

ESTIMATION THE ANTIBACTERIAL EFFECT OF CURCUMIN AND ROSEMARY AMONG PATIENTS WITH DENTAL CARIES *IN VITRO*

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ABSTRACT

Objective: The aim is to estimate the effect of curcumin and rosemary as antibacterial agents among dental caries cases.

Methods: Samples of saliva were randomly collected from 40 patients in Al-Furat General Hospital who attended the hospital from July to September 2018. Swabs were cultured on blood agar at 37°C for 24 h and then subcultured in mannitol salt agar and trypticase soy broth at 37°C for 24 h. Different concentrations of aqueous extract curcumin solution (0.1, 0.3, 0.5, and 1 mg/ml) and rosemary solution (1 g/ml) were prepared and added to the bacterial culture. Later, minimum inhibition zones of the bacterial cultures were determined.

Results: The results showed that there were 25 cases of *Streptococcus mutans*, 10 cases of *Staphylococcus aureus*, 3 cases of anaerobic bacteria, and 2 cases of normal flora among 40 culturing swabs of bacteria. Aqueous extract of curcumin showed antibacterial effect with concentrations (0.1, 0.3, 0.5, and 1 mg/ml) against oral bacteria; nevertheless, these bacteria were resistant to the aqueous extract of rosemary with concentration 1 g/ml.

Conclusion: It can be concluded that curcumin can be an effective antibacterial agent against dental caries disease and its effect increases positively in relation to its concentration. On the other hand, rosemary with 1 g/ml concentration did not show any effect on oral bacteria.

Keywords: Curcumin, Rosemary, Dental caries.

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INTRODUCTION

Teeth caries and cavities are the major forms of mouth health problem approximately 36% of the population worldwide have dental caries in their permanent teeth [1]. *Streptococcus mutans* is a pathogen bacterium associated with dental caries and cavities. *S. mutans* bacteria form a colony on teeth surface as dental plaque and convert extracellular polysaccharide to lactic acid through fermentation and eventually caused teeth caries [2,3]. Toothpaste and mouthwash which are used in mouth care can contain natural components as thymol or synthetic components like chlorhexidine; however, those containing synthetic ingredients are usually associated with teeth staining [4,5].

Turmeric (the common name of curcumin) is cultivated in India and other parts of Southeast Asia. It has a long history used as an anti-inflammatory in Ayurvedic medicine [6]. Curcumin is lipophilic, but it is quite stable in acidic pH of the stomach. Due to its low rate of absorption, curcumin is often formulated with bromelain for higher absorption [7-11]. Curcumin is showed anti-inflammatory, antioxidants, antimutagenic, and antimicrobial activity [6,12,13,14,15-18]. In addition, curcumin can be applied in the dental field as in dental pain by massaging the aching teeth, mouthwash, periodontal problem by topical application, subgingival irritant, scaling, and root planning [19-22].

Rosemary (*Rosmarinus officinalis*) is a common aromatic evergreen shrub grown in many parts of the world. It showed antioxidant and anti-inflammatory effects against different types of bacteria. The fresh and dried leaves are mostly used as a food preservative and flavoring agent in traditional Mediterranean cuisine [23-25].

Herbal therapies such as curcumin and rosemary that showed powerful antioxidant and anti-inflammatory activity can be used for the prevention and treatment of dental caries disease as well as to overcome

the problem of teeth staining accompanied using a mouthwash containing synthetic substances due to their natural components.

METHODS

Sample collection

This study was conducted with 40 patients attended the Al-Furat General Hospital/Dentistry Department in Baghdad, Iraq, from July to September 2018. Protocol approval was achieved from the Al-Furat General Hospital; furthermore, the Ethical Committee Approval was achieved for the protocol before starting the study. Swabs were cultured on blood agar plates at 37°C for 24 h and then subcultured in mannitol salt agar and trypticase soy broth at 37°C for 24 h. Later, bacteria types were confirmed by typical morphologies under a dissecting microscope.

