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Research Article
Pathogenic and Antimicrobial Properties of Aquatic Extracts of *Viscus album* and *Apium graveolens*

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Abstract
Background and Objective: Wild plants had been recognized to have chief roles for many populations to provide them with many requirements, also, these plants have been applied for the treatments of various complaints, especially microbial infections. This study aimed to identify the inhibitory effects of watery extracts obtained from *Viscus album* and *Apium graveolens* against the number of clinical bacterial isolates. **Materials and Methods:** Aqueous extracts of *Viscus album* and *Apium graveolens* were applied to examine their effects on inhibition of growth and some physiological parameters such as swarming and biofilm-formation against 19 clinical bacterial isolates (Gram-positive and negative). Method of agar wells-diffusion, tissue culture plate method for biofilm inhibition test, inhibition of adherence and swarming assay were applied for estimating and evaluating the inhibitory effects. **Results:** *Viscus album* and *Apium graveolens* extracts showed significant inhibitory effects against most tested Gram-positive and negative bacterial isolates with inhibitory ranges 20-35 and 18-28 mm, respectively, also with significant strong inhibition of adhesion and biofilm formation by *Viscus album* when compared with less inhibition by *Apium graveolens.* **Conclusion:** Aquatic extracts of *Viscus album* and *Apium graveolens* can be considered beneficial prophylaxis and treated against a wide range of pathogenic bacteria.

Key words: Pathogenic bacteria, antimicrobial, aquatic extract, plant extracts, *Viscus album*, *Apium graveolen*, tissue culture

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The rapid bacterial spreading and uncontrolled usage of antibacterial agents have led to the progression of horrible drug resistance among variable virulent pathogenic bacterial species. This is resulting in the development of a huge problem of antimicrobial resistance which increases the rate of morbidity and mortality. Additionally, other problems regarding the cost and availability of different synthetic broad-spectrum antibiotics, as most of them are too costly and difficult to be got. These drawbacks abate the therapeutic convenience of available synthetic antibacterial drugs and enhance the requirement to replace them with effective, available and cheaper alternative remedies[1].

Various plants can be functional for the treatments and solving of different illnesses and problems, by serving as sources of many powerful drugs. These plants contain different bio-active composites which are responsible for the therapeutic activities of these plants with their availability and diversity of them in different ecological regions around the world. Thus, many new synthetic drugs are to be obtained from natural resources, remarkably of plant origin[2]. The antibacterial properties of aromatic and spices herbs have been detected in several types of research, as their composition, activities, applications, role as a natural food preservative and as medical treatments.

Apium graveolens belonging to the family Umbelliferae (English name Celery), is a herbaceous, biennial and branched stem medicinal plant wildly available in various countries, it is ordinarily cultured in the Europe area as a diet yield and it is also grown in Egyptian cities, regions of Kingdom of Saudi Arabia and Algeria[3]. Celery leaves contain flavonoids, tannins and saponins which have antibacterial effects, as the juice of its roots and leaves possess effective biochemical actions[4]. This extract has many medical activities like anticancer, anti-inflammatory, anti-bacterial, anti-spasmodic, anti-hypercholesterolemic, anti-hepatotoxic and analgesic[5] with many types of research that record anti-inflammatory, antibacterial and antifungal effects of essential oil of celery[6,7].

Celery can be a possible application to enhance the development and production of commercially used insect repellents and to be applied as alternatives for the commonly used synthetic chemicals in the community for controlling insects mainly the vector ones. In past years, a certain substance called CAH had been detected in the ethanolic extract of celery seed, which had been checked as an active reagent for *Helicobacter pylori* infection treatment[8]. Additionally, Celery has powerful antimicrobial activities against *Saccharomyces cerevisiae*, *Bacillus subtilis* and *E. coli*, the oil of celery seeds was detected to be effective against *Campylobacter jejuni*, with potent inhibitory effects against various saprophytic and pathogenic microorganisms[9,10]. Moreover, celery contains two senkyunolides and sedanolide which are effective against mosquito larvae, nematodes and fungi, furanocoumarins inhibited *Micrococcus luteus*, *Listeria monocytogenes* and *E. coli* O157:H7[11,12].

