Evaluation of Antibacterial Activity of *Boswellia Serrata* Extract Against some of the Oral Pathogenic Bacteria

Khawlah A. Salman[1], Sahar M. Jawad[2], Saddam Hussein Abbas[3]

[1]Scholar Researcher, Department of Food Sciences, Faculty of Agriculture, University of Kufa, Al-Najaf, Iraq 540011, [2]Scholar Researcher, Department of Soil and Water Sciences, Faculty of Agriculture, University of Kufa, Al-Najaf, Iraq 540011, [3]Scholar Researcher, Department of Field Crops, Al-Qasim Green University, Iraq51013

Abstract

Boswellia serrate is one of traditional remedy used for a long time to cure many diseases. Due to Hence, there is a motivation of medicinal and cosmetics applications. In this paper, the crude aqueous extracts from *Boswellia serrate* bark were screened for in vitro antibacterial properties against clinical isolates of Periodontitis bacterial causative agents *(Streptococcus orails, Gemella morbillorum, Rothia dentocariosa)*. The antibacterial test was carried out following the method done by Perez and others. The tested extract from this medicinal plant with the different concentrations (100 mg/ml ,250 mg/ml,500 mg/ml) were screened. The standard antibiotics Ciprofloxacin (5 µg/ml) and Cefotaxime (30µg/ml) were used as controls. The extract of 250 mg/ml being more effective in action as compared to the others. Furthermore, *Streptococcus orails* showed the most isolate affected by the extract. This research has revealed the active inhibitory effect of *Boswellia serrate* against all the tested isolates. This extract contains active chemical components that contribute to biological activity thereby assisting to combat bacterial infections and the potential for maintaining and promoting toral health. However, many studies need to be carried out to recognize the accountable components for growth inhibition.

Keywords: *Boswellia serrate, aqueous extract, antibacterial activity, oral pathogenic bacteria.*

Introduction

Recently, the attention of Oral cavity hygiene has grown rapidly. Antimicrobial agents are frequently established to be crucial elements to contribute to oral hygiene products for the cure and avoidance of plaque and gingivitis [11]. Dental caries is an important oral health issue that involves an inequality of interactions between the tooth exterior/interior and the close adjacent bacterial biofilm [7]. Most individuals may not perform the exact mechanics to remove plaque adequately, thus rinsing the mouth with antimicrobial products that are used daily may provide an effective way to remove or control bacterial plaque and thus prevent gingivitis and periodontitis [7]. Due to the continuous emergence of microbial species resistant to antibiotics as a sequence of indiscriminate, misuse and excessive use, the urgent need has arisen by researchers and scientists to develop biological compounds derived from natural sources [3]. Such compounds might be the alternative antimicrobial agents, that could be fruitful for mankind not only their natural source, but also other distinctive characteristics such as lacking side effects and cost-effectiveness [15]. *Boswellia serrate* known frankincense that grows in the Middle East, India, and Africa [13]. This herb was established to be one of the most important medicinal plants that has proven to be highly effective in many therapeutic aspects [9]. *The chemical composition of Boswellia serrate* is mainly consists of oil (60%). It contains mono- (13%) and diterpenes (40%) as well as ethyl acetate (21.4%), octyl acetate (13.4%) and methylanisole (7.6%) [4]. The most biologically active substance against oral cavity pathogens is 11-keto-ß-acetyl-beta-boswellic acid [13,16]. The activity of this

Corresponding author:
Sahar M. Jawad,
Email: saharm.alkarawy@uokufa.edu.iq

remedy has been investigated and pharmacological outcomes have been revealed in many therapeutic applications. This included many diseases such as anti-inflammatory, antitumor, immunomodulatory, and inflammatory bowel diseases in addition to an antioxidant agent [1,2]. It has been well established that this plant possesses anti-bacterial, antifungal activity [8]. It has been revealed that the aqueous extract of *Boswellia serrate* produced a large zone of inhibition against a panel of clinical isolates Gram-positive (*Bacillus subtilis, Staphylococcus aureus and Streptococcus pneumonia*) and Gram-negative (*E.coli, Klebsiella pneumonia, Enterobacter aerogenes, Pseudomonas aeruginosa and Proteus vulgaris*). The researchers concluded that extract showed a large zone of inhibition [5].

