
Evaluation of antimicrobial activity of Curcumin against two oral bacteria

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Abstract: Oral pathogenic gram-positive bacteria, *Streptococcus pyogenes* (*Strept.pyogenes*) are caused severe human diseases with complications, including rheumatic fever, sepsis, severe soft-tissue invasion, and toxic-shock-like syndrome (TSLs). Whereas, *Streptococcus mutans* (*Strept. mutans*), described a key causative agent of caries and infective endocarditis. Therefore, the aim of this study was to evaluate the inhibitory effect of Curcumin on *Strept. mutans* and *Strept.pyogenes* growth in comparison with the antibiotic Ciprofloxacin using well diffusion method. Minimum Inhibitory Concentration (MICs) showed that curcumin-mediated growth inhibition of *Strept. mutans* and, *Strept. pyogenes*. Results showed that curcumin significantly inhibited the activity of *Strept. mutans* and *Strept. pyogenes* growth with inhibition zones, 9.7mm and 10.2 mm, respectively while the inhibition zone of Ciprofloxacin 15.52mm to 13.4mm against *Strept. mutans*, *Strept. pyogenes* respectively. The antimicrobial activity of curcumin on *Strept. mutans* and *Strept. pyogenes* growth suggests that the curcumin may be useful for controlling dental biofilms, subsequently dental caries formation. This study provides significant insights into the therapeutic effect of curcumin against pathogenic gram positive bacteria, suggesting its potential as an alternative therapy, and opens the way for further studies on identification of novel antimicrobial targets of curcumin.

Keywords: *Curcumin*, Antibacterial Activity, Minimum Inhibitory Concentration (MIC)

1. Introduction

Curcumin is the most explored of the so called curcuminoids, a family of chemo-preventive substances present in the spice, which increasingly being mentioned in health journals and scientific papers as research on it grows (1,2, 3). Recent studies suggest that it may help to ease digestive discomfort, reduce inflammation and hereby to increase resistance to disease (2). Oral pathogenic bacteria are involved in periodontal, vascular diseases(4), and respiratory infections(5). Researchers were found that the plant extracts have potential antimicrobial effects against microorganisms [6, 7]. The increasing failure of chemotherapeutics and antibiotic resistance on pathogenic microbial growth has led to the screening of new tools that have a potential antimicrobial activity [7,1]. Indeed, plants are natural sources of antibacterial agents. Plant-derived medicines have been a part of our traditional health care system, and the antimicrobial properties of plant derived compounds are well documented. Herbal medicines are more

effective and less harmful as they have negligible side effects. They exhibit low mammalian toxicity and can be handled [9, 10]. In addition, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. Therefore, there is a significant demand to develop an alternative antimicrobial drugs for the treatments of infectious diseases from medicinal plants [11, 12]. Curcumin is the most abundant polyphenol present in the dietary spice turmeric. Turmeric powder, derived from the rhizome of *Curcuma longa*, which is associated to the Zingiberiaceae family [13], commonly used as a spice, food preservative, and food-coloring agent [14,15]. Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione; Diferuloylmethane], a yellow bioactive pigment, is the major component of turmeric. The dried rhizome is a rich source of beneficial phenolic compounds known as the turmeric [16]. Curcumin described as prebiotic, which have beneficial effects on human health (17). Prebiotics and probiotics have diverse effects on human health (1,19,20). Turmeric as a

part of prebiotics has a long history for therapeutic use. It has been used for various purposes and through different routes of administration. It has been used topically on the skin for wounds, blistering diseases such as pemphigus and herpes zoster, for parasitic skin infections, and for acne. It has been used via oral administration for the common cold, liver diseases, urinary tract diseases, and as a blood purifier [3]. For chronic rhinitis and coryza, it has been used via inhalation [21, 23]. Throughout the most important effects, curcumin had shown a wide spectrum of biological actions such as anti-inflammatory agents, thereby a wide range of pharmacological uses [24, 26]. The meta analysis data showed that the curcuminoids may be effective in controlling dental biofilms and dental cavity formations, suggested that turmeric extracts can be extensively used in the treatment premalignant lesions in oral cavity [27]. Turmeric can be used in relief from pain and bleeding of gingival in gingivitis and periodontitis, as colorant in pit and fissure sealant or in dental-plaque detection system. Chemopreventive activity of curcumin is observed when it is administered prior to, during, and after carcinogen treatment as well as when it is given only during the promotion/progression phase of colon carcinogenesis [27, 1]. In this study, curcumin showed a potential effect on oral gram positive bacteria.

