# Antibacterial Effect of Aqueous and Alcoholic Propolis Extracts on Aggregatibacter Actinomycetemcomitans in Patients with Chronic Periodontitis (An *In-vitro* Study)

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## ABSTRACT

Background: Propolis has received great interest because of its wide range antimicrobial activity. Propolis also called (bee glue) due to its collection by (*Apismellifera*) honeybees from various plants resinous substance. The aim of this study was to determine the antibacterial effect of propolis extracts (aqueous and alcoholic) on anaerobic periodontal pathogen namely *Aggregatibacteractinomycetemcomitans.*

Materials and Methods: Strains of *Aggregatibacter actinomycetemcomitans* wasisolated from pockets of systemically healthy patients aged between 35-55 years old suffering from chronic periodontitis with pocket depths of 5-6 mm, the bacteria cultured on special blood Agar plates solid media. Propolis was extracted by using water and alcohol. Agar well technique was used to study the sensitivity of Aggregatibacter actinomycetemcomitans to different concentrations of propolis extracts (70, 80, 90, 100, 125 and 150) mg/ml and other control agents (distilled water and chlorhexidine 0.2%).

Results: *Aggregatibacter actinomycetemcomitans* was sensitive to propolis extracts; alcoholic extract was more effective than aqueous extract.All concentrations of propolis extracts showed smaller inhibition zones than 0,2% CHX except 150 mg/ml concentration of aqueous extract ,(100, 125 and 150)mg/ml concentrations of alcoholic extract showed larger inhibition zones than 0,2% CHX.

Conclusion: Propolis extracts were effective against anaerobic periodontal pathogens (Aggregatibacter actinomycetemcomitans).

Key words: Propolis, antibacterial activity, anaerobic periodontal pathogen. (J Bagh Coll Dentistry 2015; 27(4):115-118).

## INTRODUCTION

Periodontal disease is an infectious condition started as microbial plaque accumulates at the gingival margin of the tooth surface and provokes an inflammatory reaction [(1).] Although the inflammatory process protects the host; it may lead to tissue destruction [(2)]. *Porphyromonasgingivalis, Treponemadenticola, Tanerellaforsythensis, Actinobacillusactinomycetemcomitans (A.A), Fusobacteriumnucleatum, Eikenellacorrodens* are considered to be associated with Chronic Periodontitis [(3)]. During the last two decades, it has been shown that *Aggrecatibacter actinomycetemcomitans* can be regarded as a major pathogen in destructive periodontal diseases [4)], it was also found that A.A is associated with systemic diseases [(5)].

Several studies suggested that the outcome of periodontal treatment is better if particular pathogens especially *Aggrecatibacter actinomycetemcomitan*s can no longer be detected after therapy [(6)].

Clinical treatment of periodontal diseases is initiated by controlling the accumulation of dental plaque associated with scaling-root planing that allows the elimination of biofilm and calculus.

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However, sometimes this treatment is not enough to control the severity of the disease, needing the antibiotic use. Development of effective strategies for treatment of chronic periodontitis has posed a challenge, considering the increase in opportunistic bacterial infections. Some of the drugs used in the treatment of periodontitis, are limited because of the high rate of allergy, resistance of periodontopathic bacteria and elevated cost. Thus, Searching for alternative antibacterial compounds has been a major concern in recent years [(7,8)].

Herbs are being widely explored to discover alternatives to synthetic antibacterial agents [(9)]. Propolis is a resinous complex material formed by bees from bee's (wax, secretions) and plant exudates[(10)]. Propolis is responsible of honeycombs safety against microorganisms [(11)]. Honeybee's propolis has wide range of biological actions including: (antimicrobial, antitumor, anti-inflammatory, antioxidative, and hepatoprotective activities) which attracted researcher's attention [(12)]. It is composed of 5% pollen 50% vegetable balsam and resin, 30% wax, 10% essential and aromatic oils and 5% other components like organic remnants, but this composition vary according to the vegetal source [(13)].

The aim of the present study was to determine the antibacterial effect of propolis extracts (aqueous and alcoholic) on anaerobic periodontal pathogen namely *Aggregatibacteractinomycetemcomitans.*

# MATERIALS AND METHODS

**Patient Selection and Sampling:**

Fifteen systemically healthy patients of age range between 35-55 years old participated in this study; they had chronic periodontitis with at least four pockets of 5-6mm depth. A sample of plaque from subgingival periodontal pocket was excavated by gracey curette without touching adjacent tissue.Plaque sample was spread on Colombia blood agar solid media supplied with selective materials in the plates then plates were transported into an anaerobic jar with anaerobic gas pack incubated anaerobically for 72 hours.

