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Antibacterial activity, anti-adherence and anti-biofilm activities of plants extracts against *Aggregatibacter actinomycetemcomitans*: An in vitro study in Hilla City, Iraq

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ABSTRACT

The current study was designed to establish the antibacterial efficacy of various extracts from medicinal plants. The inhibition effects of six plants including *Viscus album, Apium graveolens, Melissa officinalis, Plantago ovata, Senna acutifolia*, and *Vitis vinifera* were examined against *Aggregatibacter actinomycetemcomitans*, which obtained from dental caries patient. The output showed that the tested bacterial isolates were greatly sensitive to *M. officinalis* and *S. acutifolia* and their maximum inhibition zones were 35 mm and 33 mm respectively. The tested bacterial isolates were greatly sensitive to *M. officinalis* and *S. acutifolia* in comparison with the antibiotic. According to the well-diffusion test results, the crude aqueous extracts from the plants showed varying degrees of inhibition of bacterial growth. Moreover, these medicinal plant extracts were a promising group of natural product sources that can be examined to continue developing products through the use of oral medicines and health care.

Keywords: Antibacterial activity, *Aggregatibacter actinomycetemcomitans*, Plant extracts, Inhibition zone.
Article type: Research Article.

INTRODUCTION

Medicinal plants play an important role in meeting demand from distant markets for the manufacture of new drugs. In fact, it is possible to trace the use of medicinal plants to treat diseases back to human history (Fatemeh *et al.* 2018). More than one-tenth of the various plant species are used in medicinal and beautifying processes. Old medicinal plants are described by the World Health Organization (WHO) as materials natural of plant which are used for local-scale treatment of diseases (Tilburt & Kaptchuk 2008). Nowadays, according to the World Health Organization, which serves as the main source of medical treatment, over 80%, of the world's population mostly relies dependent conventional medicines. Traditional medicine has been used for thousands of years in developed and developing countries because it is natural, and exhibits relatively less complications (Kaliyaperumal *et al.* 2013). Overcome resistance to antibiotics is the biggest challenge to the WHO for the next millennium. Plant selection for antimicrobial agent have acquired significant because WHO supports and facilitates the production of resources for medicinal plants in the conventional medicine (Ali *et al.* 2019). *Actinomycetemcomitans* is an Gram-negative coccobacillus that has been associated with localized periodontitis pathogenesis in children and has also been recognized for its ability to cause severe out-of-mouth infections,

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especially endocarditis (Wilson *et al.* 1989). *Aggregatibacter actinomycetemcomitans*, one of the putative pathogens associated with periodontitis, especially in young adults and adolescents, is commonly known (Henderson *et al.* 2000). Periodontitis pathogenesis is highly complex, including immunogenic factors, lifestyle, and periodontitis-associated bacterial species development, including actinomycetes in oral biofilms. In the last decade, the investigation of plants as a vector for the control of infectious diseases has increased. Not only did old medicine gain popularity and recognition, but also it is the only system available in most of the rural areas (Foster 2000; Ekor 2014). Exploration of medicinal plants become important because capable of inhibiting different microbial pathways. Therefore, the aim of the study was to determine antibacterial activity, anti-adherence and anti-biofilm activities of different medicinal plants against *A. actinomycetemcomitans*.

MATERIAL AND METHODS

For preparation of aquatic extract from *Viscus Album, Apium graveolens*, *Melissa officinalis, Plantago ovata, Senna acutifolia* and *Vitis vinifera,* related aquatic extracts were obtained according to (Hindi & Chabuck 2013).

Bacteria and isolates

A total of 50 samples were taken from pockets of periodontic patients including 30 males and 20 females between the ages of 20 and 66 years (periodontic department, teaching clinics for oral and dental surgery), then cultivated on blood agar plates, followed by incubating aerobically and anaerobically (within the anaerobic jar) for 24 to 72 h at 37 ºC and 10% CO_2. Then, the samples were subjected to cultural property recognition, such as black pigmented colonies, microscopic reviews, such as enzyme tests for organic chemistry and capsule, indole, catalase biochemical checks, antibiotic sensitivity (vancomycin; 30 µg) and vatic test (Forbes *et al.* 2007).

Antimicrobial activity test by agar-well diffusion assay (in vitro)

Antibacterial activity assay: According to Forbes *et al.* (2007), antimicrobial activity has been observed by diffusion of agar-discs (the test were performed in triplicates).