Viscum album (Mistletoe) is a member of the Loranthaceae family. It is a familiar evergreen parasitic plant, growing on deciduous trees as a ball-like scrub. The evergreen plants are considered obligate parasites, depending partly on their host to get minerals and water but can process photosynthesis. Thus it is considered a semi-parasite that can live in most regions of the world, either on edible or nonedible trees, remembering that only those that grow on edible plants can be used for medicinal purposes[13].

It contains resin, saponin, organic acids and alkaloids and also contains hydrophilic cytotoxic substances and immune-modulatory proteins such as viscotoxins and mistletoe lectins. They have the ability of immune system stimulation via leukocytes activation leading to the release of cytokines, inhibiting cellular proliferation with *in vitro* and *in vivo* apoptosis induction[14,15].

The certain study applied *in vivo* on rats infected by *S. aureus* with *B. cereus* and *E. coli* with *P. aeruginosa*. Haematological, bacterial and histopathological studies, 7 days after treatment with extract of *Viscum album* exhibited its therapeutic effect[1].

This work was done to examine the antibacterial inhibitory activity of aquatic extract of both *Viscus Album* and *Apium graveolens* against a group of clinically isolated bacteria, whether by direct inhibition or inhibition of adhesion and biofilm formation.

MATERIALS AND METHODS

Plant collection: Fresh dried plant materials of *Viscus album* (mistletoe) and *Apium graveolens* (celery) were purchased from a local market in Hilla city/ Iraq. Preparation of aquatic extract from fresh *Viscus album* and *Apium graveolens* was prepared by soaking 50 gm of each one as a dried powder in 100 mL distilled water and allowed to stand for about 72 hrs then sterilized by filtration (by Millipore filter paper-0.45). The above-prepared extracts were cleaned, peeled, heat dried and cut into small pieces, The electric blender was used to make the ground and placed in clean containers[16].

Bacterial and isolates: Nineteen bacterial isolates obtained from clinical specimens (Gram +ve and Gram –ve isolates)

Table 1: Gram-positive and Gram-negative bacteria involved in this study

Gram-negative bacteria	Gram-positive bacteria
Salmonella typhi	*Staphylococcus aureus*
Salmonella typhimurium	*Staphylococcus epidermidis*
Pseudomonas aeruginosa	*Staphylococcus saprophyticus*
Pseudomonas fluorescens	*Streptococcus pyogenes*
Proteus vulgaris	*Streptococcus pneumoniae*
Proteus mirabilis	*Streptococcus mutans*
Klebsiella pneumoniae	*Streptococcus faecalis*
Enterobacter aerogenes	*Streptococcus agalactiae*
Acinetobacter	
Escherichia coli	
Serratia spp.	

Table 2: Bacterial adherence and biofilm formation by the method of TCP (20)

Mean of OD value at 630 nm	Adherence	Biofilm formation
0.120>	Non	Non
0.120-0.240	Moderate	Moderate
>0.240	Strong	High

were used in the work (Table 1). Bacterial isolates were activated three continual times on nutrient agar and then stored at 4°C as nutrient agar slant. The documentation of isolates was established by various biochemical tests (Api system (Biomerieux, France) in microbiological laboratory/college of Pharmacy/the University of Babylon[17].

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

Antibacterial activity assay: According to CLSI[17], The screening of antimicrobial activities of each aquatic extract on the tested bacteria was determined on Muller Hinton agar media by using the agar well diffusion method. Wells of 8 mm diameter were made on the solid agar using a sterile glass borer. Approximately 20 µL of each extract was inoculated onto wells and were made in the spread plate culture of each microbial isolate, then incubated at 37°C for 24 hrs. The examination of the diameter of the inhibitory zone by measuring it in millimetres and comparing them with the inhibitory zone of Ciprofloxacin (2 µg mL^{-1}) (the test was performed in triplicates)[16,18].

