

Evaluation and modulation of bactericidal potential of different antibacterial agents against bacterial pathogens from conjunctivitis infections

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Abstract

Conjunctivitis is one of the most common disorders seen in hospitals. Youngsters under the age of seven are most likely to be diagnosed. The second highest point in the distribution is reached by women at age 22 and men at age 28. The goal of the current study was to identify and characterize the pathogens that cause conjunctivitis and determine their sensitivity against various antibiotics (such as metronidazole, ciprofloxacin, azithromycin, and levofloxacin), as well as aqueous plant extracts (*Ficus religiosa*, *Syzygium cumini*, *Azadirachta indica*, *Allium cepa*, *Eucalyptus camaldulensis*, *Syzygium aromaticum*, *Aloe barbadensis*, and *Citrus limon*) and their green synthesized silver nanoparticles. The samples were taken at Fatima Memorial Hospital, Lahore. The blood agar test was used for screening of pathogenic bacteria after samples were isolated. Types of hemolysis that the bacteria displayed were observed in order to assess the pathogenicity of the bacterial isolates. To monitor the antibacterial activity of particular pathogenic strains, the well diffusion method was employed. The outcomes demonstrated that every antibiotic was considerably more effective against isolated bacteria. Significant antibacterial activity against bacterial strains was demonstrated by *E. camaldulensis*, *S. cumini*, and *C. limon* (13.66 ± 1.20 to 9 ± 0.57 , 15.5 ± 0.76 to 10.33 ± 1.45 , and 21.33 ± 0.88 to 12.66 ± 0.33 respectively) out of all aqueous plant extracts. Green synthesized silver nanoparticles from *A. barbadensis*, *S. aromaticum*, *C. limon*, *A. indica*, *F. religiosa* and *A. cepa* displayed more noteworthy outcomes, with the zones of inhibition measuring 11.66 ± 0.66 to 8.83 ± 0.72 ; 12.5 ± 0.62 to 9.83 ± 0.72 ; 16.16 ± 1.09 to 10.83 ± 1.01 ; 13.33 ± 1.20 to 8.83 ± 0.72 ; 12.16 ± 1.16 to 7.33 ± 0.66 , and 13.16 ± 0.59 to 8.33 ± 0.88 respectively. Bacterial strains were identified as *Bacillus thuringiensis*, *Bacillus cereus*, *Bacillus paramycoides*, *Bacillus coahuilensis* and *Pseudomonas aeruginosa*. This study showed that due to the rise in bacterial resistance to drugs, antibiotic medication may be replaced by biological antibacterial tools such as plant extracts and green synthesized silver nanoparticle formulations.

1. Introduction

Conceivably the most delicate organ in the human body is the eye (Astley *et al.*, 2023). Conjunctivitis is a common condition found in ophthalmology clinics worldwide (Azari *et al.*, 2020). Conjunctivitis is typified by inflammation of the conjunctival tissue, ocular discharge, and vascular enlargement. It could be infectious or not, acute or chronic, etc. Allergies, viruses, and bacteria can all cause conjunctivitis (Hashmi *et al.*, 2023). The months with the highest frequency of bacterial conjunctivitis are December through April (Alfonso *et al.*, 2015; Ramirez *et al.*, 2017). Common bacterial infections are usually the cause of bacterial conjunctivitis. These pathogens include *Streptococcus pneumoniae*, *Staphylococcus* spp., *Haemophilus* spp., *Moraxella* spp., (Arrfa, 1997) and. Most cases of bacterial conjunctivitis resolve on their own in 1 to 2 weeks. Children are impacted by bacterial conjunctivitis far more frequently than adults (Hvding *et al.*, 2008). Youngsters under the age of seven are most likely to be diagnosed, and the age range between 0 and 4 years old is of greatest importance. The second highest point in the distribution is reached by women at age 22 and men at age 28 (Adebayo *et al.*, 2010).

Since antibiotic resistance causes millions of deaths worldwide, it poses a serious threat to public health. Antibiotic resistance has become frighteningly widespread in recent years (Hetta *et al.*, 2023). Antibiotic resistance is a major global health concern, since it is estimated to have caused at least 1.27 million deaths worldwide and roughly 5 million deaths in 2019 (CDC, 2022). The urgent need for safer and more effective agents stems from the growing burden of microbial resistance and the adverse effects of synthetic medications on global health and death rates. The pursuit of this project has intensified the hunt for alternatives to plant extracts and their nanoparticles (Chukwuma *et al.*, 2023).