After incubation, bacterial identification was based on (the microscopic appearance and colonial shape and size, gram stain, biochemical tests like Catalase, hemolytic capability, urease test, and antibiotic susceptibility tests). Colonies were subcultured again on the same media anaerobically for 72 hours under the same condition, using the same method, to obtain pure cultures of *Aggregatibacter actinomycetemcomitans* for detection of inhibition zone.

**Extraction Procedures to Obtain Propolis Extracts:**

**1- Aqueous Extract:**

1000 ml of distilled water were added to100 grams of propolis in a dark glass which was left at room temperature for one to two weeks with shaking two to three times daily with shaker, then filtration was done first using gauze to get rid of the large particles, then the resultant liquid was filtered using a sterile Whitman filter paper No1. The filtered extract was concentrated under vacuum 45ºC using a rota evaporator for five hours). Then put in clean and dark container in warm place until use [(14)].

**2-Alcoholic Extract:**

The preparation was done by the same procedure of aqueous extract except we use (96% ethanol alcohol) instead of distilled water [(14)].

**Sensitivity of A.A to Different Concentrations of Alcoholic and Aqueous Propolis Extracts in Vitro:**

The concentrations of alcoholic propolis extract used in this experiment were: (70, 80, 90, 100, 125, and 150) mg/ml.

The concentrations of aqueous propolis extract used in this experiment were: (70, 80, 90, 100%, 125, and 150) mg/ml.

CHX gluconate (0.2%) and D.W (distilled water) were used in this experiment as a positive control and negative control respectively.

Agar well diffusion method was used, using a sterile loop, three colonies were picked up and spread on blood agar plate in a mattress fashion, then wells of equal size and depth will be prepared in the agar, afterwards each well was filled with the selected agent (100 microliter) then the plates were incubated anaerobically for 48 hours. The inhibition zones were measured in millimeters using a ruler.

# RESULTS

The mean values and standard deviation (SD) with the maximum (Max) and minimum (Min) values of the inhibition zones in millimeters (mm) of the alcoholic propolis extract against *Aggregatibacter actinomycetemcomitans* (A.A) are presented in table (1).Alcoholic extract showed increase in the diameter of the inhibition zone as the concentration increased, 125 mg/ml concentration and chlorhexidine show approximately the same results (mean of inhibition zone for 125mg/ml concentration was 14.6 mm and the for chlorhexidine was 14 mm).150 mg/ml concentration showed larger inhibition zones than chlorhexidine (positive control); while distilled water (negative control) showed no inhibition zone.

The mean values and standard deviation (SD) with the maximum (Max) and minimum (Min) values of the inhibition zones in millimeters (mm) of the aqueous propolis extract against *Aggregatibacteractinomycetemcomitans* (A.A) are presented in table (2).

Aqueous extract showed increase in the diameter of the inhibition zone as the concentration increased, 125 mg/ml concentration and chlorhexidine show approximately the same results (mean of inhibition zone for 125 mg/ml concentration was14.3 mm and for chlorhexidine was 14.1mm) .150 mg/ml concentration showed larger inhibition zones than chlorhexidine (positive control); while distilled water (negative control) showed no inhibition zone.

By using t-test, the differences between alcoholic and aqueous extract for all concentrations were: highly significant difference in 70 mg/ml and 80 mg/ml concentration, no significant difference in (90, 100 and 125) mg/ml concentrations and significant difference in 150 mg/ml concentration. As shown in table (3).

The means of inhibition zones of all concentrations of alcoholic and aqueous extracts are presented in figure (1) it clearly shows that alcoholic extract showed higher inhibition zones than aqueous extract in all concentrations.