Biofilm formation assay: Semi-quantitative microtiter plate test or tissue culture plate method assay (TCP) designated by previous studies[19] was assumed as the gold standard method for detection of biofilm formation[20]. Results can be compared in Table 2.

Adherence test: Bacterial adhesion to the epithelial cell of the mouth is one of the chief and important virulence criteria of those bacteria and can be detected using the method suggested by authors[21,22].

Statistical analysis: To analyze the data, Bonferroni test[23] was applied, ($p \leq 0.05$) to show significant differences between the types of extracts and with ciprofloxacin by one-way ANOVA. All statistical analysis was performed with the Software Prism 5.01 (GraphPad Inc., La Jolla, CA).

RESULTS

The results of the two aquatic extracts were compared with the Ciprofloxacin (2 µg mL^{-1}) against the same Gram-positive and negative bacteria and it showed less inhibitory effect against most tested bacteria than those two aquatic extracts (Fig. 1a-k and Fig. 2a-h). The two aquatic extracts showed a significant antibacterial effect. *Viscus album* observed high efficacy against both Gram-negative and Gram-positive bacteria with an inhibition zone ranging from 20 mm when used against *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* (Fig. 1c and Fig. 1e, respectively) to 35 mm on use against *Streptococcus agalactiae* (Fig. 2h). While the antibacterial effect of *Apium graveolens* revealed a slight antibacterial effect against both Gram-negative and positive bacteria at a range of 18 for *Pseudomonas aeruginosa* (Fig. 1c) to 28 mm for *Streptococcus faecalis* (Fig. 2g).

Regarding the detection of anti-adherence and anti-biofilm activities of aquatic extracts of *Viscus album* against Gram-negative bacteria, it was showing strong inhibition to the growth with the adhesion of bacterial isolate on a glass plate (Table 3), with strong inhibition of adhesion of epithelial cell for all using bacteria (Table 4). In contrast to moderate inhibition for both anti-adherence and anti-biofilm activities by using *Apium graveolens* extract.