According to their diameters, nanoparticles are the end product of technologically modifying matter and are a few orders of magnitude larger than an atom as a result of molecular processing of matter (Yusuf *et al.*, 2023). Au, Ag, Cu, Ni, Si, and Se are examples of metal NPs. AgNPs are a well-researched nanomaterial that can be produced chemically or biologically, primarily using plants and microbes (Husain *et al.*, 2021). The medical field has shown that AgNPs' antibacterial and anti-inflammatory properties are helpful in managing microbial infections (Barrasa *et al.*, 2010; Mie *et al.*, 2013). AgNPs' effects on microorganisms are explained by a number of theories, including as the inhibition of enzymes required for cell life, the activation of enzymes required for cell survival, the increase in cell permeability, and the penetration of silver ions into cells. AgNPs interact with the phosphate and sulfur of bacterial DNA to form a byproduct of microbial death. According to Wang *et al.* (2022), nanoparticles stop signal transduction and stop bacteria from growing. The utilisation of diverse bio-based materials sourced from bacteria, fungi, plants, and algae for the manufacturing of nanoparticles has stimulated the development of pragmatic, eco-friendly, economical, and easily expandable techniques (Bhandari *et al.*, 2023). The WHO recommends incorporating herbal medicines into national health care programs since they are safer, more widely available, and less expensive than modern synthetic treatments (Gandhimathi *et al.*, 2022).

In affluent countries, conventional medicine derived from medicinal plants is used by about 80% of the population (Hindi & Chabuck, 2013; Solanki *et al.*, 2022). Certain antibacterial substances can be found in plants, spices, and herbs. Aloe Vera leaves have an inside gel that may be rich in bioactive materials such as vitamins, minerals, enzymes, amino acids, and polysaccharides. Aloe vera is a multipurpose plant with numerous medical applications because of its anti-inflammatory, antioxidant, antibacterial, and antiviral qualities (Bhargava & Singh, 2023). Citric acid (Kundu *et al.*, 2020), secondary metabolites (Ghasemi *et al.*, 2009), phenolic derivatives (Ortuno *et al.*, 2006), and a host of other chemicals that exhibit broad antibacterial activity comprise the majority of citrus fruits (Solanki *et al.*, 2022).

Flavonoids are found in citrus fruits like lemons in addition to alkaloids (Ortuno *et al.*, 2006). *Syzygium aromaticum*, the clove tree, is native to Indonesia (Rojas *et al.*, 2014). Cloves are mostly composed of the phenolic chemicals flavonoids, hydroxoxibenzoic acid, hydroxicinamic acid, and hydroxoxiphenyl propense. Additionally, higher gallic acid concentrations were found (Patel *et al.*, 2022). Due to their high phytonutrient content, which includes flavonoids and anthocyanins (Al-Khafaji, 2019), onions have antibacterial, anti-inflammatory, antifungal, anticancer, and antioxidant qualities (Albandary, 2023). The majority of jamun leaves' health benefits are attributed to phytochemicals such gallic acid, flavonoids,

tannins, mallic acid, jambolin, essential oils, jambosine, ellagic acid, betulinic acid and antimellin that are present in jamun leaves (Kumari *et al.*, 2023). The leaves of *F. religiosa* contain tannins, terpenoids, flavonoids, and other phytochemicals (Sharma *et al.*, 2023). It is well recognized that Eucalyptus members serve as significant repositories of a diverse array of secondary metabolites, numerous of which possess several biological functions (Zriouli *et al.*, 2023).

2. Materials and Methods

2.1. Sampling

Five samples of conjunctivitis eye infection were collected from Fatima Memorial Hospital (FMH), Shadman Lahore by using sterilized culture sticks. Further study was done in microbiology research laboratory of Zoology department in Government College University, Lahore.

2.2. Bacterial Isolation

For isolation of bacteria from culture sticks, 2.8% Nutrient agar medium (2.8g nutrient agar/100ml distilled water) was prepared and autoclaved at 121 °C temperature and 15 psi pressure for 60 minutes. Autoclaved nutrient agar medium was poured into sterile petri plates inside sterilized laminar air flow. Spread plate method was used to isolate bacteria. After solidification of nutrient agar in petri plates, collected samples were spread on nutrient agar plates with the help of culture sticks and sterilized glass spreader and plates were incubated for 24 hours at 37 °C. After isolation, bacterial isolates were purified by using streak plate method (Figures 1 &2).