2. Material and Methods

2.1. Curcumin Extraction

The dried rhizomes of curcumin were crushed with pestle and mortar. The powder was weighed and extracted with methanol. They were soaked in the solvent for 3 days and then filtered with Whatman filter paper (pore size 0.2µm). The residue of extraction was re extracted by soaking in the same solvent for three times. The combined filtrate was concentrated and weighed. The solvent was added to make final concentration of the extract as 100 mg/ml; same amounts of extracts were used for further studies such as antibacterial activity, and minimum inhibitory concentration (MIC).

2.2. Screening for Antibacterial Activity

Firstly, oral bacteria isolated (from 56 saliva patients) suffering from caries and pharyngitis on mitis salivarius plus bacitracin medium and blood agar for *Strept. mutans* and *Strept. pyogenes*, respectively and identified by API-20 Strep. System (bioMérieux, France). Secondly, the antibacterial activity was determined by using micro well dilution methods, in addition to using the Kirby-Bauer method [29]. Bacterial cultures were first grown on nutrient broth at 37°C for 18-24 h, incubated till turbidity became equivalent to McFarland 0.5 turbidity standard was obtained. The inoculates of the particular bacteria were streaked on the Muller Hinton Agar (MHA) (Oxoid, UK) plates using a sterile swab in order to ensure a uniform thick lawn of growth following incubation. Wells of 6mm in diameter were formed on Muller Hinton agar plates. The dried plant extract was

dissolved in dimethylsulfoxide (DMSO) to assume a concentration of 30 mg/ml. Wells were cut into the agar and filled with 75µl of the plant extracts and one well with (DMSO) as negative control. Inoculated plates were incubated at 37°C for 18- 24hrs, and resulting diameters of inhibition zones were measured. These studies were performed in triplicate for each plant extract. The diameter of the inhibition zone around each well was taken as a measure of antibacterial activity. The antibacterial activity results were expressed in term of the diameter of inhibition zone and <9mm zone was considered as inactive form; 9-12 mm as partially active form; while 13-18 mm as active form and >18mm as very active form [30]. The mean and standard deviation of the diameter of inhibition zones were calculated.

2.3. Determination of Minimum Inhibition Concentration (MIC) of the Extract

The MIC was defined as the lowest concentration that completely inhibited the growth of microorganisms for 24 hours. Bacterial strains were grown overnight on MHA plates at 37°C before being used. The antimicrobial activity of extract was examined using the standard broth dilution method. The MIC was determined in MHA broth using serial two-fold dilutions of extract in concentrations ranging from 200 to 12.5% (V/V) initial bacterial inoculums of 10⁶ CFU/ml and the time and temperature of incubation being 24 h at 37°C, respectively. The MIC is the lowest concentration of antimicrobial agents that completely visually inhibits growth of the microorganisms. The MIC measurement was done in triplicate to confirm the value of MIC for each tested bacteria [31].

2.4. Minimal Bactericidal Concentration (MBC)

After MIC determination of the extract tested, aliquots of 50 µl from all tubes in which no visible bacterial growth was observed were seeded in MHA plates and were incubated for 24 h at 37°C. The MBC endpoint is defined as the lowest concentration of antimicrobial agent that kills 100% of the initial bacterial population. The number of plates without colonies was noted.

2.5. Statistical Analysis

Data were analyzed using analysis of variance (General Linear Model, Minitab version 16).

All means are presented with the appropriate standard deviation. Significance was set at the confidence intervals of 0.05.

