



Effect of *Allium sativum*, *Zingiber officinale*, *Mentha pamiroalaica*, and *Curcuma longa* on Oral Bacteria

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Abstract

This study aimed to assess the antimicrobial activity of *Allium sativum*, *Zingiber officinale*, *Mentha pamiroalaica*, *Curcuma longa* aqueous extracts against gram-positive and gram-negative bacteria found in supragingival plaque of periodontitis patients. Thirty-five samples were collected from the supragingival plaques. Using disk diffusion and agar well diffusion methods, the antimicrobial activity of amoxicillin and aqueous extracts of *Allium sativum*, *Zingiber officinale*, *Mentha pamiroalaica*, *Curcuma longa* were examined. The antimicrobial activity of amoxicillin was compared to that of these plants. Seventeen bacterial isolates were obtained, 11 were G-negative (64.7%) and 6 were G-positive (35.3%). All bacterial isolates responded to *Allium sativum*, *Zingiber officinale*, *Mentha pamiroalaica* and *Curcuma longa* extracts, with inhibition zones (20 to 35 mm). In contrast, most bacterial isolates were resistant to amoxicillin, and some were responsive to amoxicillin. *Allium sativum*, *Zingiber officinale*, *Mentha pamiroalaica* and *Curcuma longa* extracts appeared to be effective in inhibiting various microorganisms and may raise the hope of being good alternatives to antibiotics.

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Introduction

The main causative factor of periodontitis is bacterial biofilms which aggregates near the gingival margin [1]. It is estimated that approximately 500 or even more bacterial species can be found in dental biofilms. At the time when gram-positive bacteria predominate the supragingival area, anaerobic gram-negative bacteria predominate the subgingival area. It had been shown the development of periodontitis is associated with different types of pathogens (*Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum* and *Porphyromonas gingivalis*) [2,3]. For the destruction of dental plaque and inhibition of its maturation by brushing, the use of

interdental aids and professional prophylaxis are the targets of almost all periodontal treatments [4]. Despite the effectiveness of mechanical treatment, the need for new classes of antimicrobial products is extremely high especially with the terrifying increase in the resistance of microorganisms to antibiotics. *Allium sativum*, the binomial name of garlic, is a plant that has numerous antiviral, antifungal and antibacterial activities. It has been used in medicine since ancient times and has long been known to have antibacterial, antifungal, and antiviral properties [5]. With a broad spectrum of activities, garlic has antimicrobial effects on: *Streptococcus*, *Staphylococcus*, *Salmonella*, *Klebsiella*,

Mycobacterium, *Clostridium*, *Helicobacter*, *Escherichia* and *Proteus species* [6].

The most frequently used herbal component in medical treatment is *Zingiber officinale* which is the binomial term for ginger. As an alternative to medication in the treatment of several infectious diseases, ginger has been used and examined for its antioxidant, antimicrobial and bioactivity properties [7]. China, India, Mexico and Southeast Asia are the main producers of *Zingiber officinale*. The *Zingiberaceae* family has anti-inflammatory, antioxidant and antimicrobial activities against a wide range of oral bacteria that play an important role in the determination of dental biofilm ecology [8].

The Lamiaceae family includes a plant genus called *Mentha* which represents a mint plant. With its predominance in North Africa, North America and Europe; the mint has become popular worldwide. Not only were leaves and flowers used but the mint plant as a whole was used to prepare the extract of the essential oil used as a medicine. An essential oil with a concentration of 0.1–1.0% can be produced from mint leaves; this oil consists of (20–31%) methane and about (29–48%) of menthol [6]. Mint has a wide range of antimicrobial, anti-inflammatory, antioxidant and anticancer activities, and is considered as a bioactive substance against periodontal pathogens [9].

