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Silver Anoparticles Ynthesized by Hree Pecies of Enus *Streptococcus* and Valuate Heir Ynergistic Ffects with the Il Xtracted from *Syzygium Aromaticum* on Ome Linical Acterial Solates

Suaad Ali Ahmed*, Hussam Mahmood Hasan
Department of Biology College of Science University of Baghdad, Baghdad, Iraq

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Abstract

The nanoparticles biosynthesis is disclosing a new route of exploration concerned with nanotechnology. Silver nanoparticles (AgNPs) are integrated in familiar techniques via chemical routs, for having utterly toxic natures. *Syzygium aromaticum* is an aromatic plant which belongs to the genus Eugenia. Three species of the *genus Streptococcus* were tested using LB for their ability to produce AgNPs and all of them had a brown appearance that confirmed their involvement in AgNPs production. AgNPs were estimated by utilizing a spectrophotometer. The antimicrobial enterprise (AgNPs) of the three species was tested against several types of dangerous bacteria. To determine the MIC, three separate concentrations (v/v) of 0.5%, 5% and 10% of clove oil with dimethyl sulfoxide (DMSO) were prepared (well diffusion technique). Clove oil extract together with AgNPs biosynthesized from three kinds of *Streptococcus*, were shown to have antimicrobial effects on unusual clinical microorganisms.

Keywords: *Streptococcus*, *Syzygium aromaticum*, Nanoparticles, Antibacterial activity

جزيئات الفضة النانوية المنتجة من ثلاث انواع لجنس ***Streptococcus*** وتاثيرها التأزري مع مستخلص زيت القرنفل على بعض العزلات البكتيرية السريرية

سعاد علي احمد*، حسام محمود حسن
قسم علوم الحياة ، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

كشف التصنيع الحيوي للجسيمات النانوية عن مسار جديد في تقنية النانو. يتم دمج جزيئات الفضة النانوية (Ag NPs) في تقنيات مألوفة عبر مسارات كيميائية ، لها طبيعة سامة تمامًا.. *Syzygium aromaticum* نبات عطري ضمن جنس Eugenia (عائلة Myrtaceae)، يعرف عادة باسم القرنفل. يستخدم بشكل رئيسي في التحميص ، الصيدلة ، العطور والمراهم. تم اختبار ثلاثة أنواع من جنس *Streptococcus* لمعرفة قدرة إنتاج جزيئات الفضة النانوية باستخدام وسط LB ، وظهرت جميع الأنواع باللون البني الذي يشير إلى التخليق الحيوي لـ AgNPs. تم استخدام مقياس الطيف الضوئي المرئي بالأشعة فوق البنفسجية لتقدير AgNPs. تم اختبار مضادات الميكروبات AgNPs المنتجة من الأنواع الثلاثة ضد

*Email: suaad.ali@sc.uobaghdad.edu.iq

أنواع مختلفة من البكتيريا المسببة للأمراض. تم تحضير ثلاث تراكيز مختلفة (حجم / حجم) 0.5٪ ، 5٪ و 10٪ من زيت القرنفل مع ثنائي ميثيل سلفوكسيد (DMSO) لتحديد MIC باستخدام طريقة الانتشار الجيد. تم إثبات التأثير التآزري لـ AgNPs الذي تم تصنيعه حيوياً من ثلاثة أنواع من *Streptococcus* مع مستخلص زيت القرنفل ضد الأنواع المتطرفة من البكتيريا السريرية.

Introduction

Nanotechnology technique has been used in several fields [1]. It is a method of administration of elements on an atomic, molecular and magnificent molecular scale, [2]. Nanotechnology may be adept to develop many modern materials and apparatus with an immense range of applications such as in energy construction, electronics, buyer products, biomaterials and medicine [3]. The use of atoms, molecules, or compounds is the basic idea of nanotechnology for the production of materials and devices with special properties for the construction of functional structures [4] .