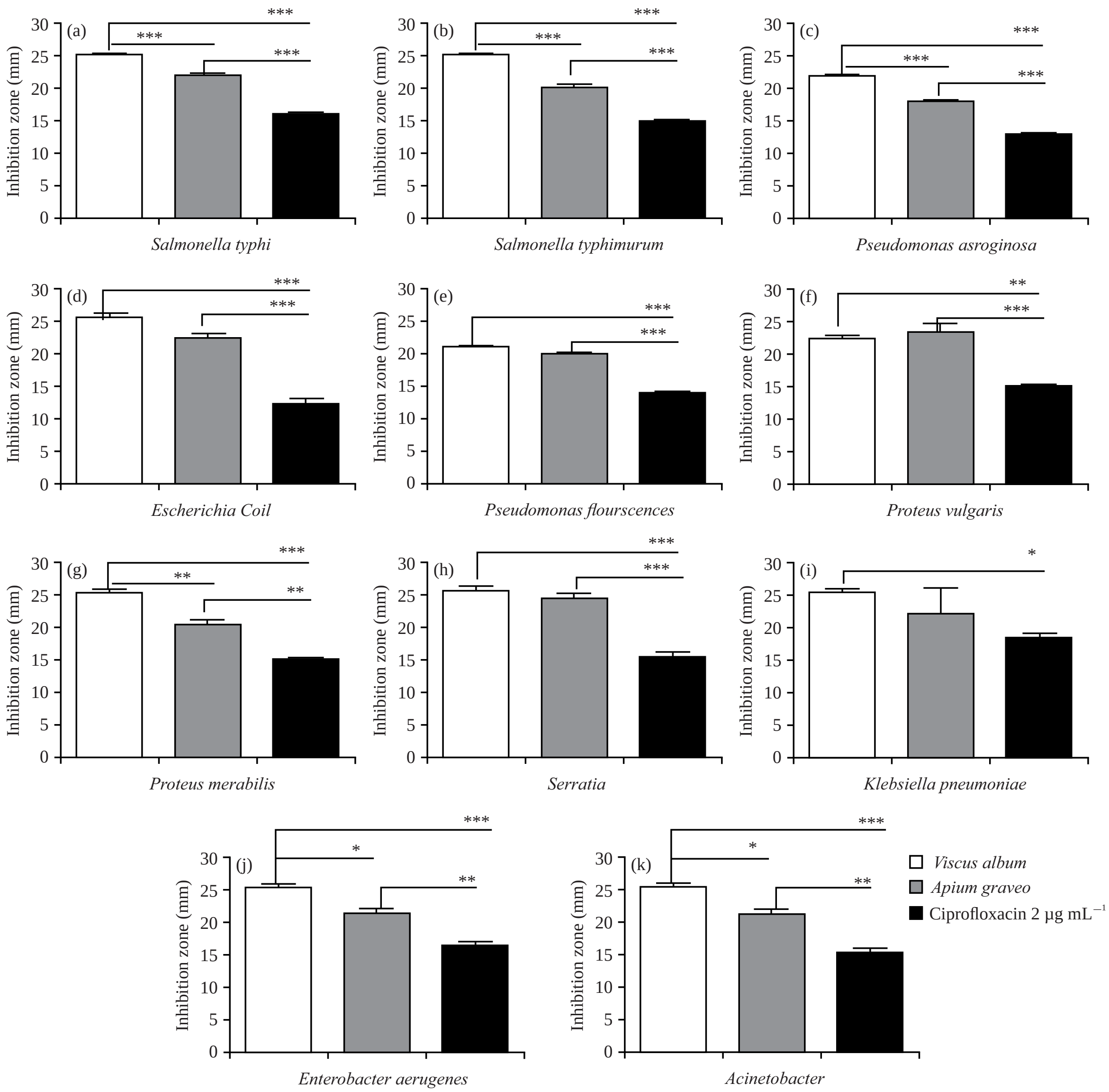

Fig. 1(a-k): Antibacterial effect of *Viscus album* and *Apium graveolens* against several clinical isolates of Gram-negative bacteria by agar well diffusion method in comparison with Ciprofloxacin (2 µg mL^{-1}), (a) *Salmonella typhi*, (b) *Salmonella typhimurium*, (c) *Pseudomonas aeruginosa*, (d) *Escherichia coli*, (e) *Pseudomonas fluorescens*, (f) *Proteus vulgaris*, (g) *Proteus mirabilis*, (h) *Serratia*, (i) *Klebsiella pneumonia*, (j) *Enterobacter aerogenes* and (k) *Acinetobacter*

Number of asterisks (*) corresponds to the level of the statistical significance (*$p<0.05$, **$p<0.01$ and ***$p<0.001$)

Table 3: Anti-adherence activity of aquatic extracts of *Viscus album* and *Apium graveolens* against Gram-negative bacteria

Gram-negative bacteria	*Viscus album*	*Apium graveolens*
Salmonella typhi	High	Moderate
Salmonella typhimurium	High	Moderate
Pseudomonas aeruginosa	High	Moderate
Pseudomonas fluorescens	High	Moderate
Proteus vulgaris	High	Moderate
Proteus mirabilis	High	Moderate
Klebsiella pneumoniae	High	Moderate
Enterobacter aerogenes	High	Moderate
Acinetobacter	High	Moderate
Escherichia coli	High	Moderate
Serratia spp.	High	Moderate