The aim of the present study was to evaluate the antimicrobial activities of aqueous extracts of *Boswellia serrate* against three species of oral pathogenic bacteria especially the ones that cause periodontitis.

Material and Methods

Collecting plant samples and preparing them for extraction

The raw gum was bought from the local market, processed and crushed. Prepare the working solution by dissolving 1 mg of the powder in 1 ml of distilled water to obtain the following dilution: (100,250, 500 mg/ml). Filter the plant extract with Whatman filter paper and then the filtrate was used for the biological assay.

Specimens Collection

20 samples were collected from the Faculty of Dentistry, University of Kufa, from periodontitis patients. Samples were collected from patients using cotton rolls and carefully cleaned with sterile cotton. For each site, two drops (30-40) were inserted into the gingival pocket for 30 seconds and the pocket depth was equal to or greater than (3.5-7) mm and placed in a sterile container with saline solution (2 mL) and cultured on plates agar.

Preparation of the Bacterial Suspension

The turbidity of each of the bacterial suspensions was prepared to match the standard of 0.5 McFarland (1.5x108 CFU / ml). Turbidity was measured with a spectrophotometer at turbid suspension at 625 nm as per Bauer-Kirby Method (1966).

Determination of antimicrobial activity

Streptoccocus oralis, *Gemella morbillorum* and *Rothia dentocariosa* which were isolated and diagnosed t by the Vitek 2 system, On Muller Hinton Agar spread 0.1ml of the culture with a sterile swab, dry at room temperature for (10-15) minutes. Inhibitory activity was detected by the agar-well diffusion method [12], after sterilizing with the cork borer, four wells were made on the surface of the culture media with a diameter of 10 mm then add (100 µl) to each well in different concentrations *Boswellia serrata* extracts, In the center of the plate, the antibiotic tablets of ciprofloxacin (5 micrograms) and cefotaxime (30 micrograms) were placed for comparison with the plant extract. The plate was incubated for 18-24 hours at 37 ° C. The diameter of the inhibition zones was measured. The experiment was repeated three times and the mean values were validated.

Statistical Analysis

The data gathered and exported to a Microsoft Excel spreadsheet where descriptive statistics were performed. The data was analyzed processed using SAS version 9.1. Two-way ANOVA was also carried to determine if there was any interaction between the effect of extracts concentration and the pathogenic bacteria. $P \leq 0.05$ is considered significant in both tests (Tukey test). Furthermore, the analysis was done to find the difference between the means (4 replicates). One-way ANOVA (Analysis of variance) was carried out to demonstrate statistical difference using the varying zones of inhibition when the extracts from *Boswellia serrata* was used against the isolates included in this study.

Results

The antimicrobial assay of the aqueous extract of *Boswellia serrata* different concentrations of 100 mg/ml, 250 mg/ml, and 500 mg/ml was carried out against most of the tested isolates (*Streptococcus orails, Gemella morbillorum, Rothia dentocariosa*. Figure 1 shows that the average zone of inhibition 15.3 mm in case of a concentration of 250 mg/ml, while a concentration of

250 mg/ml exhibited the average of growth inhibition 16.11mm. A concentration of 100 mg/ml revealed the average zone of inhibition 12.11mm.