C. longa has been widely used in cosmetics and medicine because of its antioxidant, antimicrobial, anticarcinogenic, and antimutagenic properties. In dentistry it had been examined to check its effects in treatment of dental caries, gingivitis, halitosis and oral lichen planus. Sharma et al. showed that the use of *Curcuma longa* extract was effective in the treatment of periodontal diseases, regardless of whether it was used with or without periodontal prophylaxis [10]. As an alternative to dental medication used in the treatment of periodontal diseases, curcumin is considered an economical, not toxic, and safe choice [11]. The anti-inflammatory activities of curcumin are summarized by its suppression of a wide range of inflammatory mediators that are important in the development of periodontal diseases (i.e. Interleukins, tumor necrosis factor, cyclooxygenase-2 and nitric oxide synthase). By the inhibition of the toll like receptor 2, 4 and 9, curcumin can inhibit the loss of connective tissues associated with periodontitis [12]. This study aimed to evaluate the antibacterial activities of various plants extracts on dental biofilms derived from patients with periodontitis as an excellent alternative to traditional antibiotics.

Materials and Methods

By using of tap water followed by distilled water, the leaves and roots of the tested plants were rigorously washed. The roots and leaves were allowed to dry before being ground to 100 mg powder in a blender. Aquatic extracts from *Allium sativum*, *Zingiber officinal*, *Mentha pamiroalaica*, *Curcuma longa*-related aquatic extracts (25%), and amoxicillin were prepared according to a previously described method [13]. The duration of this study from 20-3-2024 to 1-7-2024.

The culture medium selected for the current study was nutrient agar. Approximately 500 ml of distilled water was mixed with nutrient agar (14 g) and the latter was dissolved under heat. At a temperature of approximately

121^o C for 15 minutes, this mixture was autoclaved and allowed to set after pouring it in the culture plates. Supragingival plaque samples were collected from periodontitis patients [14], after which, these samples were incubated and cultured for approximately 24 h. For the aqueous extract, approximately four wells were prepared in each plate using the wells diffusion technique and inhibition zones were determined. Hindi and Chabuck method was used as a reference for the in vitro antimicrobial activity test [15].

Amoxicillin antibacterial activity test

By using amoxicillin disk diffusion method, the antibacterial action of amoxicillin was determined using the amoxicillin disk diffusion method, and the assay was performed in a triplicates [16].

Samples Collection

In this study, 35 samples were collected from supragingival plaques of patients with periodontitis who visited the educational clinics of the College of Dentistry / Babylon University.

Isolation and Identification of Bacterial Isolates

The samples were cultured on different media (Nutrient, Blood and MacConkey agar) and incubated at 37 C^o for 24 h, pure cultures were kept in a sterile refrigerator for future work. Isolates were detected and identified using morphological, biochemical testes and the Vitek 2 Compact System [16].

Ethical statement

The study proposal agreed with the ethical standard of Helsinki and was approved by the local medical ethics committee according to document number 47 involving the number and date in 16-2-2024 to get approval.

Statistical Analysis

Data analysis was performed using SPSS 27 and statistical significance was set at $p < 0.05$.

Results

In the current study, 17 different species of bacteria were isolated from the supragingival plaque of patients with periodontitis. Bacterial isolates were stored as agar slant at four °C after being activated on nutrient agar three times. 11 G-negative bacterial isolates (64.7%) and six G-positive bacterial isolates (35.3%) were detected in the present study as shown in Table 1

The Antibacterial effects of *Allium sativum*, *Zingiber officinal*, *Mentha pamiroalaica*, *Curcuma longa* at 25% concentration against bacteria were studied using the agar well method. All bacterial isolates of gram-positive and gram-negative bacteria were responsive to these plants' substances and the

range of inhibition zone (27 to 34 mm) for *Allium sativum* is shown in Figure 1. The range of inhibition zones (27 to 35 mm) for *Zingiber officinal* is shown in Figure 2. Furthermore, the range of inhibition zone (20 to 28 mm) for both *Mentha pamiroalaica* and *Curcuma longa* is shown in Figure 3 and 4 respectively.

The activity of amoxicillin against selected species of gram-positive bacteria and gram-negative bacteria was detected using the disc diffusion method. Most bacterial isolated from gram-negative and gram-positive bacteria were resistant to these antibiotics and some bacterial isolates were sensitive to amoxicillin as shown in Figure 5.