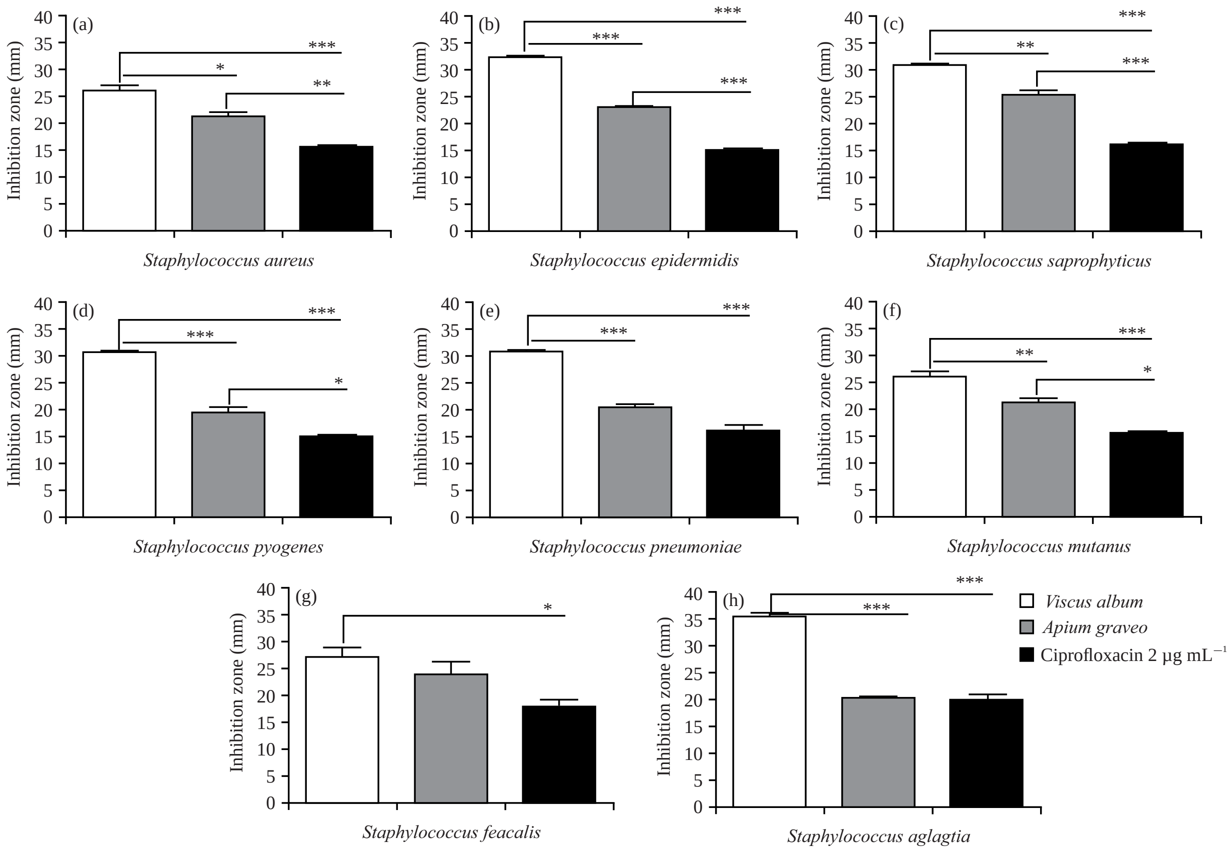

Fig. 2(a-h): Antibacterial effect of *Viscus album* and *Apium graveolens* against the number of a clinical isolate of Gram-positive bacteria by agar well method in comparison with Ciprofloxacin (2 μg mL^{-1}), (a) *Staphylococcus aureus*, (b) *Staphylococcus epidermidis*, (c) *Staphylococcus saprophyticus*, (d) *Streptococcus pyogenes*, (e) *Streptococcus pneumoniae*, (f) *Streptococcus mutans*, (g) *Streptococcus faecalis* and (h) *Streptococcus agalactiae*

Number of asterisks (*) corresponds to the level of the statistical significance (*p<0.05, **p<0.01 and ***p<0.001)

Table 4: Anti-biofilm activity of aquatic extracts of *Viscus album* and *Apium graveolens* against Gram-negative bacteria

Gram-negative bacteria	*Viscus album*	*Apium graveolens*
Salmonella typhi	High	Moderate
Salmonella typhimurium	High	Moderate
Pseudomonas aeruginosa	High	Moderate
Pseudomonas fluorescens	High	Moderate
Proteus vulgaris	High	Moderate
Proteus mirabilis	High	Moderate
Klebsiella pneumoniae	High	Moderate
Enterobacter aerogenes	High	Moderate
Acinetobacter	High	Moderate
Escherichia coli	High	Moderate
Serratia spp.	High	Moderate

DISCUSSION

Medicinally-used plants in particular display a prolonged history in the management of different diseases with a foremost role in the development and production of novel drugs today. While medicine and technology have been developed widely, with an increment in poverty, decrement in natural richness and other obstacles, all of these enhance the obligatory usage of natural products for many goals.