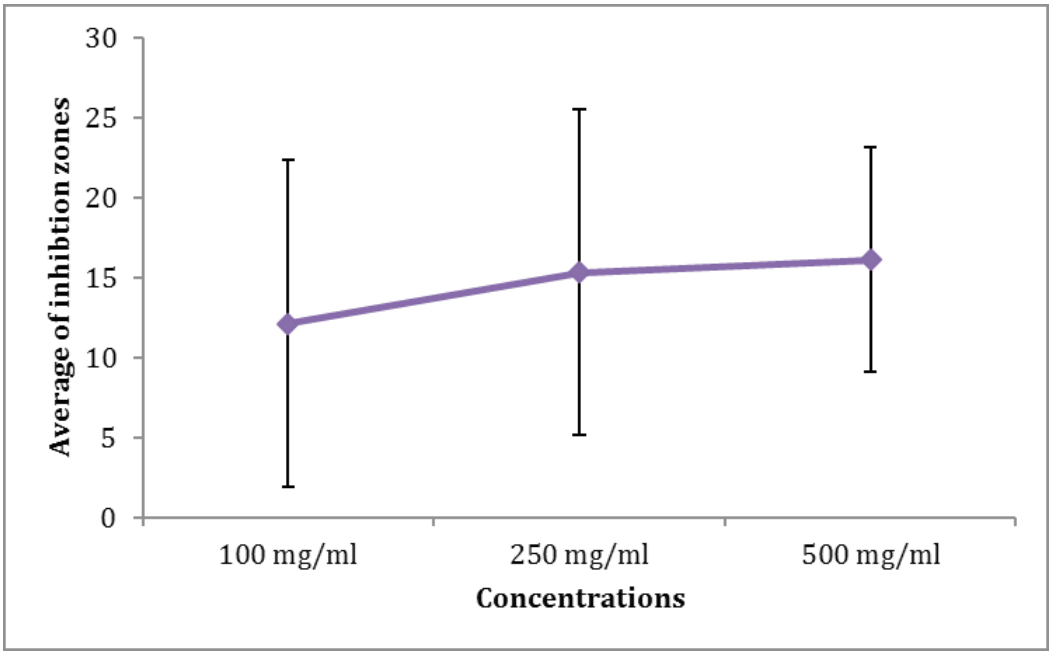

Fig. 1 **The inhibitory effect of three different concentrations of** *Boswellia serrata* **against three treated isolates. The error bars represent ± standard deviation (SD). Bars with different letters demonstrate significant differences (ANOVA, two-way, Tukey, P<0.05, n=4).**

Regarding the sinsivity of the examined isolates towards the aqueous extract of *Boswellia serrate, Rothia dentocariosa* isolate showed the most resistant activity against the extract, however, the inhibition was slightly increased by increasing the concentration from 100 mg/ml to 500 mg/ml. The highly sensitive isolate was *Streptococcus orails,* figure 2. From the same figure and figure 3, the isolate *Gemella morbillorum* showed that by increasing the concentration the increase in the inhibition activity was observed.

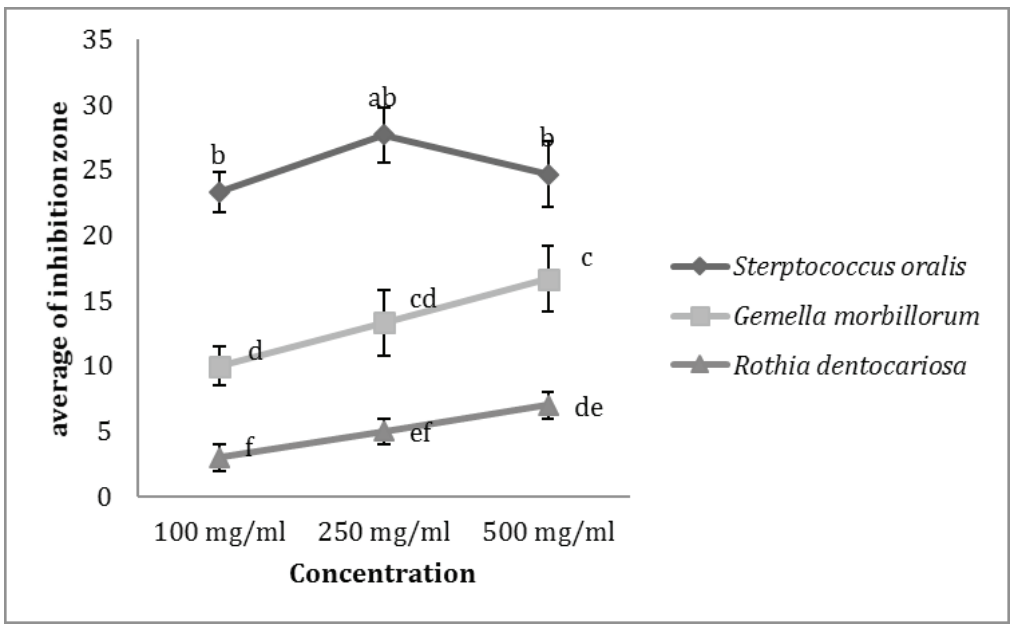

Fig. 2 Inhibition rates for three different concentrations of the extract of *Boswellia serrata* **as average isolates. The error bars represent ± standard deviation (SD). Bars with different letters demonstrate the significant differences (ANOVA, one way, Tukey, P<0.05, n=7).**