Discussion

Several studies have shown that periodontal diseases represented a status of increased inflammation [17,18], however the bacteria remain the main cause of these diseases. Plants are a good source for the evolution of new medications that mission. Plants were tested and used from viral infections to cancer treatment. Different biologically active substances (for example: essential oils, phenolics, tannins and saponins), had been shown to be responsible for the antibacterial action of the plants [19]. The current study showed that the antimicrobial actions of all selected plants were comparable to those of amoxicillin. As almost all the selected plants showed successful eradication of bacterial species, it is possible to use these substances in the treatment of nosocomial infections and multidrug resistant bacterial infections [20].

Several studies have shown that plants have considerable antimicrobial activity. Although amoxicillin bacterial inhibition is better than that of most plant extracts, this antibiotic is resistant to antibiotic [21].

Various phyto-chemicals are present in plants metabolites, and these products can aid in new medication development [22,23]. To decrease the adverse effects of medications used in dentistry, awareness of the use of the natural products should be encouraged. This concept has been studied previously, and different parts of plants have been tested including flowers, seeds, bark and leaves for their antimicrobial effects [24,25].

It is also effective against *lactobacillus spp*, *P. aeruginosa*, *S. sanguis*, *S. salivarius* and *S. mutans*, garlic is considered an effective antibacterial plant [5,26], and it has been suggested that garlic can be used as a medication for periodontitis, as it has the ability to inhibit the most important pathogens in periodontitis (*A. actinomycetemcomitans* and *P. gingivalis*) [27,28]. It has been shown that *Leptotrichia buccalis*, which belongs to

gram-negative species, had the least sensitivity to garlic, with scales like the membrane covering the surface of these bacteria [29]. Atae et al revealed that 100% of methicillin-resistant bacteria and erythromycin-resistant bacteria were sensitive to garlic [30]. Additionally, *S. aureus* derived from hamburgers is sensitive to garlic and other garlic products [31]. It has also been shown that *Lactobacillus spp.*, *S. sanguis*, *S. salivarius*, *S. mutans* and *P. aeruginosa* are sensitive in a manner like garlic [32]. Additionally, the antimicrobial activity of garlic is comparable to that of chlorhexidine [33,34]. It had been shown that the increase in concentrations of the plant extracts associated with concomitant increase in the inhibition zone of these products [35,36]. *Staphylococcus aureus*, *E. Coli*, *Streptococcus faecalis* and *Bacillus subtilis* are sensitive to ginger [35]. Various components present in ginger, including gingerols, shogaols and volatile oils are responsible for the antimicrobial activity of ginger [37,38]. Similar studies have also reported that plants extracts were effective in inhibiting the growth of various bacterial isolates [39,40].

Conclusion

The extracts of *Allium sativum*, *Zingiber officinale*, *Mentha pamirolaica*, and *Curcuma longa* appeared to be of high therapeutic value against a wide range of gram-negative and gram-positive bacteria in dental plaques from periodontitis patients and are considered an excellent alternative to antibiotics to overcome antibiotic resistance.

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None.

Conflict of Interest

None.

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Table 1. Gram positive and Gram negative bacterial isolates obtained.

G+ bacteria	G- bacteria
<i>Staphylococcus aureus</i>	<i>Aggregatibacter actinomycetemcomitans</i>
<i>Staphylococcus epidermidis</i>	<i>Prevotella intermedia</i>
<i>Streptococcus mutanus</i>	<i>Salmonella typhi</i>
<i>Streptococcus pneumonia</i>	<i>Pseudomonas fluorescences</i>
<i>Streptococcus pyogenes</i>	<i>Pseudomonas aeruginosa</i>
<i>Streptococcus feacalis</i>	<i>Escherichia coli</i>
	<i>Proteus merabilis</i>
	<i>Proteus vulgaris</i>
	<i>Acinetobacter</i>
	<i>Enterobacter aerugenes</i>
	<i>Klebsiella pneumonia</i>