2.3. Pathogenicity test

Blood agar test was performed to check the pathogenicity of isolated bacterial strains. 6ml human blood was added in 2.8% autoclaved nutrient agar. Bacterial strains were streaked on already prepared blood nutrient agar plates and incubated at 37 °C for 24 hours. After incubation, blood hemolysis around bacterial streaks were observed. Greenish zone indicated α -hemolysis, clear zone indicated β -hemolysis and no zone indicated γ -hemolysis (non-pathogenic). After pathogenicity test, glycerol stocks of pathogenic bacteria were prepared for future use (Figure 3).

2.4. Antibacterial activity of antibiotics, aqueous plant extracts and green synthesized silver nanoparticles (AgNPs)

To check antibacterial activity of antibiotics, aqueous plant extracts and green synthesized silver nanoparticles, and resistance of bacteria against them, well diffusion method was used. For this purpose, four antibiotic (metronidazole, levofloxacin, azithromycin and ciprofloxacin) solutions were prepared by solubilizing 10mg antibiotic in 50ml distilled water. Eight different types of aqueous plant extracts were synthesized by heating 40g of plant medium (leaves/seeds/peel/gel/bulb) in 200ml distilled water, at 50 °C for 20 minutes. These extracts included *A. indica*, *S. cumini*, *E. camaldulensis*, *F.*

religiosa (leaf extract), *C. limon* (peel extract), *A. barbadensis* (gel extract), *S. aromaticum* (seed extract) and *A. cepa* (bulb extract). After heating, extracts were filtered (whatman filter paper) and stored in refrigerator (Figures 4 &5). For preparation of green synthesized AgNPs, biological method was used. 100ml silver nitrate solution (0.017g AgNO₃/100ml distilled water) was prepared. After that, 10ml of already prepared aqueous plant extract was mixed with 90ml of AgNO₃ solution, covered the opening of flask and placed in sunlight for 10 minutes to observe colour change. Colour change from extract colour to dark reddish brown was the indication of AgNPs formation. All nanoparticles were covered with aluminum foil and stored in refrigerator. For the confirmation of nanoparticle formation, UV-visible spectrophotometry was performed. Peak should be in the range of 350-600nm (Figure 6).

For well diffusion method, nutrient agar plates were prepared by pouring autoclaved nutrient agar. 5 wells were formed on a single plate and bacteria were spread on the agar plate. 50µl of antibiotic solutions, aqueous plant extracts and AgNPs were poured in the well. Distilled water was used as a negative control. After incubation at 37 °C for 24 hours, zones of inhibition were observed and measured. Clear zones indicated susceptibility of bacteria while no zones indicated resistance of bacteria (Figures 4').

3. Results

In the present study, different bacteria were isolated from samples of conjunctivitis infection, cultured in microbiology laboratory of Government College University, Lahore and observed their resistance against different antibiotics, aqueous plant extracts and green synthesized silver nanoparticles. These bacterial strains were identified by biochemical characterization as *Bacillus thuringiensis* (strain-H), *Bacillus paramycoides* (strain-I), *Pseudomonas aeruginosa* (strain-J), *Bacillus coahuilensis* (strain-K), and *Bacillus cereus* (strain-L).

Bacillus is a rod-shaped, gram-positive, motile bacterium that is widely found in nature and forms spores (Astley *et al.*, 2023). *B. thuringiensis* and *Bacillus cereus* are food pathogens and can cause vomiting and diarrhea (Okamoto & Okutani, 2024), systemic infections like meningitis and bacteremia, as well as localized infections such infections of the ear canal and eyes (Baindara & Aslam, 2023). *P. aeruginosa* is a gram negative, lactose-fermenting and non-fermenting bacteria (Ayehubizu *et al.*, 2021; Manuel *et al.*, 2023). It is a common rod-shaped (Pachori *et al.*, 2019) opportunistic pathogen possessing a broad range of adaptable virulence factors. The 16s rRNA has also been used to identify *Pseudomonas* sp. as a component of the conjunctival microbiome. Acute conjunctivitis (Pandey *et al.*, 2020), dacryocystitis (Luo *et al.*, 2021), post-surgical and post-traumatic endophthalmitis are all brought on by *P. aeruginosa* (Astley *et al.*, 2023).

Table 1 indicates antibacterial activity of 4 different kinds of antibiotics i.e. azithromycin, levofloxacin, ciprofloxacin and metronidazole with 50µl concentration against isolated bacterial strains. All bacteria show resistance against metronidazole. All bacterial strains are sensitive to levofloxacin, azithromycin and ciprofloxacin. All the strains show least sensitivity against levofloxacin except *P. aeruginosa* with

39mm zone of inhibition (ZOI). *B. thuringiensis* is highly susceptible to azithromycin with 33mm ZOI followed by *P. aeruginosa* with 29mm ZOI then *B. coahuilensis* and *B. paramycoides* with 28mm ZOI and *B. cereus* is least sensitive with 25mm ZOI. In case of ciprofloxacin, *P. aeruginosa* is highly sensitive with 43mm ZOI while *B. coahuilensis* is least sensitive with 28mm ZOI (Figures 7 & 10).