AgNPs are considered among the most important and charming nanoparticles amid definite metallic nano-materials that are involved in biomedical operations [5]. Seeing their exclusive chemical and physical tracts, there are multifunctional utilizations of AgNPs; for instance, as antimicrobial (bacteria, fungi and viruses), anti-inflammatory, and anti-cancer ministers [6]. These cover visual, electrical, and melting, conductivity, high biological goods [7]. Nano-sized mineral particles are particular and can noticeably be divergent biological, chemical and physical goods over their surface-to-volume scale [8]. In order to render the demand of AgNPs, assorted techniques have been taken up for their generation. Typically, traditional methods (chemical and physical) glance to be very fancy and precarious [9, 10]. Attractively green-prepared AgNPs display huge output, solubility and huge balance. Biological practice impliesy to be non-toxic, straightforward, quick, resilient, and green approaches that can produce precise size and morphology under changing settings. [11].

The antimicrobial effects of aromatic plants against variety of M.O have been thoroughly documented in the drug industry. The primary medical benefits of these plants are closely related to the fundamental oils they generate [12]. In actuality, the extracts' constituents serve as possible reducing and capping agents [13]. *Syzygium aromaticum*, frequently known as clove, is typically used in food arrangement, cancer drug, in regular therapy for gastrointestinal tract muddle, respiratory confusion and hassle [14]. Clove oil is also used as insecticide, scattering disease-being mosquitoes and other insects. Clove oil is a main oil extorted from clove plants, notably from its flowers, stems and leaves. The trait of clove oil is ordinarily indicated by its eugenol and caryophyllene innards [15]. The requisite oil of *Syzygium aromaticum* parades anti-inflammatory and cytotoxic action nearby antimicrobial and insecticidal farms [16].

Materials and Method

Plant Preparation and Oil Extraction

The dehydrated flower was cleaned first with water and then with D.W. The process was repeated 2-3 times. The obtained material was converted to powder by blender. For oil extraction, Clevenger (University of Baghdad) was used. 10g of ground cloves and 150 mL of D.W were mixed. The cloves were allowed to be wetted in the water for about 15 min, and then the mixture was distilled and transferred to the separator funnel before extracting oil twice with 2.0 mL of dichloromethane (DCM) (BDH, England). The DCM extracts were united, adding Na_2SO_4 to be dried, and then gently evaporated to get eugenol as pale yellow oil [15].

Cultivation of Bacteria and Biosynthesis of AgNPs

Three pure isolates of *Streptococcus* (*S. salivarus*, *S. mitis* and *S. agalactiae*) were obtained from Department of Biology, College of Science, University of Baghdad, and then refined in activation broth for 24h. For AgNPs biosynthesis, bacterial species were inoculated in LB, and then shaken at 200 rpm at 37^0C, before being harvested by centrifuge. Later on 10 ml of supernatant was mixed with 5ml of $AgNO_3$ (10 mM), incubated at 30°C. Purified AgNPs was gathered for subsequent work [17].

Estimation of AgNPs

Silver nano-particles were characterized by spectrophotometer (400 -800nm). The colored AgNPs showed at ~400 nm [18].

Antibacterial Activity of AgNPs and MIC Determination for Clove Oil

AgNPs effect from three species of *Streptococcus* was dogged by using well diffusion against different clinical bacteria (*S. aureus, E. faecalis, K. pneumoniae, E. coli, P.aeruginosa* and *P.luteola*). And then three concentrations (0.5%, 5% and 10%) of clove oil were prepared with DMSO (v/v) to reveal MIC [19]. Reticence zones were measured for all.

Results and Discussion

Biosynthesis and Estimation of AgNPs from Three Species of *Streptococcus*

The three bacterial species of *Streptococcus* exhibited brown color when the bacterial supernatant was mixed with silver nitrate solution, thus revealing the biosynthesis of AgNPs (Figure 1).