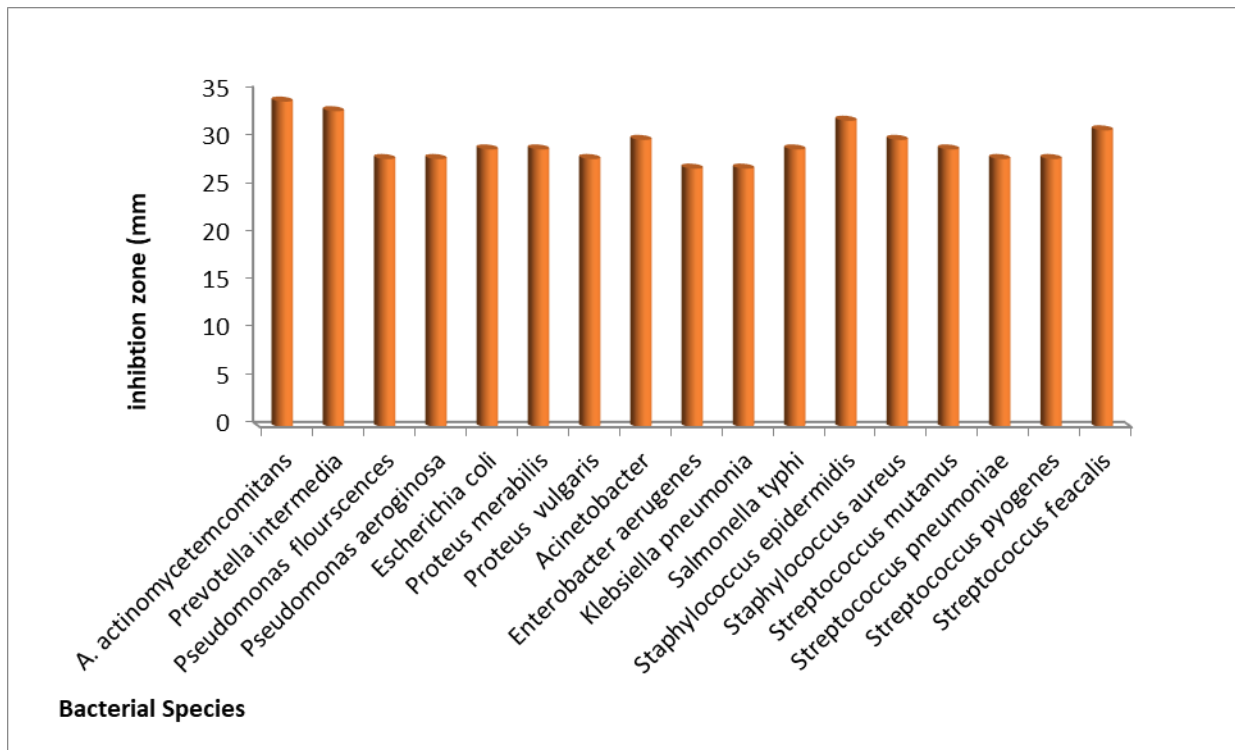


Figure 1. Activity of *Allium sativum* against selected species by agar well method.