Table 1. Antibacterial activity of antibiotics

Antibiotics	Zone of inhibition (mm)±S.E				
	<i>Bacillus thuringiensis</i>	<i>Bacillus paramycoides</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus coahuilensis</i>	<i>Bacillus cereus</i>
Metronidazole	R	R	R	R	R
Levofloxacin	32±0.72	34±0.57	39.66±0.5	28.33±0.33	29±0.57
Azithromycin	33±0.28	28±0.88	29±0.57	28.66±0.88	25.66±0.88
Ciprofloxacin	30±1.01	33±0.33	43.33±0.88	28±0.57	29.66±0.88

R=Resistant

Table 2 shows antibacterial activity of aqueous plant extracts against isolated bacterial strains. All bacterial strains are resistant to aqueous extracts of *A. indica*, *F. religiosa* and *A. barbadensis*. *P. aeruginosa*, *B. coahuilensis* and *B. cereus* show resistance against aqueous extracts of *A. cepa* and *S. aromaticum*. Out of *S. cumini*, *E. camaldulensis* and *C. limon* aqueous extracts, *C. limon* shows maximum antibacterial activity against *B. thuringiensis* (21mm), *B. coahuilensis* (14.33mm) and *B. paramycoides* (14mm). While *S. cumini* aqueous extract is most effective against *P. aeruginosa* (13mm), *B. coahuilensis* (15mm) and *B. cereus* (13mm).

Table 2. Antibacterial activity of aqueous plant extracts

Aqueous plant extracts	Zones of inhibition (mm)±S.E				
	<i>Bacillus thuringiensis</i>	<i>Bacillus paramycoides</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus coahuilensis</i>	<i>Bacillus cereus</i>
<i>Azadirachta indica</i>	R	R	R	R	R
<i>Syzygium cumini</i>	14.66±0.88	12.16±0.60	12.83±0.6	15.5±0.76	13.16±0.60
<i>Eucalyptus camaldulensis</i>	13.66±1.20	11±1.15	9.83±0.44	10.66±0.88	11.16±0.44
<i>Ficus religiosa</i>	R	R	R	R	R
<i>Aloe barbadensis</i>	R	R	R	R	R
<i>Allium cepa</i>	9.33±0.33	11.66±0.66	R	R	R
<i>Syzygium aromaticum</i>	10±0.57	R	R	R	R
<i>Citrus limon</i>	21.33±0.88	14±0.57	12.66±0.33	14.33±0.33	12.66±0.88

R=Resistant

Table 3 indicates antibacterial activity of green synthesized silver nanoparticles (AgNPs) against all isolated bacterial strains. According to results, *A. indica*, *F. religiosa*, *S. aromaticum* and *C. limon* green synthesized AgNPs are effective against all isolated bacterial strains. *C. limon* AgNPs show maximum antibacterial activity against all bacterial strains. *B. thuringiensis* (16mm) is highly sensitive to *C. limon* AgNPs followed by *B. coahuilensis* (14mm), *B. paramycoides* (13mm), *P. aeruginosa* (12mm), and *B. cereus* (11mm). While *E. camaldulensis* AgNPs gave significant positive results against *B. cereus* (7mm). Out of all bacterial strains, *P. aeruginosa* is resistant to *A. barbadensis* and *A. cepa* AgNPs. All bacterial strains are resistant to only *S. cumini* AgNPs (Figures 8 & 11).

Table 3. Antibacterial activity of green synthesized silver nanoparticles (Figures 9 & 12)

AgNPs	Zones of inhibition (mm)±S.E				
	<i>Bacillus thuringiensis</i>	<i>Bacillus paramycoides</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus coahuilensis</i>	<i>Bacillus cereus</i>
<i>Azadirachta indica</i>	13.33±1.20	11.66±0.27	10±1.01	10.66±1.20	8.83±0.72
<i>Syzygium cumini</i>	R	R	R	R	R
<i>Eucalyptus camaldulensis</i>	R	R	R	R	7.5±0.28
<i>Ficus religiosa</i>	12.16±1.16	12±0.47	13±1.15	12.33±1.45	7.33±0.66
<i>Aloe barbadensis</i>	11.66±0.66	9.66±0.72	R	8.83±0.72	9.83±0.60
<i>Allium cepa</i>	13.33±0.88	13.16±0.59	R	9.33±0.33	8.33±0.88
<i>Syzygium aromaticum</i>	10.83±0.92	12.5±0.62	12±1.01	11.66±1.20	11.66±0.88
<i>Citrus limon</i>	16.16±1.09	13±0.47	12±0.57	14±1.15	10.83±1.01