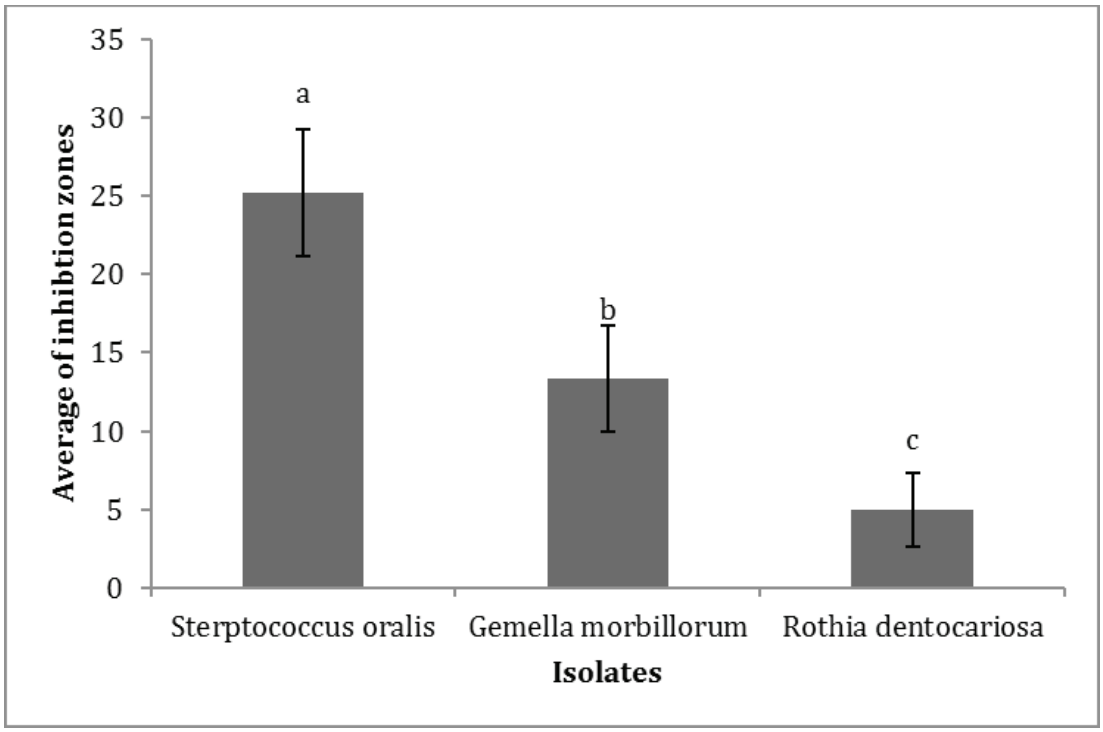

Fig. 3 **The response of the average treated isolates (***Granulicatella adiacens, Staphylococcus sciuri and Kocuria spp.) towards the* **average concentrations of aqueous extract Boswellia serrate.. The error bars represent ± standard deviation (SD). Bars with different letters demonstrate the significant differences (ANOVA, one way, Tukey, P<0.05, n=7).**

The inhibition activity formed by the standard antibiotics (Ciprofloxacin, Cefotaxime) was also observed in different degrees of inhibition according to the sensitivity of the tested bacteria towards the extract. As can be noticed from Table 1 there was a significant antigrowth activity of both antibiotics used 40mm of Ciprofloxacin and 35mm of Cefotaxime against *Streptococcus orails* isolate, whereas the lowest inhibition zones were detected on *Rothia dentocariosa*. The data suggested that this bacteria was not highly sensitive neither to the bark extracts nor to the typical antibiotics.