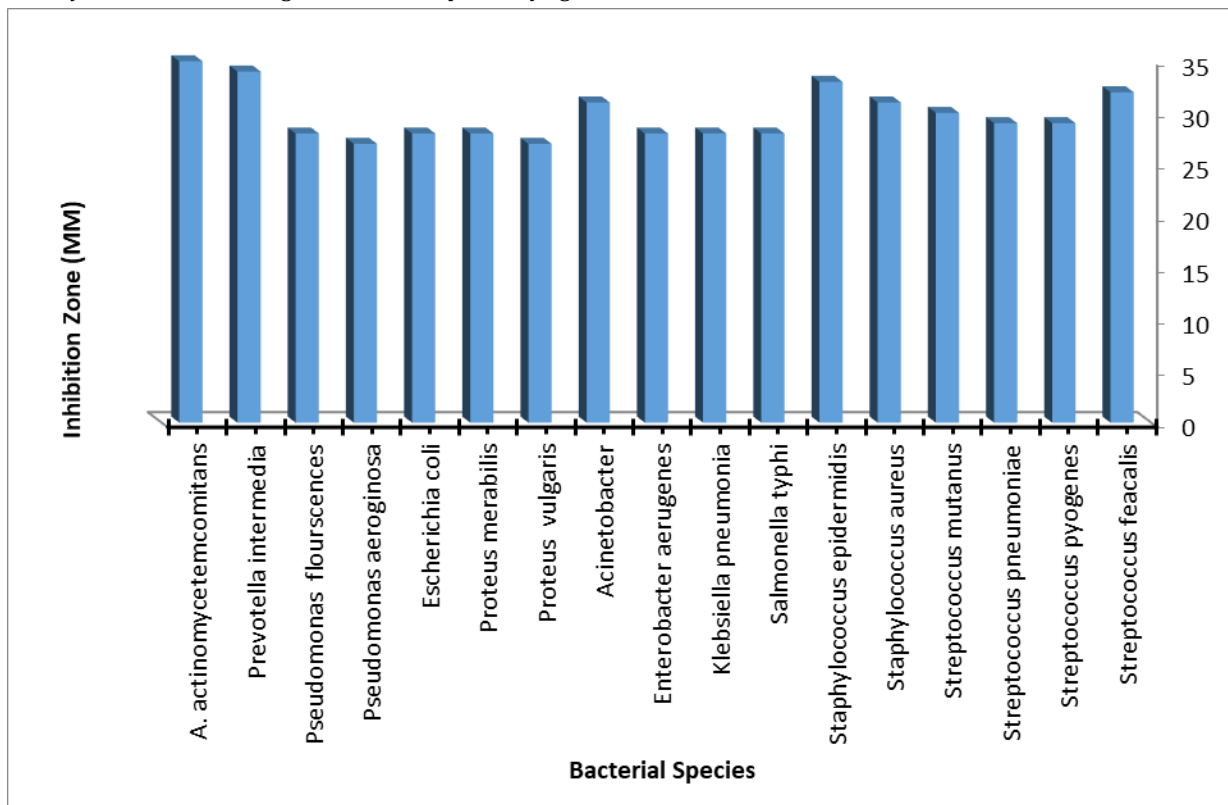


Figure 2. Activity of *Zingiber officinal* against bacterial isolates by agar well method.

