

Several strains of oral pathogenic bacteria such as streptococci and lactobacilli are responsible for initiating the formation of dental plaque, which plays an imperative role in the onset of dental caries and other periodontal related diseases in humans [3]. Dental plaque has been said to one of the important etiologic factor in dental caries [4]. The composition of dental plaque, a complex bacterial biofilm is governed by various factors including cell adherence, coaggregation, and growth and survival in the environment [5]. Increasing resistance to these microorganisms against present antimicrobial agents and adverse effects of these drugs is of major cause of concern [6]. Hence, there is an urgent need to develop alternative antimicrobial

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drugs for the treatment of infections obtained from medicinal plants to counteract the resistance and to minimize the adverse effects. Accordingly, several plant derived compounds have been investigated as promising agents to control and prevent oral ailments, in particular plaque-related diseases such as dental caries [7,8].

Silymarin (SIL) is a standardized extract from the dried seeds of the milk thistle (*Silybum marianum* L. Gaertn.), family Asteraceae. Silymarin contains approximately 70–80% flavonolignans and 20–30% polyphenolic compounds. The mixture of flavonolignans mainly consists of Silibinin (SBN), the major bioactive component of the extract, and isosilybin, silychristin, and silydianin and two flavonoids taxifolin and quercetin [9,10]. Both Silymarin and Silibinin have been reported to have wide spectrum of biological activities. These compounds have been tested for their *in vitro* and *in vivo* antiproliferative, antimicrobial, antiinflammatory, antioxidant, anticancer, free radical scavenging, membrane stabilizing properties and came out with promising results [11–13]. In light of the above reports it is reasonable to assume that the above flavonoids could have antimicrobial activity against oral pathogens. Hence, in this study, we investigated the antibacterial activity of Silymarin and Silibinin against oral bacteria.

METHODS

Drugs

Silymarin (C₂₅H₂₂O₁₀; CAS No. 65666-07-1) and Silibinin (C₂₅H₂₂O₁₀; CAS No. 22888-70-6) was purchased from Sigma Chemicals, India. It is a mixture of two diastereomers (silybin A and silybin B).

Clinical Isolates

Clinical isolates were obtained and experiments were approved by the institutional human ethical committee of Saveetha Dental & Medical College and Hospitals, Saveetha University, Chennai, India.

Preparation of Stock Solution and Test Solutions

Stock solutions of Silymarin and Silibinin were prepared at a concentration of 10 mg/ml with dimethylsulfoxide.

Determination of Minimal Inhibitory Concentration (MIC)

Antimicrobial activity of SIL and SBN were tested by minimum inhibitory concentration method (MIC) using micro broth dilution method. Briefly, 100 μ l of BHI (Brain Heart Infusion, HiMedia) broth was added to each well of 96-well microtitre plate and 100 μ l of the stock solutions of SIL and Silibinin were serially diluted to achieve the concentrations ranging from 16 to 0.5 μ g/ml. Ten μ l of the 0.5 McFarland standard turbidity adjusted bacterial or yeast suspensions were added to all wells [14]. Well without microorganism served as a control. Then, the plates were incubated at 37° C for 24 h. The assays were done in triplicate. Followed by the next day, 2–3 μ l of suspensions from each well were aseptically transferred to the sterile BHIA (Brain Heart Infusion Agar, HiMedia) plates corresponding to the dilutions. The lowest concentration of the Silymarin and Silibinin that completely inhibited the Silymarin growth of isolates in the agar plate was considered as MIC.

RESULTS

Antimicrobial effect of silymarin against oral dental pathogens

The antimicrobial activity of silymarin showed good inhibitory against *Streptococcus mutans*, *Lactobacillus acidophilus*, *Candida albicans*. Silymarin shows MIC value of 1 and 2 μ g/ml against *S. mutans* and *L. acidophilus*, respectively. While *Staphylococcus* and *Enterococcus* did not show any change in the maximum concentration tested i.e., 16 μ g/ml (Table 2).

Antimicrobial effect of silibinin against oral dental pathogens

In this study, Silibinin shows high sensitivity towards the *S. mutans* and *L. acidophilus*. The antimicrobial efficacy against *S. aureus* and *C. albicans* was almost similar i.e., 16 μ g/ml. The inhibitory efficacy of Silibinin against *E. faecalis* was not that much prominent with the maximum concentration tested in this study i.e. 16 μ g/ml (Table 2).

Comparative antimicrobial efficacy of SIL and Silibinin against oral dental pathogens

In this study, we evaluated the antibacterial efficacy of Silymarin and Silibinin against different oral pathogens. We found that Silymarin has high sensitivity against *S. mutans*, *L. acidophilus*, and *C. albicans* with the MIC of 1, 2, 4 µg/ml, respectively. On the other hand, Silibinin shows MIC values of 4, 8, and 16, respectively. These results clearly indicate the fact that minimal concentration of Silymarin has higher efficacy than that of Silibinin against organisms we tested.

Table 1. List of clinical isolates used.

Sample No	Clinical Isolates
1	<i>Candida albicans</i>
2	<i>Enterococcus faecalis</i>
3	<i>Lactobacillus acidophilus</i>
4	<i>Staphylococcus aureus</i>
5	<i>Streptococcus mutans</i>

Table 2. Antimicrobial effect of Silymarin and Silibinin against different clinical isolates.