Table 1 Average of inhibition zones of *Boswellia serrate* **extract on tested isolates caused by the antibiotics and the extract. The presented values are the average of 4 replicates of inhibition zones (mm). The average of inhibition zones of the standard antibiotics are shown.**

Isolates	Control		Concentration (mg/ml)			
	Ciprofloxacin (5 µg/ml)	Cefotaxime (30µg/ml)	100	250	500	
Streptococcus orails	40	35	23.3	27.7	24.7	Average of inhibition zones (mm)
Gemella morbillorum	26	20	10	13.3	16.7	
Rothia dentocariosa	11	5	3	5	7	

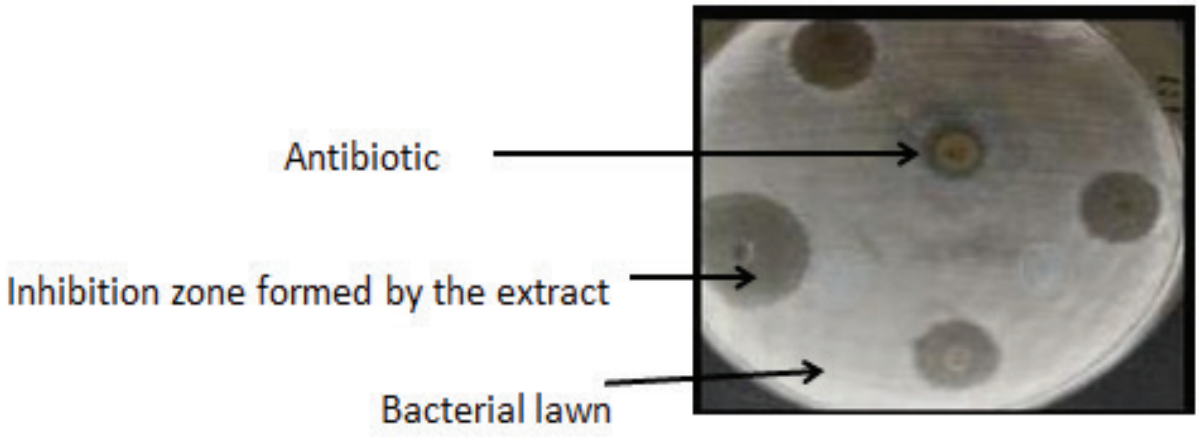

Fig. 4 Inhibition zones formed by the extract of *Boswellia serrata* at concentration 250 mg/ml.

Discussion

Medicinal plants for a long time have been applied as substitutable agents to medicine in many societies [3]. The natural products derived from medicinal plants are crucially contributed to the medical system to cure some of the serious infections in the world. The growing emphasis on the investigation of the Pharmacological effect of natural plants has been extensively expanded during the last two decades to discover medicinal valuable, novel and active molecules for the development of new therapeutic materials [15]. In this research, the extract of *Boswellia serrata* pr was assessed for their antimicrobial activity. The outcomes of antimicrobial screening assay of *Boswellia serrata extract* gave excellent antigrowth action against the tested isolates. The extract from *Boswellia serrata* showed to be considerably active against *Streptococcus orails* at (250, 500 mg/ml) concentrations. The second susceptible strain was *Gemella morbillorum* that being affected by the extract with a diameter of inhibition (16.7 mm) at (500 mg/ml) concentration, whereas the inhibition zones were increased gradually by increasing the concentrations from 100 mg/ml to 500 mg/ml in the case *Rothia dentocariosa*. However, the inhibition zones formed by the standard antibiotics were greater than the extract. *Streptococcus orails Gemella morbillorum* isolates were highly sensitive to antibiotics, comparatively smaller zones were caused by the antibiotics on *Rothia dentocariosa.* Correspondingly, The results of the present investigation were undertaken for detection of the action of the *Boswellia serrata* extract and these outcomes are comparable to many researchers that studied the *Boswellia serrata* antibacterial activity [10,5]. There is some indication stated in the literature that many components of *Boswellia serrata* are active antibacterial agents that showed activity against oral bacterial infections [13,10,5].

Conclusion

The present findings of Antimicrobial activity have been brought forward as one of the resources of achieving promising effects. The medicinal plant applied in the study has been specified to be of therapeutic significance. The extracts of *Boswellia serrate* displayed a significant inhibition activity against a wide range of Gram-positive bacteria with a significant inhibition zone ranging from 27.7 to 3 against all the tested strains. The outcomes highly encourage the use of remedies as a natural source to tackle the antibiotic resistance issue. Undeniably, this medicinal plant has the possibility to treat oral bacterial infections due to its bioactive components. However, additional investigations and identification procedures need to be undertaken so that to recognize the constituents with a medicinal value.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

References

[1] Alluri V, Dodda S, Kilari E, Golakoti T, Sengupta K. Toxicological Assessment of a Standardized Boswellia serrata Gum Resin Extract. *International Journal of Toxicology*, 2019. 38(5), pp.423-435.