R=Resistant

4. Discussion

One major supply of antimicrobials is natural items. Identification of substances that function as suitable antibacterial agents has been the subject of extensive research (Ramalivhana *et al.*, 2014). Plant products containing phyto-chemicals and antibacterial agents are the most valuable resources for producing antibiotics that are both less harmful and more effective (Meher *et al.*, 2017). The previously mentioned plant extracts demonstrated noteworthy or moderate activities against clinical isolates of *B. thuringiensis*, *B. paramycoides*, *P. aeruginosa*, *B. coahuilensis* and *B. cereus*. These plant extracts have excellent antibacterial activity. Moreover, conjunctivitis, which is typically brought on by resistant bacteria, can be treated and/or cured with these plant species.

Nsofor *et al.* (2023) evaluated the aqueous and methanolic gel extracts of Aloe vera against gram-negative bacteria (*P. aeruginosa*) and gram-positive bacteria (*B. cereus*) by well diffusion method. According to Nsofor *et al.* (2023), aqueous extract of Aloe vera showed the least antibacterial activity against *B. cereus* and *P. aeruginosa*. While ethanolic extracts of Aloe vera displayed a high antibacterial activity against *B. cereus* and *P. aeruginosa*. However, in our present study, aqueous gel extract of Aloe vera showed no antibacterial activity against *P. aeruginosa* and *B. cereus*. Similar to this, there are several reports on the antibacterial effects of various Aloe vera kinds (Akinniyi *et al.*, 2013; Etusim *et al.*, 2013).

Aqueous extracts of *S. cumini*, *E. camaldulensis* and *C. limon* exhibited inhibition against all isolated bacterial strains. Extract of *S. cumini* showed maximum inhibition percentage against *B. coahuilensis* followed by *B. thuringiensis*. Extract of *E. camaldulensis* demonstrated maximum inhibition percentage against *B. thuringiensis* followed by *B. paramycoides* and *B. cereus*. And extract of *C. limon* displayed maximum inhibition percentage against *B. thuringiensis* followed by *B. coahuilensis* and *B. paramycoides*.

Aqueous and methanolic leaf extracts of *Syzygium cumini* (Jamun) exhibited antibacterial activity against gram-positive bacteria (*Bacillus subtilis* and *S. aureus*) and gram-negative bacteria, such as *P. aeruginosa* and *Salmonella* spp. Methanol extracts outperformed aqueous extracts in terms of potency (Gowri & Vasantha, 2010). In present study, aqueous leaf extract of *S. cumini* inhibited both gram-negative and gram-positive bacteria. Antibacterial activity was maximum against *B. coahuilensis* and minimum against *P. aeruginosa* and *B. paramycoides*. According to Gowri & Vasantha (2010), flavonoids, alkaloids, steroids, glycosides, tannins, phenols, and saponins were abundant in the leaves of *S. cumini*.

Tshabalala *et al.* (2021) and Patel *et al.* (2022) found that clove exhibit high antibacterial activity. Furthermore, Elisha *et al.* (2022) prepared cold water, hot water and ethanolic extracts of *S. aromaticum* to observe the antibacterial activity against different bacterial isolates including *S. typhi* by disc and well diffusion method. The plant extracts showed antibacterial activity against all tested isolates, including *S. typhi*. According to my present research study, aqueous extract of *S. aromaticum* (clove) showed significant antibacterial results against *B. thuringiensis* only while other bacterial strains were resistant to it. And green synthesized AgNPs of *S. aromaticum* aqueous extract showed antibacterial activity against all bacterial strains. They were most effective against *P. aeruginosa* and *B. paramycoides* while least effective against *B. thuringiensis*.

Ethanolic and aqueous leaf extracts of *F. religiosa* were shown to have antibacterial action against pathogenic bacterial strains such as *B. subtilis*, *E. coli*, *S. aureus*, and *P. aeruginosa* and *S. typhi*. The leaf extracts significantly and varyingly inhibited most of the examined bacteria (Walia *et al.*, 2022). While in this present research, aqueous leaf extract of *F. religiosa* didn't show any significant inhibitory activity against any of bacterial strains. Rather than aqueous extracts, green synthesized AgNPs of *F. religiosa* aqueous leaf extract showed antibacterial activity against all bacterial strains. Maximum inhibitory percentage was against *P. aeruginosa* and minimum inhibitory percentage was against *B. cereus*.