Biofilm Formation Assay

Semi quantitative microtiter plate test or method assay (TCP) of tissue culture plate by (Hindi *et al.* 2014)

Table 1. Bacterial adherence and formation of biofilm by the TCP process (Hindi *et al.* 2014).

Mean of OD value at 630 nm	Adherence	Biofilm formation
0/120>	non	Non
0/240-0/120	Moderately	Moderate
>0.240	Strong	High

Adherence test

One of the key and essential virulence properties of these bacteria is bacterial adherence to the epithelial cell (Mateveki *et al.* 2004; Avila Campos *et al.* 2000).

Statistical analyses

The test for Bonferroni was used for data processing as $p < 0.05$ to show significant differences between the types of extracts (Danial 1988).

RESULTS

Antibacterial activities of plant extracts and antibiotics

The inhibition effects of six plants including *Viscus album, Apium graveolens , Melissa officinalis, Plantago ovata, Senna acutifolia, and Vitis vinifera* were tested against *Aggregatibacter actinomycetemcomitans*, which obtained from dental caries patient. The results showed that the bacterial isolates were greatly sensitive to *M. officinalis* and *S. acutifolia*, their maximum inhibition zones were 35mm and 33mm respectively (Fig. 1). According to the well-diffusion test results, the crude aqueous extracts from the plants showed varying degrees of inhibition on bacterial growth.

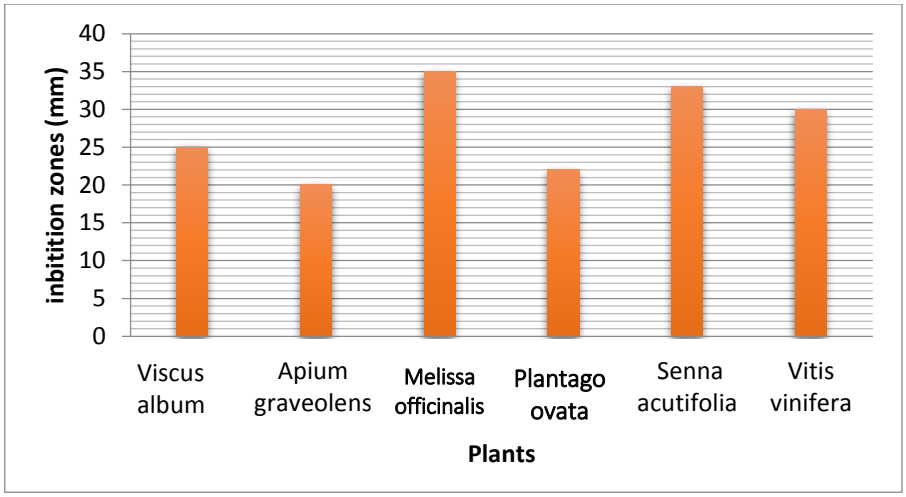

Fig. 1. Antibacterial effect of *V. album*, *A. graveolens*, *M. officinalis*, *P. ovata*, *S. acutifolia* and *V. vinifera* against *A. actinomycetemcomitans* by agar well method.

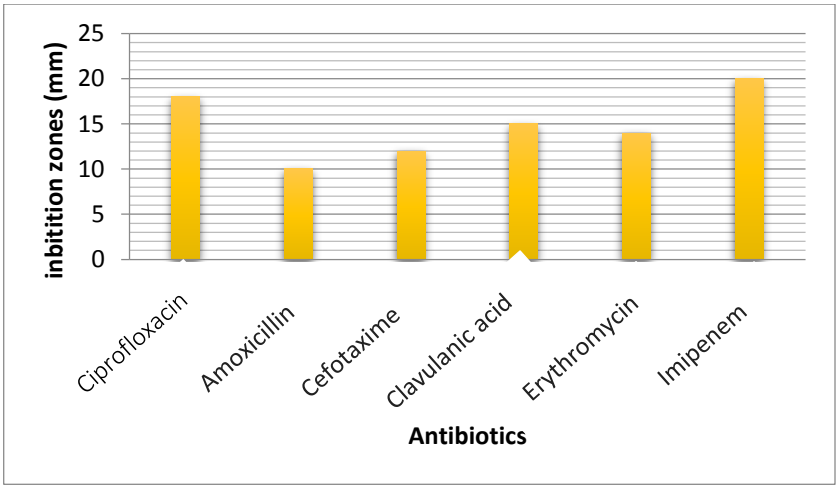

Fig. 2. Antibacterial effects of some Antibiotics against *A. actinomycetemcomitans* by disc well method.