Medium color advance was related to bio-integration of AgNPs in *Pseudomonas, Bacillusmethylotrophicus, Actinobacteria Fusarium semitectum, Aspergillus fumigatus* cultures [20], *E. coli* [17], *Proteus mirabillis Pseudomonas aeruginosa* and *Klebsiella pnuemoniae* [16]. The conversion of the Ag to AgNPs was measured by using spectrophotometer (400 -800 nm). This practice has been exposed to be duly sensitive to check AgNPs intense surface plasmon vibrations [20]. The results offered that the highest peak was observed at 432, 435, 436 nm for the mixture solution of the species *S. agalactiae, S. mitis* and *S. salivarus* respectively (Figure 2).

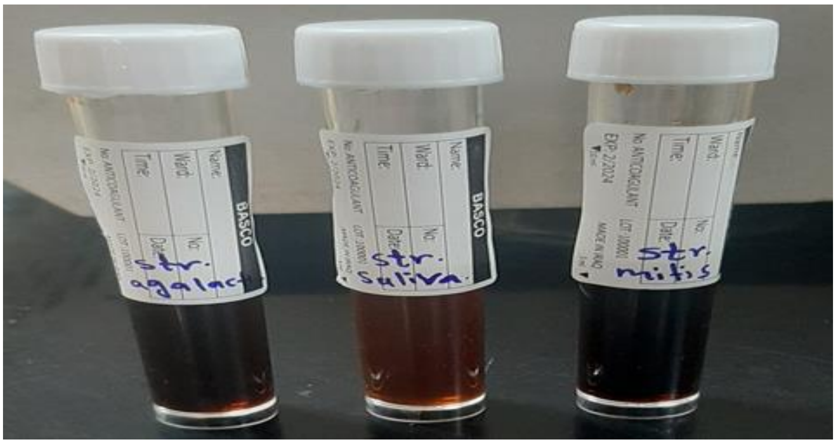

Figure 1: Biosynthesis of AgNPs by three streptococcal species

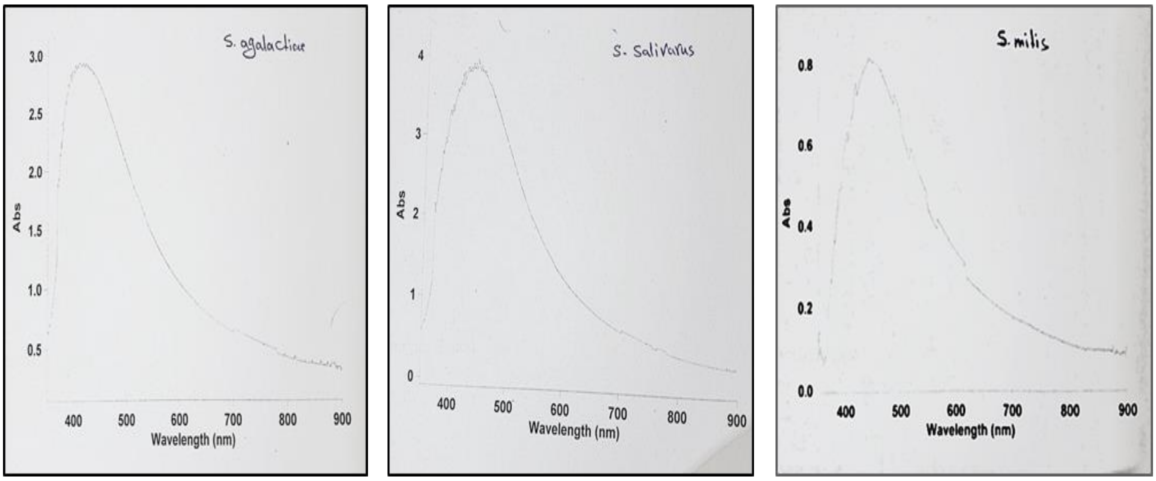

Figure 2: characterization of AgNPs by spectrophotometer

Antibacterial Action of AgNPs and MIC Determination for *Syzygium aromaticum*

The crucial clove oil is extracted from the flower buds of *Syzigium aromaticum* and its chemical ingredients are ß-caryophyllene, tannins along with phenols. The famous relevant component of the oil is eugenol which is liable for the virtue smell of the plant and is a major composing. The traditional uses of clove oil have been reported in numerous scientific articles, focusing on its antioxidant, hypotensive, dental analgesic, antibacterial, anti-inflammatory and antifungal activity [21].