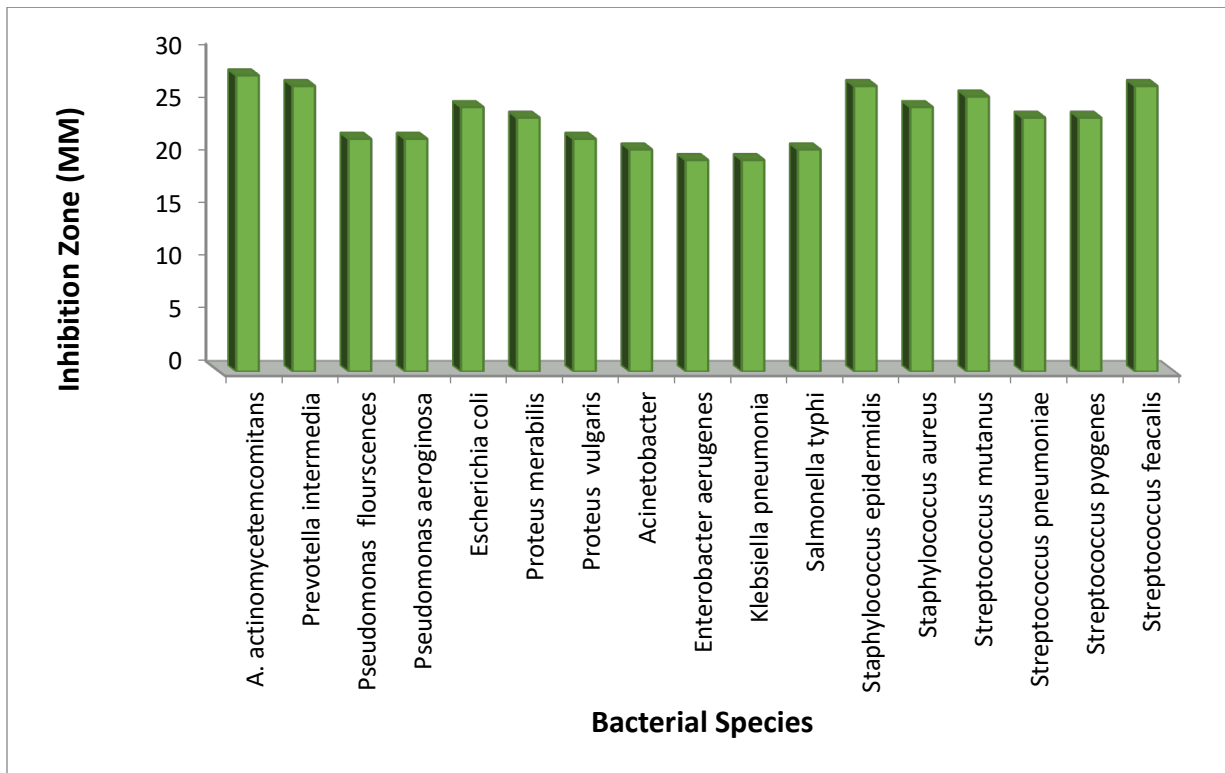


Figure 3. Activity of *Mentha pamiroalaica* toward bacterial isolates.