Sample No	Organism	MIC (µg/ml) Silymarin	MIC (µg/ml) Silibinin
1	<i>S. aureus</i>	>16	16
2	<i>E. faecalis</i>	>16	>16
3	<i>C. albicans</i>	4	16
4	<i>L. acidophilus</i>	2	8
5	<i>S. mutans</i>	1	4

DISCUSSION

Oral microbiota is linked with various systemic ailments such as cancer, diabetes, rheumatoid arthritis, cardiovascular diseases, and preterm birth and it is a potential biomarker for these diseases [2]. Regardless of age dental caries is

one of the common chronic diseases affects people worldwide and oral bacteria such as *L. acidophilus*, *E. faecalis* and *S. mutans* are main factor that initiates caries [15].

The incidence of fungal infection has augmented significantly in the past two decades. Among different fungal species *Candida* is one of the most widespread and threatening fungal pathogens in HIV-infected individuals and it is also responsible for the majority of other invasive and non-invasive fungal infections. Oral candidiasis remains the most common oral lesions in HIV infected patients [16]. Among the *Candida* species *C. albicans* is responsible for the cause of invasive oral candidiasis, superficial and deep tissue fungal infections [17].

Development of resistance of microorganism against existing antimicrobial agents is one of the causes of concern among scientists and physicians worldwide. Accordingly, various pathogenic microorganisms responsible for diseases are more difficult to treat with the existing drugs [6]. Moreover, some commonly used narrow and broad spectrum antibiotics are associated with various adverse effects on host including hypersensitivity, immune suppression and allergic reactions, drug-drug interaction, photosensitivity, teratogenicity etc., [18]. To overcome the above obstacles in current antimicrobial drugs and to obtain more efficacious drugs with minimal or without any adverse effect, an antimicrobial drug having a novel mode of action should be warranted [19].

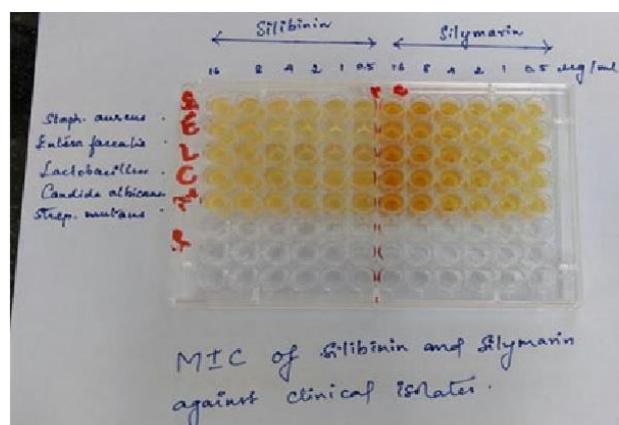
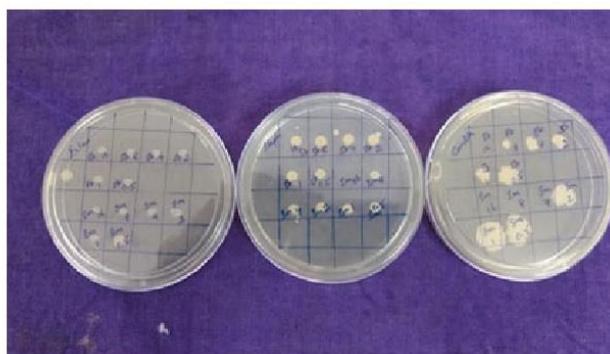


Figure 1. Methodology of experiment.



On the other hand, flavonoids are class of plant constituents that have received increasing interest over the last decades. These polyphenols and flavonoids compounds obtained from various medicinal plants have been reported for their beneficial antimicrobial efficacy [20]. It has been reported that some flavonoids compounds are formed in plants as antimicrobial barriers response to microbial infection. Hence, it is not surprising that these flavonoid compounds found in vitro to be effective as an antimicrobial agents against various pathogenic microorganisms [20].

Silymarin, such a plant derived flavonoid compound shows high efficacy against oral dental pathogens tested in this study. It shows more sensitivity against *C. albicans*, *L. acidophilus* and *S. mutans* in its low concentrations than Silibinin. Silymarin has MIC with <5 µg/ml vs *C. albicans*, *L. acidophilus* and *S. mutans*, these microorganisms play pivotal role in the onset of dental caries and oral candidiasis. It is reported that these flavonoids have capability to complex with extracellular, soluble proteins and cell wall of microorganism, due to high lipid soluble nature of flavonoids also disrupts bacterial cell wall and fungal membranes [21-23]. The synergistic effect of flavonoids combined with commonly utilized antibiotics is also reported previously [13]. In light of the above reports, it is suggested that the profound antimicrobial efficacy of Silymarin and Silibinin found in this study could be attributed due to their interference with the cell wall of the above tested microorganisms.

It has been reported that systemic *C. albicans* infections are fatal in 42% of cases [24,25], despite the use of antifungal therapies, and *C. albicans* is the fourth most common infection in hospitals [26,27]. Fluconazole is a potent and broad-spectrum antifungal agent. It is active against many *Candida* species. However, it has few side effects and this drug has developed resistance to *C. albicans* over time [28]. In the present study, Silymarin inhibits the growth of *C. albicans* in its low concentration indicate the fact that this drug can be a therapeutic alternative for *C. albicans* infection. However, studies are needed in detail to prove the exact mechanism of action of these plant derived compounds used in this study.

In conclusion, the present study highlights the antimicrobial efficacy of Silymarin and Silibinin. Silymarin and Silibinin can be used as an appropriate drug candidate to control dental caries, endodontic infections.

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Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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