Abd & Hasan (2023) proved that silver nanoparticles of Aloe Vera leaf extract were effective against both gram-positive (*S. epidermidis*) and gram-negative (*P. aeruginosa*) bacteria. While, according to our study, green synthesized AgNPs of Aloe Vera showed significant antibacterial activity against all gram-positive bacteria while there was no activity against gram-negative bacteria (*P. aeruginosa*). In related study, Anavil *et al.* (2023) exhibited that silver nanoparticles of *A. barbadensis* (Aloe vera) showed maximum antibacterial activity against *E. coli* than *S. aureus*. Rossos *et al.* (2021) demonstrated that silver nanoparticles of eucalyptus leaves extract were highly active against *P. aeruginosa*, *S. epidermidis* but they performed poorly against *S. aureus*. In this present work, these AgNPs were slightly active against

B. cereus while no activity was observed against other bacterial strains including *P. aeruginosa*, *B. thuringiensis*, *B. paramycoides* and *B. coahuilensis*.

Similarly, antibacterial properties of lemon (*C. limon*) have been determined against variety of bacteria (Ali *et al.*, 2017; Mustafa *et al.*, 2021; Solanki *et al.*, 2022). This present study showed that aqueous extract and green synthesized AgNPs of *C. limon* were significantly active against all identified bacterial strains. Other than *C. limon*, *A. indica*, *F. religiosa* and *S. aromaticum* showed antibacterial activity against all identified bacterial strains. Aqueous extracts of some plants such as *A. indica*, *F. religiosa* and *A. barbadensis* and AgNPs of plant *S. cumini* didn't exhibit any antibacterial activity against isolated identified bacterial strains. The medicinal plant species included in this study that showed efficacy against the bacterial isolates may be studied in more detail to find naturally occurring bioactive compounds.

Conclusion

Significant antibacterial activity against isolated bacterial strains has been demonstrated by a few aqueous plant extracts (*C. limon*, *S. cumini*, *E. camaldulensis*, *A. cepa*, and *S. aromaticum*) and green synthesized silver nanoparticles (*A. indica*, *F. religiosa*, *C. limon*, *S. aromaticum*, *A. cepa*, and *A. barbadensis*). Due to the adverse effects that chemically manufactured antibiotics have on the human body at high dosages. Antibiotic medication may be replaced by biological antibacterial tools such as plant extracts and green synthesized silver nanoparticle formulations. In order to prepare these antibacterial agents for their therapeutic use against ocular infections, additional study on them can be undertaken.

Declarations

Ethics approval and consent to participate

The current study is approved by board of studies and the sampling was done by ophthalmologist.

Consent for publication

All authors gave their consent for publication.

Availability of data and material

Available

Competing interests

No competing interests among authors

Funding

None

Authors' contributions

S.A and B.M carried out experiment work. N.A wrote the first and final draft of manuscript and supervised the experimental work. M.C, S.M and M.D did the analysis. MAR provided resources and helped in rough draft. all authors approved final draft.

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References

1. Abd, E. M., & Hasan, A. Y. (2023). Biosynthesis and characterization of silver nanoparticles by *Aloe vera* leaves extract and determination of its antibacterial activity. In *American Institute of Physics Conference Proceedings*, 2475(1).
2. Akinniyi, A. P., Oluwaseun, E., Mopelola, D. A. A., Mosunmola, O. J., Remi, R. A. R. & Afolabi, O. (2013). Susceptibility of multi-antibiotic resistant bacteria strains in Abeokuta, Nigeria to Aloe vera juice. *American Journal of Research Communication*, 1(9), 56-64.
3. Albandary, N. A. (2023). Phenolic compounds content, antioxidant, antibacterial and antifungal activities of red onions skin. *Iraqi Journal of Agricultural Sciences*, 54(4), 1050- 1057
4. Alfonso, S. A., Fawley, J. D., & Alexa, Lu. X. (2015). Conjunctivitis. *Prim Care*, 42(3), 325-345.
5. Ali, J., Das, B., & Saikia, T. (2017). Antimicrobial activity of lemon peel (*Citrus limon*) extract. *International Journal of Current pharmaceutical Research*, 9(4), 79–82.
6. Al-Khafaji, A. M. H. H. (2019). Stimulation growth, yield, and accumulation of antioxidant compounds of onion hybrids by colored shades of poly ethylene covers. *Iraqi Journal of Agricultural Sciences*, 50(6), 1580-1587.
7. Anavil, P., Onsri, P., Panprivech, S., Chuenchom, L., & Watcharin, W. (2023). Green and facile synthesis of silver nanoparticles using plant extract of *Aloe Barbadensis Miller* and their antibacterial activity assessment. In *American Institute of Physics Conference Proceedings*, 2720(1).
8. Arrfa, R. C. (1997). *Grayson's diseases of the cornea*, 4th ed. St Louis: Mosby-Year Book, 138.
9. Astley, R. A., Mursalin, M. H., Coburn, P. S., Livingston, E. T., Nightengale, J. W., Bagaruka, E., Hunt, J. J. & Callegan, M. C. (2023). Ocular Bacterial Infections: A Ten-Year Survey and Review of Causative Organisms Based on the Oklahoma Experience. *Microorganisms*, 11(7), 1802.
<https://doi.org/10.3390/microorganisms11071802>
10. Astley, R. A., Mursalin, M. H., Coburn, P. S., Livingston, E. T., Nightengale, J. W., Bagaruka, E., Hunt, J. J., & Callegan, M. C. (2023). Ocular Bacterial Infections: A Ten-Year Survey and Review of Causative