In this work MIC of clove oil was determined against pathogenic bacteria. It was 0.5% for all bacterial isolates (Figure 3, Table 1). Rodríguez, O, et al. (2014) reported that clove oil exhibited boss antimicrobial action at 1000, 500 and 250µg/ ml concentrations against planktonic cells of *Streptcoccus mutans* ATCC700611 which cause dental disease [21]. Clove oil was prepared in three concentrations (100%, 50%, 25%). It showed antimicrobial activity versus *Stap. auras*, *S. typhimurium L. monocyogenes*, and *E. coli* were secure and confined from corrupt food [13].

Table 1: MIC (mm) of clove oil

Bacteria	0.5%	5%	10%
S. aureus	18	19	22
E. faecalis	12	17	21
E. coli	15	17	20
K. pneumoniae	16	18	22
P. earuginosa	11	16	17
P. luteola	16	17	20

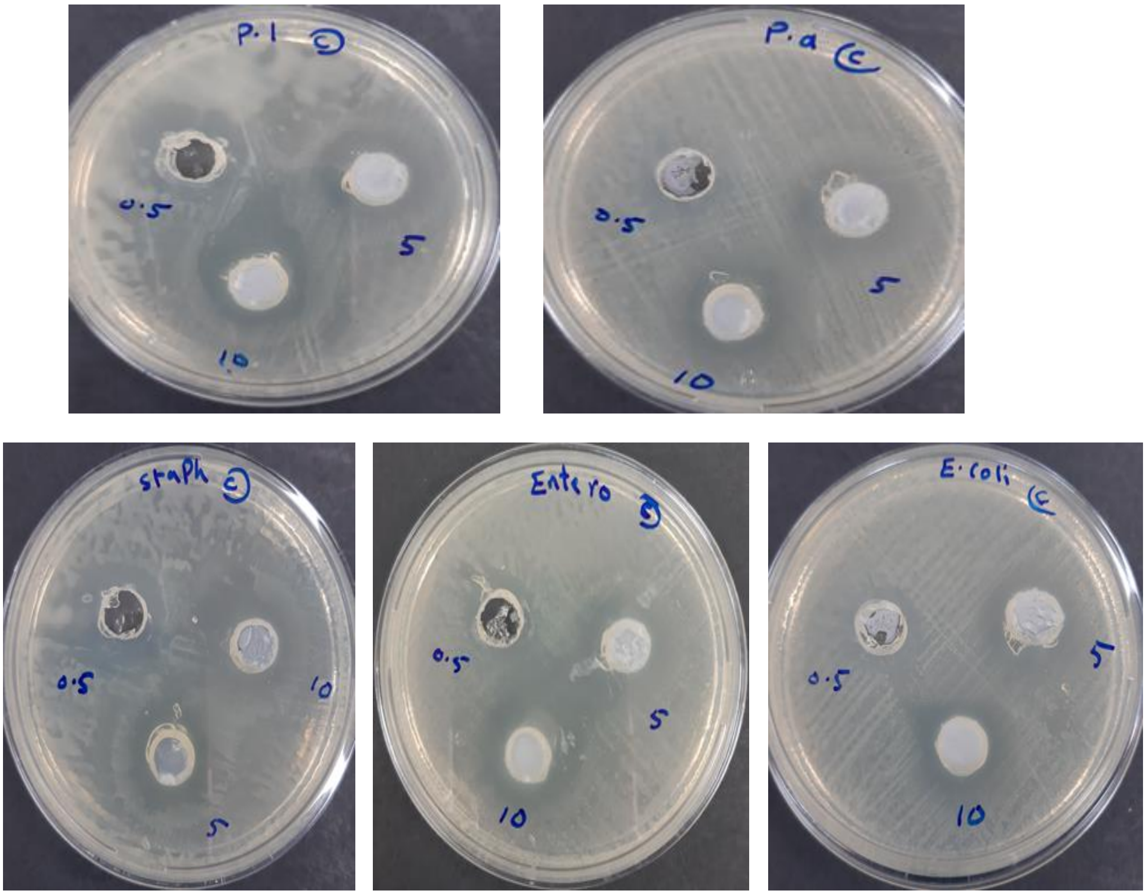

Figure 3: MIC of cloveoil

The presence of pathogens, characterized by multidrug refusal, have enlarged the number of contagious illnesses in the world. Thus, the progress of antimicrobial deputy is desired as there is an expanding burden in multidrug contrary pathogens which are derived from food [22]. Silver nanoparticles have showed significant antibacterial action versus G-ve pathogens in food. Thus, AgNPs