**Table (1): The Inhibition Zone (mm.) of AA Bacteria Using Different Concentrations of Alcoholic Propolis Extract and +ve and –ve Control.**

| | Inhibition zone with Alcoholic extract of Propolis with +ve and –ve control | | | |
|---|---|---|---|---|
| | Mean | S.D. | Min. | Max. |
| 70 mg/ml | 10.45 | 0.50 | 10 | 11 |
| 80 mg/ml | 11.2 | 0.48 | 10.5 | 12 |
| 90 mg/ml | 12.4 | 0.52 | 12 | 13 |
| 100 mg/ml | 13.4 | 0.52 | 13 | 14 |
| 125 mg/ml | 14.6 | 0.52 | 14 | 15 |
| 150 mg/ml | 16.2 | 0.79 | 15 | 17 |
| CHX | 14 | 0.67 | 13 | 15 |
| D.W. | 0 | 0 | 0 | 0 |

**Table (2): The Inhibition Zone (mm.) of AA Bacteria Using Different Concentrations of Aqueous Propolis Extract and +ve and –ve Control.**

| | Inhibition zone with Aqueous extract of Propolis with +ve and –ve control | | | |
|---|---|---|---|---|
| | Mean | S.D. | Min. | Max. |
| 70 mg/ml | 8.6 | 0.57 | 8 | 9.5 |
| 80 mg/ml | 10.3 | 0.67 | 9 | 11 |
| 90 mg/ml | 12 | 0.47 | 11 | 13 |
| 100 mg/ml | 13.2 | 0.63 | 12 | 14 |
| 125 mg/ml | 14.3 | 0.67 | 13 | 15 |
| 150 mg/ml | 15.3 | 0.67 | 14 | 16 |
| CHX | 14.1 | 0.74 | 13 | 15 |
| D.W. | 0 | 0 | 0 | 0 |

**Table (3): The Differences between Alcoholic and Aqueous Propolis Extract for all Concentration on A.A by using T-test.**

| | Difference (d.f.=18) | |
|---|---|---|
| | t-test | p-value |
| 70 mg/ml | 7.753 | 0.000 (HS) |
| 80 mg/ml | 3.429 | 0.003 (HS) |
| 90 mg/ml | 1.809 | 0.087 (NS) |
| 100 mg/ml | 0.775 | 0.449 (NS) |
| 125 mg/ml | 1.116 | 0.279 (NS) |
| 150 mg/ml | 2.741 | 0.013 (S) |
| CHX | -0.318 | 0.754 (NS) |
| D.W. | - | - |

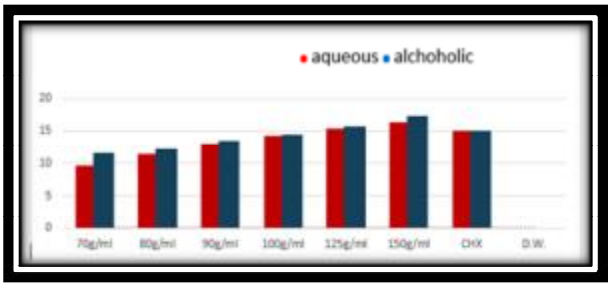


**Figure (1): Histogram Showed the Mean Values of Inhibition Zones of Alcoholic and Aqueous Extracts with +ve and –ve Controls Against A.A**

## DISCUSSION

Sensitivity of A.A to different concentrations of alcoholic and aqueous extracts of propolis by agar well diffusion method had been tested in this study. Results showed that alcoholic and aqueous propolis extracts were able to inhibit the growth of A.A, the diameters of inhibition zones were found to increase when the concentration of the extracts (aqueous and alcoholic) increased, this may be due to the amount of the dissolved active constituents of the extract will be more abundant as the concentrations increase causing increased antimicrobial activity of the extract and also showed that alcoholic extract had more antibacterial activity than aqueous extract this is because the amount of active component in the extract and polarity of the solvent (ethanol alcohol) which has great ability to dissolve the biologically active component of Propolis [15].

These findings were in coincidence with Al-Ammar [16] study of the activity of propolis extracts against pathogenic bacteria include gram positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes*) and gram negative bacteria (*Pseudomonas aeroginosa, Escherichia coli*) and found that propolis extracts (both aqueous and alcoholic) were effective against gram positive and gram negative bacteria, the activity of both extracts were more on gram positive bacteria than gram negative one and that alcoholic extract was more effective than aqueous extract.The solvent used for propolis extract like ethanol, methanol, chloroform, propylene glycol and others can affectits antimicrobial activity [17].

Mahmood and Abdul Hadi studied the effect of Turkish propolis (water and methanol) extracts against gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis and Bacillus cereus*) and gram negative bacteria (*Salmonella enteritidis, Escherichia coli, Klebsiella pneumonia*) and found that alcoholic propolis extract was active against both types of bacteria s but they found that the watery extract

had no antibacterial activity which is not in agreement with this study, this may be due to: difference in the source of propolis, methodology of extract and type of target microorganisms [18].

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