Organisms Based on the Oklahoma Experience. *Microorganisms*, 11(7), 1802.

<https://doi.org/10.3390/microorganisms11071802>

11. Ayehubizu, Z., Mulu, W. & Biadglegne, F., (2021). Common bacterial causes of external ocular infections, associated risk factors and antibiotic resistance among patients at ophthalmology unit of Felege Hiwot Referral Hospital, Northwest Ethiopia: a cross- sectional study. *Journal of Ophthalmology Inflammation and Infection*, 11(7). <https://doi.org/10.1186/s12348-021-00238-2>
12. Azari, A. A., & Arabi, A. (2020). Conjunctivitis: A Systematic Review. *Journal of Ophthalmic and Vision Research*, 15(3), 372-395. Doi: 10.18502/jovr.v15i3.7456.
13. Baindara, P. & Aslam, B., (2023). Editorial: *Bacillus* spp. - Transmission, pathogenesis, host-pathogen interaction, prevention and treatment. *Frontier Microbiology*, 14, 1307723.
14. Barrasa, G. J., Luzuriaga, L. J. M., & Monge, M. (2010). Silver nanoparticles: Synthesis through chemical methods in solution and biomedical applications. *Open Chemistry*, 9, 7–19.
15. Bhandari, G., Chaudhary, P., Gupta, S., Gangola, S., Gupta, A., Rustagi, S., Shende, S. S., Rajput, V. D., Minkina, T., Malik, S., & Slama, P. (2023). Opportunities for sustainable agricultural practices & environmental remediation. *Agriculture*, 13(3), 668; <https://doi.org/10.3390/agriculture13030668>
16. Bhargava, K., & Singh, D. K. (2023). Aloe Vera Medical Plant. *European Chemical Bulletin*, 12(5), 1755-1764.
17. CDC, (2022). Antimicrobial (AR) Threats Report. Available online: <https://www.cdc.gov/drugresistance/biggest-threats.html>.
18. Chukwuma, I. F., Uchendu, N. O., Asomadu, R. O., Ezeorba, W. F. Z., & Ezeorba, T. P. C. (2023). African and Holy Basil - a review of ethnobotany, phytochemistry, and toxicity of their essential oil: Current trends and prospects for antimicrobial/anti-parasitic pharmacology. *Arabian Journal of Chemistry*, 16 (7), 104-870.
19. Elisha, E. Ajobiewe, H. F., Ibrahim, A. E., Alau, K. K., Umeji, L. C., Salami, A. O., Udefuna, P. A., Yashim, A. N., & Ajobiewe, J. O. (2022). Antimicrobial Activity of Clove Plant Flower Bud Extract (*Syzygium aromaticum*) on *Salmonella typhi*. *Scholars Journal of Applied Medical Sciences*, 10(5), 698-708.
20. Etusim, P. E., Okafor, E. E., Nwachukwu, N. C., Melariri, P. E., & Ogbonnaya, C. I. (2013). A study on antibacterial activities of *Aloe vera* leaves, stems and roots on some selected organisms. *Asian Journal of Research in Chemistry*, 6(6), 570- 572.
21. Gandhimathi, R. A., Saravanakumar, M., & Aanandhi, V, (2022). Current Herbal drugs trends: A Review. *Current Trends in Biological Sciences*, 11(1), 383-391.
22. Ghasemi, K., Ghasemi, Y., & Ebrahimzadeh, M. A. (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pakistan Journal of Pharmaceutical Sciences*, 22, 277-281.
23. Gowri, S. S., & Vasantha, K. (2010). Phytochemical Screening and Antibacterial Activity of *Syzygium cumini* (L.) (Myrtaceae) Leaves Extracts. *International Journal of Pharm tech Research*, 2(2) 1569-1573