Plant extraction

Preparation of aqueous extract of curcumin

Curcumin leaves were bought from the local market. 100 g of curcumin crushed and converted to powder and added to 1000 ml of boiling water in a sterilized conical flask over low flame for 15 min. The flask was then plugged and removed from the heat and allowed to cool. Later, the contents of the flask were filtered to prepare the required concentrations of 0.1, 0.3, 0.5, and 1 mg/ml [26].

Preparation of aqueous extract of rosemary

Rosemary leaves were bought from the local market, crushed and converted to powder. 50 g of leaves powder were extracted for 3 h in 500 ml of the sterilized distilled water using the Soxhlet device, and the source of heating was a boiling water bath 100°C. The leaf extract solution was then evaporated at 45°C using a rotary evaporator, and the resultant crude extract was frozen at -20°C until use to prepare the required concentration of 1 g/ml [27,28].

Minimum inhibitory concentration determination

Minimum inhibition concentration was determined for bacterial cultures after the addition of different concentration of curcumin and rosemary aqueous extract solution.

Data analysis

Data were expressed as mean \pm standard deviation with $p < 0.01$ to be considered as highly significant where $p < 0.05$ to be considered as significant. Chi-square and unpaired *t*-test were used to compare the concentrations of curcumin and minimum inhibition zone.

RESULTS

A total of 40 swabs were cultivated on blood agar at 37°C for 24 h and then were subcultured in mannitol salt agar and trypticase soy broth at 37°C for 24 h. Fig. 1 reveals that the most frequently isolated bacteria by culture was *S. mutans* (62%), followed by *Staphylococcus aureus* (25%), anaerobic bacteria (8%), and normal flora (5%). Fig. 2 reveals the number of different types of bacteria that were identified from the 40 swabs.

According to the effects of curcumin concentrations on bacterial growth, it has been found that the inhibition zone diameter of bacterial growth increases with increase in the curcumin concentration (Fig. 3). On the other hand, the rosemary did not show any effect on the bacterial growth with the concentration 1 g/ml.

DISCUSSION

Dental caries is the most common oral disease worldwide. It can be caused by different multifactorial, most of them are the presence of endogenous bacteria such as *S. mutans* [29]. Natural herbs such as curcumin and rosemary showed antibacterial and antioxidants and can be used in the prevention and treatment of disease. They can be used instead of chemotherapeutics due to their natural constituents. In addition, they can be used alternatively of antibiotics treatment to overcome the problem of high bacterial resistant [30]. In our study, mutans Streptococci was the most bacteria species found among dental caries cases, and this is in agreement with Bowen *et al.* as this bacterium is a power producing of acid rapidly and a proximal causing of dental caries [31]. Moreover, other factors should be considered into account as pH. Although many organisms have the ability to form acid immediately, many plaque biofilms have the capacity to form alkali. Thus, it is common to observe that the pH value of plaque without exposed to sugars overnight may be more alkaline than the surrounding saliva. Furthermore, the presence of other bacteria such as lactobacilli and actinomycetes have a major role in the etiology of dental caries disease [32].

In our study, the strongest effect of curcumin as an antibacterial agent (*in vitro*) was detected at a concentration of 1 mg and a linear correlation was found between the curcumin concentration and the inhibitory zone of bacteria. This is in agreement with the study of Aday *et al.* and Song *et al.*, which supports the ability of curcumin to show antiadhesive activity as well as inhibit the growth of Gram-positive and Gram-negative bacteria [33,34]. However, curcumin cannot be used widely in the treatment of diseases due to its water insolubility and poor bioavailability in the body system according to Bharali *et al.* and Al-Musawi *et al.*; therefore, using alcoholic extract more than the aqueous extract of curcumin can improve the antibacterial activity of curcumin.[35,36].

