International Journal of Dentistry and Oral Science (IJDOS)
ISSN: 2377-8075

Antibacterial Efficacy Of Emodin From Polygonum Cuspidatum Against Oral Pathogens - In-Vitro Study

Research Article

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Abstract

Introduction: Oral diseases are among the major public health problems with dental caries and endodontic infection are the commonest affecting the mankind. The natural phytochemicals isolated from plants used as traditional medicines are considered as good alternatives than commercially available chemicals which have undesirable side effects. Emodin, a anthroquinone extracted from the roots of Polygonum cuspidatum was already proven to have antiviral, antibacterial and antifungal effects. Hence this present invitro study was conducted with the aim of determining the antibacterial efficacy of emodin against oral pathogens.

Materials and Methods: Then 25 mg Emodin was taken in a falcon tube and dissolved in 10 ml of Dimethylsulfoxide. The tube filled with this mixture was subjected to an agitator vortex mixer to mix emodin and dimethylsulfoxide effectively. After which the pipette was loaded with 25 microlitre, 50 microlitre, 100 microlitre, 200 microlitre. The prepared emodin extract was immersed in the
diffusion well of organisms Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Enterococcus faecalis, Candida albicans and zone of inhibition was measured.

Results: The zone of inhibition was measured for all the micro organisms loaded with different concentrations of emodin. Staphylococcus aureus showed activity in 100 microlitre and 200 microlitre concentrations than the other organisms. Secondary to Staphylococcus aureus, S.mutans also showed good activity with emodin in 100 and 200 microlitre concentrations. The effect of the anthroquionone emodin, was also present in rest of the organisms C.albicans, E.faecalis, L.acidophilus.

Conclusion: The study concludes that emodin had a better antibacterial efficacy against oral pathogens and hences proves that emodin can be used as a novel therapeutic agent for the prevention and control of oral diseases.

Keywords: Emodin; Polygonum cuspidatum; Anthraquinone; Phytochemicals.

Introduction

Oral diseases are among the most prevalent diseases globally and have serious health and economic burdens, greatly reduced quality of life for those affected [1]. The most prevalent and consequential oral diseases globally are dental caries, endodontic infections, periodontal disease, tooth loss, cancers of lips and oral cavity [2-4]. Dental caries is a biofilm-mediated, diet modulated, multifactorial, non-communicable, dynamic disease resulting in net mineral loss of dental hard tissues [5]. Dental biofilm is the most common cause for dental caries. Dental biofilm is a consortium of microorganisms that stick to a tooth surface [6]. The microorganisms are embedded in an extracellular polymeric matrix. Streptococcus mutans is considered as a crucial pathogen in the development of dental caries. The major factors responsible for the cariogenicity of this pathogen include its ability to produce glycosyltransferases, synthesize insoluble glucans , survive at low pH thus maintaining the oral environment acidic and prone to demineralisation of teeth [7, 8]. Staphylococcus aureus survives

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Received: April 16, 2021
Accepted: September 20, 2021
Published: September 21, 2021

Citation: Sarika Balaganesh, Jayashri Prabakar, Meignana Arumugham Indiran. Antibacterial Efficacy Of Emodin From Polygonum Cuspidatum Against Oral Pathogens - In-Vitro Study. *Int J Dentistry Oral Sci.* 2021;8(9):4414-4417. doi: http://dx.doi.org/10.19070/2377-8075-21000899

in a metabolically inactive state under harsh conditions and also contributes to the biofilm formation. S.aureus resist host defences or antibiotics [9]. Enterococcus faecalis is commonly detected in asymptomatic and persistent endodontic infections [10]. Candida albicans prevalence is significantly higher in children with early childhood caries and also has a major role in endodontic treatment failure [11]. Lactobacilli appears to be a planktonic, opportunistic settler that can gather and multiply in the oral cavity and cause dental caries.[12]