Fig. 2 summarizes the outcome of the antibiotic sensitivity of the bacteria being examined against various classes of antibiotics. Six plants were compared with antibiotics for the inhibition effect. The potential effects of six antibiotics (ciprofloxacin, amoxicillin, cefotaxime, clavulanic acid, erythromycin, imipenem) on bacteria growth were examined using disc well method. The potent antibacterial effect of imipenem extract on *A. actinomycetemcomitans* was demonstrated by this study using disc well method, however, amoxicillin and cefotaxime showed weaker antimicrobial activity than imipenem. Results document that the inhibition effects of six plants were higher than the antibiotics.

Table 1. Anti-adherence and anti-biofilm activities of some plant extracts against *A. actinomycetemcomitans*.

Plant extracts	Adherence	Biofilm formation
Viscus album	High	High
Apium graveolens	Moderate	Moderate
Melissa officinalis	High	High
Plantago ovata	Moderate	Moderate
Senna acutifolia	High	High
Vitis vinifera	High	High

Table 1 shows anti-adherence and anti-biofilm activities of *A. graveolens, M. officinalis, P. ovata, S. acutifolia*, and *V. vinifera* against *A. actinomycetemcomitans*. The results depicted that *A. graveolens* and *P. ovata* reduce

and inhibit the growth with adhesion to bacterial isolate. Moreover, the adhesion of epithelial cells to *V. album, M. officinalis, S. acutifolia* and *V. vinifera* were high.

DISCUSSION

Medicines derived from the active ingredients of plants are effective, less expensive, and readily available without secondary effects. Specialists rely on the original and traditional medicine mainly on medicinal plants for the preparation of therapeutic substances (Hindi). It can be used as raw extracts of plants directly against the activities of bacteria and fungi. Therefore, the aim of this study was to determine the antibacterial activities of different extracts of medicinal plants against bacteria (Nascimento *et al.* 2019). Medicinal plants, on the other hand, have attracted increasing interest, due to their antimicrobial sensitivity against microorganisms causing oral diseases. A wide variety of materials containing plants used in herbal medicine (Ionescu 2017; Ali *et al.* 2019). The main aim of the present study was to examine and demonstrate the antibacterial efficacy of plant extracts and antibiotics against the bacteria *A. actinomycetemcomitans*. Results from the current study revealed that the inhibition effects of the medicinal plants were higher than the antibiotics.

Complementary and traditional medicine is an important and sometimes central role of the World Health Organization to track health patterns. Not only old medicine is becoming common and approved, it is often the only accessible scheme in many rural areas (Gupta & Kumar 2017; Bali *et al.* 2014). Results of the present study clearly exhibited that the plant extracts have good antibacterial activities against *A. actinomycetemcomitans*. Our results are in agreement with (Bali *et al.* 2014) who reported the antimicrobial activity of EtAc extract against *A. actinomycetemcomitans*. In addition, a variety of studies have examined the effects of the medicinal plant extracts on oral pathogens and reported the positive efficacy of plant extracts such as *M. sylvestris* and also *B. serrata* (AKBA) on *A. actinomycetemcomitans* (Benso *et al.* 2015; Lahiri *et al.* 2019). Other study documented that plant extracts were effective against *A. actinomycetemcomitans* (Mageed 2015). The antibacterial activity of the plant extracts on *A. actinomycetemcomitans* is due to the presence of biologically active compounds and their low-toxicity characteristics (Lahiri *et al.* 2019) .In order to decide if a plant has any other antibacterial properties against oral pathogens, further experiments and clinical trials are necessary to determine in vivo antibacterial properties.

CONCLUSION

The antibacterial activity of plant species including *V. Album, A. graveolens, M. officinalis, P. Ovata, S. acutifolia,* and *V. vinifera* on *A. actinomycetemcomitans* growth has been recorded in this study for the first time.

One possible result of these findings may be the production of a mouthwash containing the extracts of plant examined, and also their high growth inhibitory properties, as part of a routine for everyday oral hygiene.

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