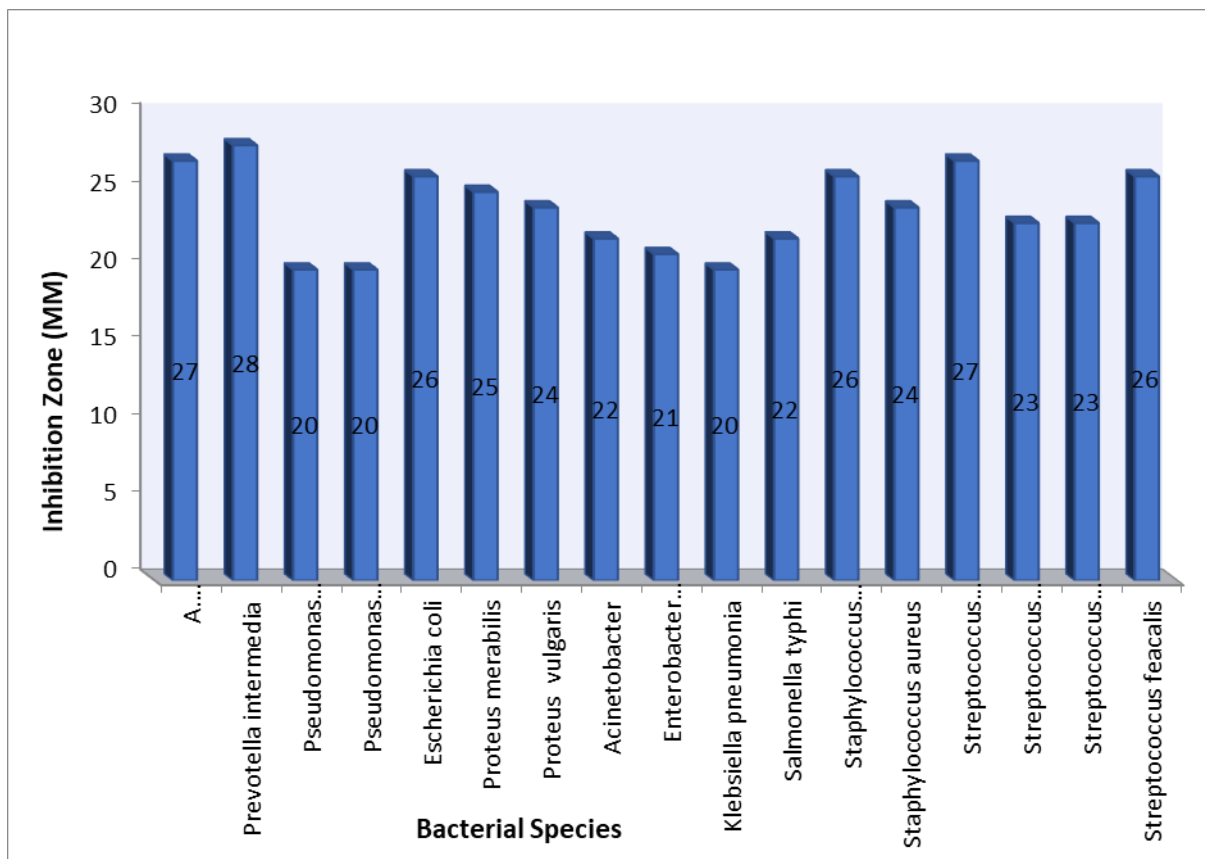


Figure 4. Activity of *Curcuma longa* toward selected species of bacteria.

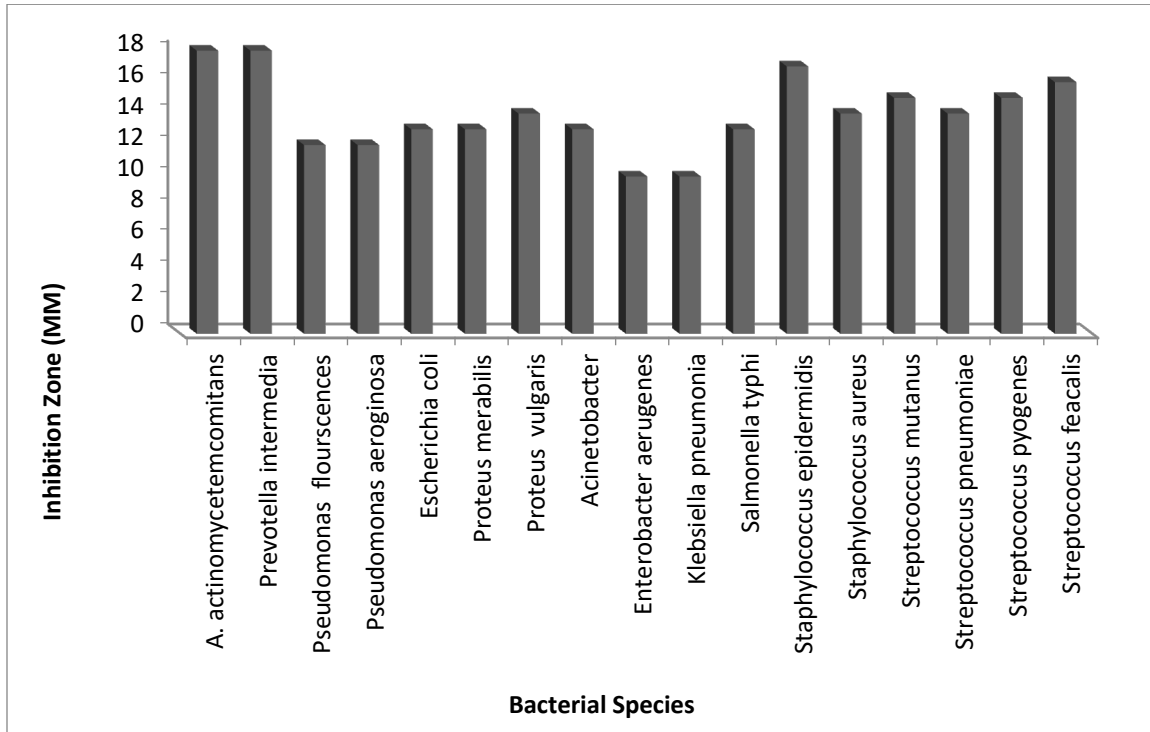


Figure 5. Antibacterial effect of amoxicillin against Gram negative bacteria by disc diffusion method.