Furthermore, the rosemary did not show any effect on bacterial growth, and this is in agreement with Jawad *et al.* that extract of rosemary showed a wide range of antibacterial activity against positive and negative bacteria like *Escherichia coli* [37]. The extract of rosemary contains phenols which have the ability of inhibition on the cell walls and cell membranes synthesis, nucleic acid metabolism and on the Gram-positive and Gram-negative bacteria and wide

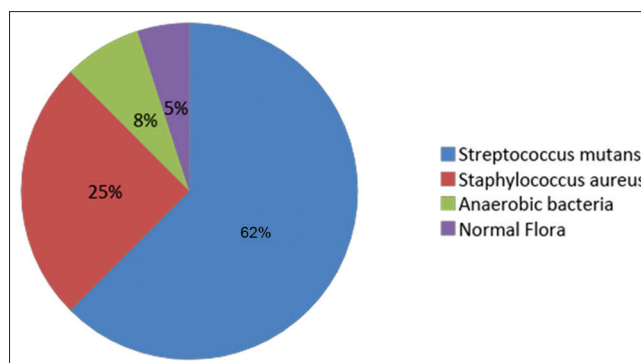


Fig. 1: Distribution of the frequency of bacteria types according to the swab culture

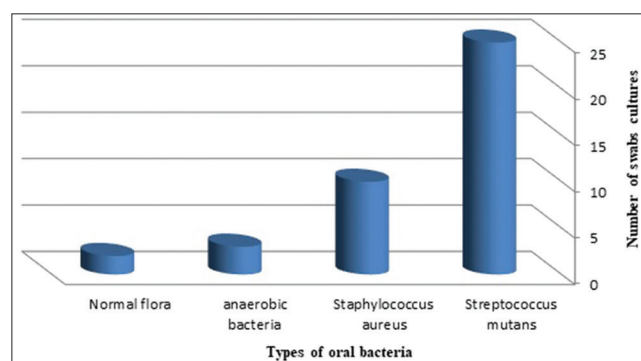


Fig. 2: Distribution of bacterial types according to oral swabs cultures. Data presented as Chi-square. Sample size (n)=40. Chi-square statistic is 15.5311. $p < 0.01$ to be considered as highly significant where $p < 0.05$ to be considered as significant

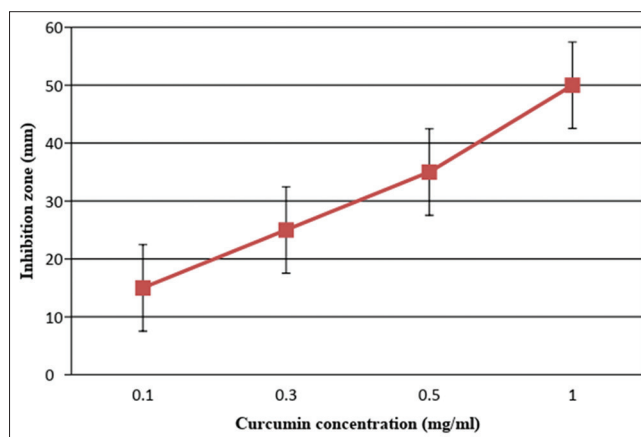


Fig. 3: The relationship between the inhibition zone diameter of oral bacteria and the curcumin's concentrations. Data presented as curcumin concentration (mg/ml) mean \pm SD (0.475 \pm 0.386) and inhibition zone (mm) mean \pm SD (31.25 \pm 14.93). Unpaired *t*-test was used to compare concentration of curcumin and inhibition zone diameters; $p < 0.01$ to be considered as highly significant where $p < 0.05$ to be considered as significant

range on bacteria and fungi inhibition. Accordingly, using alcoholic or oil extract of rosemary can give stronger antibacterial activity than the aqueous extract of rosemary [38,39]. In addition, other factors such as pH and temperature of the extract solution should be considered in determining its antibacterial activity according to Ali *et al.* [40].

CONCLUSION

Curcumin showed antibacterial activity against oral bacteria and its activity increase proportionally with its concentration; however, the rosemary with concentration 1g/ml did not show any activity against these bacteria.

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AUTHOR'S CONTRIBUTIONS

All the authors played a key role in carrying out the study to a conducive outcome. Data collection and implementation of the research were done by the first author. Study design and data analysis were done by the second and third authors. All the authors were involved in the critical revision the final approval of the version to be published.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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