might be a good substitute to develop drug versus the strains of bacteria which are defined as multidrug-resistant. The operations of AgNPs may also come to valuable discoveries in numerous fields [23].

The antimicrobial reaction of biosynthesized AgNPs from three species of *Streptococcus* were considered against disparate pathogenic bacteria by using well diffusion technique [24] (Figure 4, Table 2). Results revealed antibacterial action of AgNPs from *S. agalactiae* was the highest versus *E. coli*,*P. aeruginosa*, *P. luteola* and *K. pneumoniae*, while the antibacterial activity for the AgNPs from *S. agalactiae* was equal with *S. salivarus* against *Entero. faecalis*. The antibacterial activity for AgNPs from *S. salivarus* was the highest against *Staph. aureus*. AgNPs from *S. mitis* gave the lowest antibacterial effect against all clinical bacterial isolates that were used in this study.

Table 2: The effect of (AgNPs) synthesized from *Streptococcus* on different M.O measured by (mm)

Bacteria	AgNPs of *S. agalactiae*	AgNPs of *S. salivarus*	AgNPs of *S. mitis*
S. aureus	13	14	12
E. faecalis	13	13	12
E. coli	12	10	11
K.p neumoniae	18	15	13
P. earuginosa	17	14	13
P. luteola	14	12	13