Despite advances in the development of invasive treatment these therapeutic strategies are often unable to control the progression of Dental caries. It has been reported that the use of natural products is one of the most successful strategies for the discovery of new techniques to prevent dental caries [13]. Herbal extracts are used for the treatment of various dental disorders [14, 15]. They are the effective alternative to antibiotics and represent a promising approach to prevention and therapeutic strategies for various oral infections. Herbal medicines have less side-effect in comparison with traditional medicines [16]. Polygonum cuspidatum is a herb widely distributed in China, Japan, Korea and North America and also reported to have enormous medicinal benefits [17]. Recent studies demonstrated that the herb also had antiviral, antibacterial and antifungal effects [18, 19].

The extracted components from Polygonum cuspidatum herb showed good antibacterial property. Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is a major active component commonly present in Polygonum cuspidatum. It has been reported that emodin exhibits a wide range of biological activities including antibacterial, anticancer, anti-inflammatory, anti-diabetic and anti-oxidative activities [21]. Thus this study was conducted to make a further exploration of antibacterial compound Emodin from P.cuspidatum against oral pathogens as these pathogens are the essential contributors for poor oral health related quality of life.

Materials and Methods

Study setting

Invitro study.

Ethical approval

Saveetha Institute of review board.

Preparation of Emodin extract

Emodin is an anthraquinone derivative extracted from the herbs of polygonum cuspidatum. Emodin was purchased from a pharmaceutical company (Fig 1). Then 25 mg of this anthraquinone was taken in a falcon tube and dissolved in 10 ml of Dimethylsulfoxide. (Fig 2) The tube filled with this mixture was subjected to an agitator vortex mixer to mix emodin and dimethylsulfoxide effectively. After which the pipette was loaded with 25 microlitre, 50 microlitre, 100 microlitre, 200 microlitre.

Microbial analysis

Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Enterococcus faecalis, Candida albicans were cultured and the loaded pipette with emodin mixture was placed in each culture plate of the microorganisms. The culture plates were kept for 24 hours and checked for the activity of the microorganisms (Fig 3). After 24 hours the zone of inhibition for all the organisms were measured.

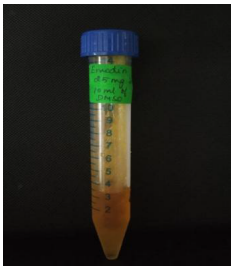
Results

In this study, the antibacterial effect of emodin on S.mutans, S.aureus, E.faecalis, C.albicans, Lactobacillus were investigated. As shown in the Fig 3, growth of all pathogens was significantly reduced in the presence of emodin. This effect was revealed to be concentration dependent.

To determine the inhibitory effect of emodin on production of acid for all pathogens, the organisms were treated with different concentrations of emodin. The zone of inhibition was measured for all organisms using vernier caliper by determining the diameter of the inhibited site. The different concentrations used were the 25 microlitre, 50 microlitre, 100 microlitre, 200microlitre and there was a significant zone of inhibition.[Fig 3]. The measured

Figure 1. Emodin - powder form.

Figure 2. Preparation of Emodin extract.



zone of inhibition was tabulated [Table 1]and graphically represented [Fig 4].

This study proved that emodin was effective against all the oral pathogens. Streptococcus mutans and Staphylococcus aureus are the predominant organisms causing dental caries. Hence emodin can be used to prevent the formation of dental caries. Future studies can be done to detect the remineralization potential of emodin so that it can be used with dentifrices to promote better oral status.

Discussion

Considering the high incidence rate of dental caries and its detrimental effects in oral cavity, the development of novel strategies for its prevention and control are required [22]. Previous studies have demonstrated that natural products are promising candidates for novel anti cariogenic substances [23]. The present study revealed that emodin,a natural product ,interfered with key cariogenic factors of *S.mutans and S.aureus*. Emodin (1,3,8 –trihydroxy-6-methylanthraquinone) has demonstrated a broad range of antibacterial effect [24]. The present sudy revealed that emodin had excellent activity against the oral pathogens in vitro.