Hildegard von Bingen explained the use of mistletoe as a treatment for splenic and hepatic diseases. Mistletoe was also used for the cure of mumps, leprosy, hepatitis, affections of the liver and lungs, deworming of children, treatment of labour pains, fractures and gout[24].

Many studies were carried out to detect its antimicrobial activities against different microbes[25-30]. Some researchers had shown that antibacterial activities of these extracts were more effective and obvious against most Gram-negative pathogenic bacteria because of their anti-biofilm activity when compared with Gram-positive bacterial species that have thin biofilms[26,31,32].

Orhue *et al.*[33] used different alcoholic mistletoe extracts against *Klebsiella aerogenes*, *P. aeruginosa E. coli* and *S. aureus*, they revealed antibacterial activity to

P. aeruginosa and the *Staph. aureus* only. While Oguntoye *et al*.[34] showed that aquatic extract produced inhibition against bacterial isolates more than the ethanolic extract.

Viscus album not only inhibits bacterial growth but also its compounds stimulate the immune system via activation of granulocytes, monocytes/macrophages, T-cells, natural killer (NK) cells and dendritic cells, with induction of various cytokines such as TNF-α, GM-CSF, IFN-γ IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10 and IL-12[24,35].

These wide different pharmaceutical applications are the result of wealthy chemical structures of *Viscum* species. The chief dynamic active compounds for these species are flavonoids, lectins, Visco toxins, phenolic acids, sterols, fatty acids terpenoids, alkaloids, phenylpropanoids and lignans[36].

Regarding *Apium graveolens*, Genatrika *et al*.[4] tried to utilize its extract as toothpaste to inhibit *Streptococcus mutans* and it showed significant antibacterial activity against these bacteria, moreover, they showed that it has a protective or disease preventive antibacterial activities. Furthermore, Venskutonis *et al*.[37] showed that celery root and leaf extracts have inhibited the growth of bacteria *in vitro*, moreover, Gram-negative bacteria were the commonest bacteria affected by these extracts with different concentrations.

Certain studies have shown that celery improves digestion, cardiovascular disorders, central nervous system, with additional anti-inflammatory, antimicrobial and other various pharmacological activities[8,38-40]. Moreover, certain work studied the effects of this plant extract on *M. tuberculosis* by the use of 200 mg mL^{-1} of whole-plant extract made using 70% ethanol, it showed inhibitory effects on MTB (only 20 colonies appeared after an incubation period of 4 weeks)[40].

Flavonoids, Saponins and Alkaloids components of celery and their role as antibacterial effects were also studied[41]. Its phytochemicals were detected in the leaves of celery[42]. Flavonoids exhibit antibacterial effects via different mechanisms such as energy metabolism, inhibition of cytoplasmic membrane function and inhibition of nucleic acid synthesis[43]. Saponins' antibacterial effects seem to have membranolytic properties[44], as it has increased the bacterial cell membrane permeability without destroying them[45]. Moreover, the mechanism of the action of highly aromatic alkaloids can intercalate with DNA[46]. Both above mechanisms may explain the suggested mechanisms of antibacterial effectivity of our plant's extracts.

CONCLUSION

The infections caused by highly resistant Gram-positive and negative bacteria are among the most difficult diseases all over the world. Medicinal plants that can be used traditionally to treat such infections are suitable to face these problems.

SIGNIFICANCE STATEMENT

Therefore, aquatic extracts of *Viscus Album* and *Apium graveolens* can be considered beneficial for prophylaxis and treatment against a wide range of pathogenic bacteria. Moreover, these plant extracts can be a source of new complementary medicines and support for the treatment of many infectious diseases and can prevent or decrease their complications.

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