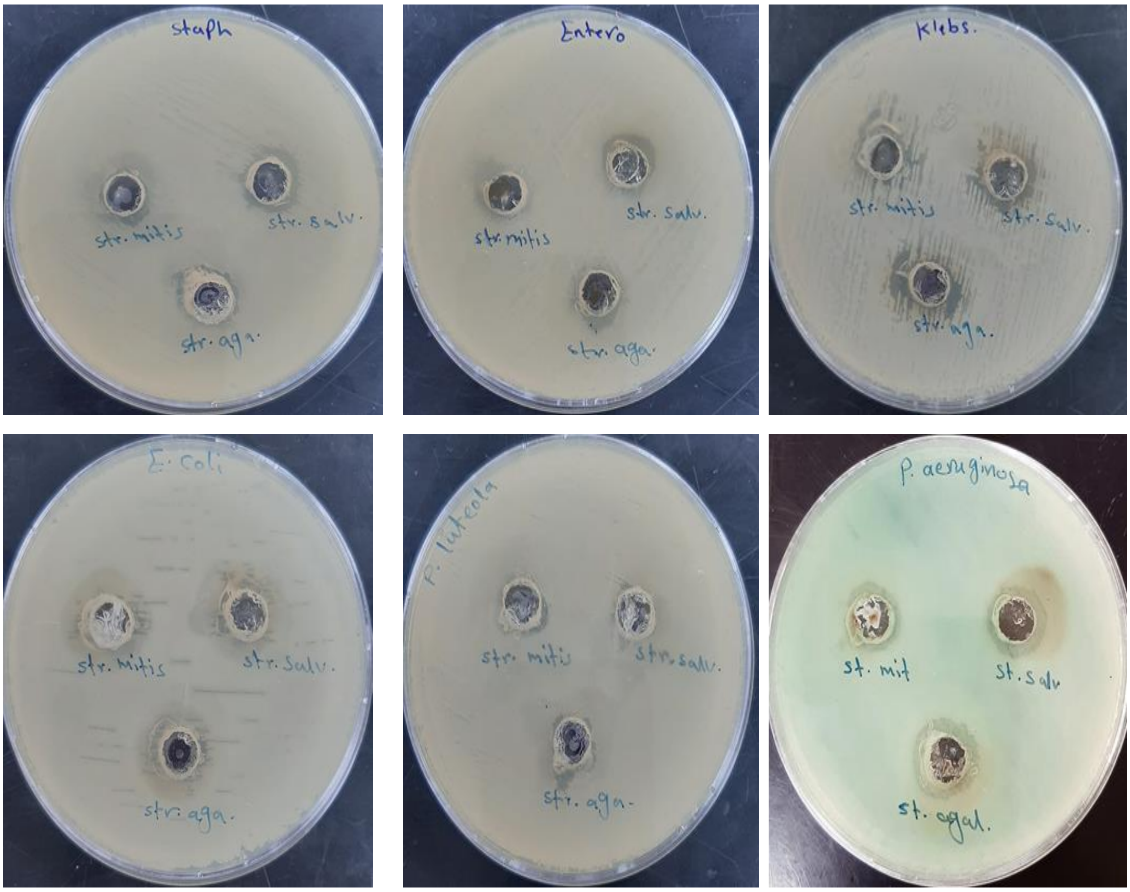

Figure 4: Action of AgNPs from streptococcal species

The perfect mechanism which AgNPs engage to develop antimicrobial implement is not certainly understood and is an oppose topic. There are despite different thesis on the action of AgNPs on microbes: 1- AgNPs have the capacity to anchor to the cell wall and afterwards penetrate bacteria 2- The construction of free radicals by the AgNPs may be advised as mean for cells death which cause destruction for cell membrane 3- Release of silver ions by the nano-particles can cooperate with the SH-groups of many urgent enzymes and inhibit them. 4- Nano-particles can act on the soft sulfur and phosphorus bases of DNA and smash it 5- Nano-particles can also modulate the signal transduction in bacteria [25].

Synergistic Effect

The resistance of pathogens to common antibiotics facing the medical community is biggest challenge. Thus, greater use of natural substances like medicinal plants as an alternative to manufactured chemical medications in the treatment of bacteria as herbal active components is of great importance. Today, in addition to the use of medicinal plants, there is a lot of interest in the medical applications of nanotechnology and metal nanostructures. Numerous NPs will be important in medicine for illness prevention [26].

In this study the synergetic effect of AgNPs produced from three species, *S. salivarus*, *S. mitis* and *S. agalactiae*, combined with clove oil extract was tested against different clinical bacterial isolates. The result showed increase in antibacterial activity against all pathogens that have been used in this report (Figure 5 and Table 3). *S. mitis* and *S. agalactiae* offer synergetic effect with clove oil extract across different clinical types of bacteria.