[2] Alluri V, Kundimi S, Sengupta K, Golakoti T, Kilari E. An Anti-Inflammatory Composition of Boswellia serrata Resin Extracts Alleviates Pain and Protects Cartilage in Monoiodoacetate-Induced Osteoarthritis in Rats. *Evidence-Based Complementary and Alternative Medicine*, 2020, pp.1-12.

[3] Álvarez-Martínez F, Barrajón-Catalán E, Micol V. Tackling Antibiotic Resistance with Compounds of Natural Origin: A Comprehensive Review. *Biomedicines*, 2020. 8(10), p.405.

[4] Al-Yasiry A, Kiczorowska B. Frankincense – therapeutic properties. *Postępy Higieny i Medycyny Doświadczalnej*, 2016. 70, pp.380-391.

[5] Bakhtiari S, Nematzade F, Hakemi-Vala M, Talebi G. Phenotypic Investigation of the Antimicrobial Effect of Organic and Hydro-Alcoholic Extracts of Boswellia serrata on Oral Microbiota. *Frontiers in Dentistry*. 2020.

[6] Bauer Kirby, Sherris Turck. Antibiotic susceptibility testing by standard single disc diffusion method. Am. J. Clin. Path. 1966. 45: 493.

[7] Colombo A, Tanner A. The Role of Bacterial Biofilms in Dental Caries and Periodontal and Peri-implant Diseases: A Historical Perspective. *Journal of Dental Research*, 2019. 98(4), pp.373-385.

[8] Di Stefano V, Schillaci D, Cusimano M, Rishan M, Rashan L. In Vitro Antimicrobial Activity of Frankincense Oils from Boswellia sacra Grown in Different Locations of the Dhofar Region (Oman). *Antibiotics*, 2020. 9(4), p.195.

[9] Hewedy W. Effect of Boswellia serrata on Rat Trachea Contractility In Vitro. *The Natural Products Journal*, 2020. 10(1), pp.33-43.

[10] Ismail1 S, Aluru KS, Bhaskar M. Antimicrobial activity of frankincense of *Boswellia serrata* Shaik Mannur. *Int. J. Curr. Microbiol. App. Sci.* 2014. 10(3) pp. 1095-1101.

[11] Jang K, Heo S. Oral Health Awareness and Oral Health Care Provision Status according to Oral Health Care Education Experience of Long-Term Care Workers. *The Korean Society of Oral Health Science*, 2020. 8(3), pp.37-44.

[12] Perez C, Paul M, Bazerque P. Antibiotic assay by agarwell diffusion method. Acta Biol Med Exp. 1990; 15: 113-115.

[13] Raja A, Ali F, Khan I, Shawl A, Arora D. Acetyl-11-keto-β-boswellic acid (AKBA); targeting oral cavity pathogens. *BMC Research Notes*, 2011. 4(1).

[14] Rajendra CE, Harish Kumar DH, Yeshoda SV, Mahaboob AN, Hanuman T. Comparative evaluation of antimicrobial activities of methanolic extract of *Curcuma longa* and *Boswellia serrata*. Int. J. Res. Pharm. Chem., 2013; 3: 534-536

[15] Saied M, Kamel N, Ward A and Abd El-kader A. Novel Alginate Frankincense Oil Blend Films for Biomedical Applications. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 2019. 90(2), pp.303-312.

[16] Sharma T, Jana S. A Validated LC–MS/MS Method for Simultaneous Determination of 3-O-Acetyl-11-Keto-β-Boswellic Acid (AKBA) and its Active Metabolite Acetyl-11-Hydroxy-β-Boswellic Acid (Ac-11-OH-BA) in Rat Plasma: Application to a Pharmacokinetic Study. *Journal of Chromatographic Science*, 2020. 58(6), pp.485-493.

[17] Shehata A.M., Quintanilla-Fend L., Bettio S., Singh C.B., Ammon H.P.: Prevention of multiple low-dose streptozotocin (MLD-STZ) diabetes in mice by an extract from gum resin of *Boswellia serrata* (BE). Phytomedicine, 2011; 18: 1037-1044.