3. Results and Discussion

Herbal plants have a source of medicinal compounds since ancient times. Plant extracts are used in different systems of medicine for the treatment of various human ailments, and for treatment of viral and fungal infections [32]. The effects of plant extracts on bacterial growth have been investigated in different parts of the world. It has been suggested that

aqueous and ethanolic extracts from plants are a potential source of antiviral, anticancer and antimicrobial agents. In the present work, the results showed that the antibacterial activity of turmeric plant. Curcumin exhibited very good antibacterial activities (zone of inhibition in mm) against *Strept. mutans* and *Strept. pyogenes*. The extract of curcumin was effective in inhibiting the two pathogenic bacteria with zone of inhibition, 9.7 mm and mm10.2 against *Strept. mutans*, *Strept. pyogenes*, respectively. While, the inhibition zone of Ciprofloxacin 15.52mm to 13.4mm against *Strept. mutans*, *Strept. pyogenes* respectively table (1) and (Figure 1, 2).

Table 1. Inhibition zone (mm) of methanolic extract of Curcumin against *Strept. mutans* & *Strept. pyogenes* compared with Ciprofloxacin.

Agent or inhibitor	<i>Strept. mutans</i>	<i>Strepto. pyogenes</i>
Curcumin	9.7±2.7*	10.2±2.1
Ciprofloxacin	15.52±3.10	13.4±3

The mean of inhibition zone mm ±SD, * significant at ≤ 0.05

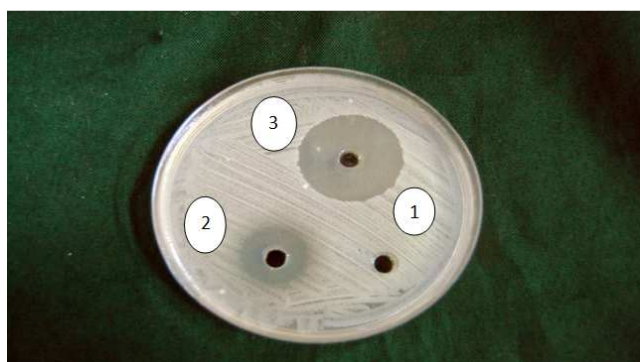


Figure 1. Inhibition zone (mm) of curcumin extract against *Strept. mutans* compared with Ciprofloxacin. 1=negative control, 2=curcumin. 3=Ciprofloxacin.

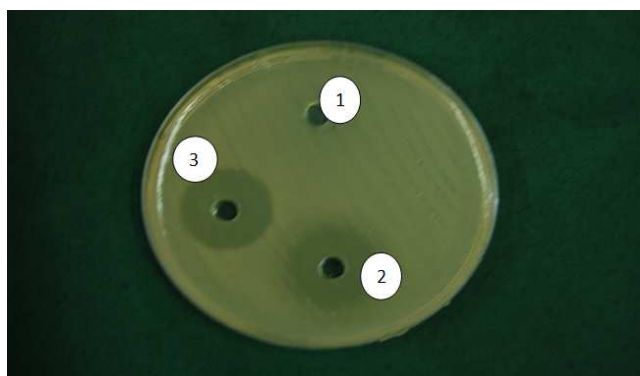


Figure 2. Inhibition zone (mm) of curcumin extract against *Strept. pyogenes* compared with Ciprofloxacin. 1=negative control. 2=curcumin. 3=Ciprofloxacin.

It is interesting to annotation that G+ve bacterial isolates were sensitive to curcumin extract. Present study is in agreement with Chattopadhyay et.al who reported that curcumin extract produce antibacterial activity against a broad range of microbes and especially G+ve strains and multiple antibiotic resistant bacteria. These results, also correlates with Shahi et.al who reported that curcumin

exhibited potent growth inhibitory effect against Gram-positive bacteria, (*Staph. aureus* and *Strept. mutans*), Gram negative (*E. coli* and *P.aeruginosa*) and pathogenic yeast *C. albicans*.

During extraction process, solvents diffuse into the solid plant material and soluble compounds of similar polarity. The polarity of solvent affects quantity and composition of secondary metabolite of an extract. Traditional healers primarily use water for extract preparation from plant extracts but organic solvents have been found to give more consistent antimicrobial activity compared to water extracts [33]. The antimicrobial activities of this plant have already been studied with different microorganisms.

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