Table 3: Synergetic effect (mm) of AgNPs with clove oil MIC

Bacteria	*S. agalactiae*	*S. salivarus*	*S .mitis*
S. aureus	19	20	21
E. faecalis	15	16	13
E. coli	18	16	16
K. pneumoniae	20	19	17
P. earuginosa	19	16	19
P. luteola	19	18	18

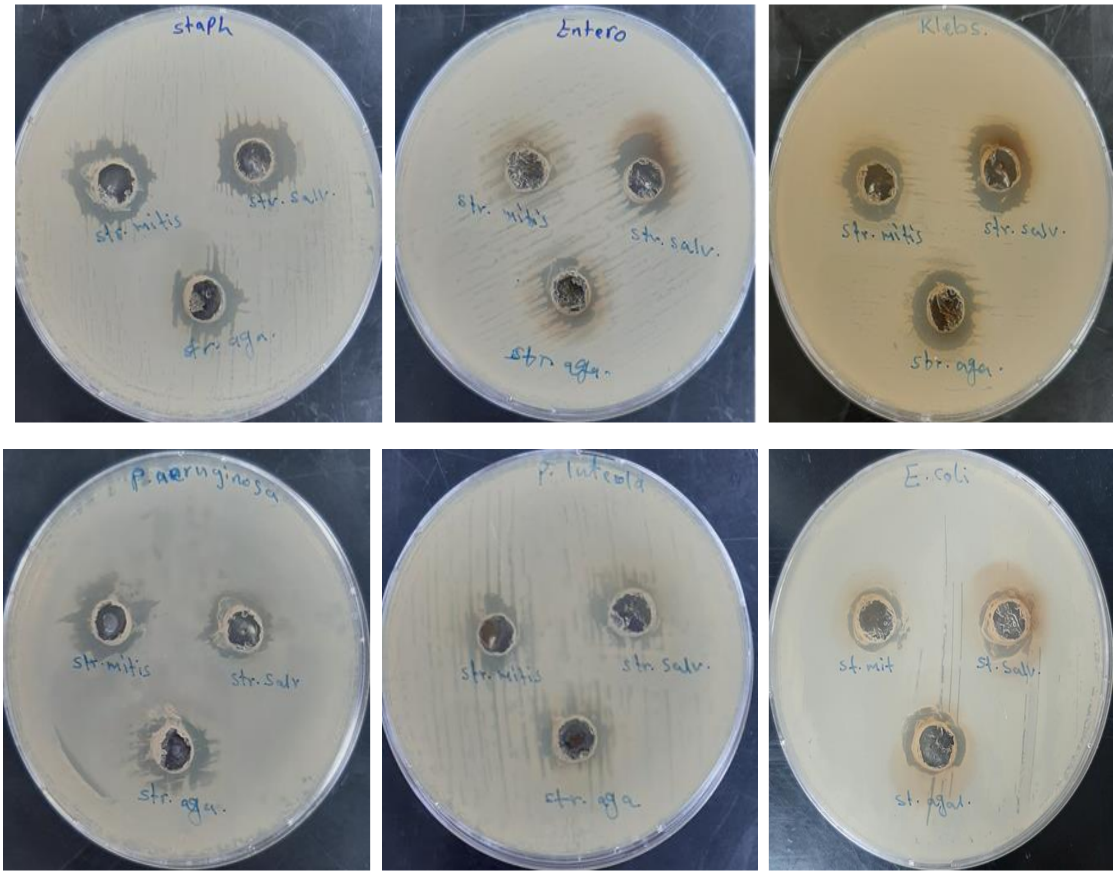

Figure 5: Synergetic effect of AgNPs from streptococcal species with clove oil

Conclusion

Metallic nanoparticles have particular abundant antimicrobial influence and, thus, they have been used within medical gadgets to avoid infection from spreading [27]. Current, plants can also be used as a remedy for diverse diseases because of their action on microbes in compared to regular antibiotics and their side effects [28]. AgNPs interact with basic components of the bacterial cells such as enzymes, ribosomes and DNA, leading to permeability changes of the membrane, oxidative stress, electrolyte imbalance, enzyme malfunction [29]. It can be concluded from this study that AgNPs produced from three species of *Streptococcus* have more antibacterial ability when combined with clove oil extract

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