Emodin, a component derived from the roots and rhizomes of a number of plants including Polygonum cuspidatum and Rheum undulatum [25]. Polygonum cuspidatum had wide range of antibacterial activites. The components of Polygonum cuspidatum are the polydatin, reverastrol,anthraglycoside B and emodin [26]. All these components was proved to inhibit the glycolytic acid production and Gtf activity of S.mutans and Streptococcus sobrinus [27]. The dichloromethane fraction from R.undulatum, composed mainly of aloe-emodin, emodin, chrysophanol and physcion, has revealed inhibitory effects on the production of glycolytic acid by S.mutans on biofilms [28]. The other study done to test the anticariogenic property of emodin of Streptococcus mutans and the development of caries in rats revealed that the topical application of emodin reduced the incidence and sever-

Figure 3a. Streptococcus mutans with emodin extract.

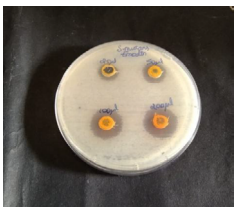


Figure 3b. Enterococcus faecalis with emodin extract.

Figure 3c. Staphlococcus aureus with emodin extract.

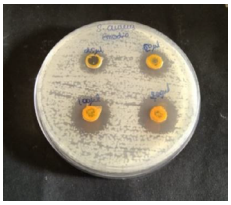


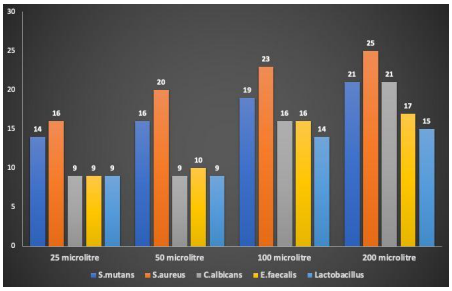
Figure 3d. Candida albicans with emodin extract.

Figure 3e. L.acidophilus with emodin extract.

Table 1. Antimicrobial activity (Zone of inhibition) of the micro organisms.

Organisms	25 microlitre	50 microlitre	100 microlitre	200 microlitre
S.mutans	14mm	16mm	19mm	21mm
S.aureus	16mm	20mm	23mm	25mm
C.albicans	9mm	9mm	16mm	21mm
E.faecalis	9mm	10mm	16mm	17mm
Lactobacillus	9mm	9mm	14mm	15mm

Figure 4. Zone of inhibition against oral pathogens.



ity of carious lesions in the rats without affecting the percentage of S.mutans in the biofilms [29]. Previous study also suggests that emodin markedly suppressed the production of acid and the synthesis of insoluble glucan by *S.mutans* ATCC25175. The most important pathogenic property of S.mutans is the synthesis of insoluble glucans. Insoluble glucans promote the adhesive interaction of bacteria with tooth surface and contribute to formation of dental biofilm [30]. Accordingly this study was done to examine the antibacterial property of emodin against S.mutans and the rest of oral pathogens.

In the current study, emodin had antibacterial efficacy against the oral pathogens tested in this study. These results suggest that emodin may be responsible for the antibacterial activity of microorganisms tested. Since the literature says that matrix metalloproteinases, are involved in the pathogenesis of dental caries [31]. Emodin has demonstrated to have inhibitory potential against matrix metalloproteinases invitro and invivo [32]. So the results obtained in this study may also suggests that emodin may have the antibacterial efficacy as they inhibit the activity of matrix metalloproteinases. Future studies may be needed to support this point on emodin is active againt the matrix metalloproteinases.

Summarizing the study, Emodin significantly attenuated the growth of the oral pathogens in vitro. Hence this result suggests that emodin may be used as a novel therapeutic agent for the oral infections threatening the mankind.

Conclusion

The results of the present study proved that emodin which is the component of Polygonum cuspidatum had better antibacterial efficacy against the oral microflora which gives an insight as this component can be used as an anticariogenic agent.

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