24. Hashmi, M. F., Gurnani, B., & Benson, S. (2023). Conjunctivitis. In: StatPearls [Internet]. Treasure Island (FL): *StatPearls Publishing*. <https://www.ncbi.nlm.nih.gov/books/NBK541034/>
25. Hetta, H. F., Ramadan, Y. N., Al-Harbi, A. I., Ahmed, E. A., Battah, B., Abd Ellah, N. H., Zanetti, S., & Donadu, M. G. (2023). Nanotechnology as a promising approach to combat multidrug resistant bacteria: A comprehensive review and future perspectives. *Biomedicines*, 11(2), 413; <https://doi.org/10.3390/biomedicines11020413>
26. Hindi, N. K. K., & Chabuck, Z. A. G. (2013). Antibacterial activity of different aqueous lemon extracts. *Journal of Applied pharmaceutical sciences*, 3(6), 074–078.
27. Hovding, G. (2008). Acute bacterial conjunctivitis. *Acta Ophthalmology*, 86 (1), 5–17.
28. Husain, S., Verma, S. K., Yasin, D., Hemlata, Rizvi, M. M. A., & Fatma, T. (2021). Facile green bio-fabricated silver nanoparticles from *Microchaete* infer dose- dependent antioxidant and anti-proliferative activity to mediate cellular apoptosis. *Bioorganic Chemistry*, 107, 104-535.
29. Kumari, N., Kumar, M., Chaudhary, N., Zhang, B., Radha, Deepak, C., Joshi, S., Singh, D., Dey, A., Rajalingam, S., Natarajan, K., Muthukumar, M., Mohankumar, P., Sheri, V., Dhumal, S. & Lorenzo, J. M. (2023). Exploring the Chemical and Biological Potential of Jamun (*Syzygium cumini* (L.) Skeels) Leaves: A Comprehensive Review. *Chemistry & Biodiversity*, 20(9)
30. Kundu, D., Das, M., Mahle, R., Biswas, P., Karmakar, S., & Banerjee, R. (2020). Citrus fruits.Valorization of fruit processing by-products, *Elsevier Inc*, 145–166.
31. Manuel, D. E. M., López, B. J. M., Bobadilla, S. A. D., León, V. D. C., Velázquez, N. N. G., Eutimio, M. M. A., Palacios, P. R., Mendoza, C. C. C. & Hernández, B. D. M. R., (2023). Biochemical Characterization of Bacterial Agents Causing External Ocular Infections Isolates of Patients in a Third Level Hospital. *Pathogens*, 12(11), 1294. <https://doi.org/10.3390/pathogens12111294>
32. Meher, S., Ali, I., Sami, A., Ismail, M., Naheed, N., Khan, S. A. & Ahmad, V. U. (2017). Screening of some medicinal plants for antibacterial activity against conjunctivitis. *JAPS: Journal of Animal & Plant Sciences*, 27(6), 2069-2074.
33. Mie, R., Samsudin, M. W., Din, L. B., Ahmad, A., Ibrahim, N., & Adnan, S. N. A. (2013). Synthesis of silver nanoparticles with antibacterial activity using the lichen *Parmotrema praesorediosum*. *International Journal of Nanomedicine*, 9, 121–127
34. Nsofor, O. U., Ogochukwu, A. P., Gabriel, A. O., Emmanuel, E., & Linus, C. M. (2023). Evaluation of antibacterial activity of aloe vera extract on some bacterial pathogens, *International Journal of Phytology Research*, 3(1), 26–29.
35. Okamoto, A. & Okutani, A., (2024). The *Bacillus cereus* group. *Molecular Medical Microbiology* (3rd ed.), 957-986.
36. Ortuno, A. A., Baidez, P., Gomez, M. C., Arcas, I., Porras, A. G., & Del, R. J. A. (2006). *Citrus paradise* and *Citrus sinensis* flavonoids: Their influence in the defence mechanism against *Penicillium digitatum*. *Food Chemistry*, 98(2), 351–358.
37. Pachori, P., Gothwal, R. & Gandhi, P. (2019). Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit; a critical review. *Genes & Diseases*, 6, 109–119

38. Pandey, C., Kumari, S. & Tiwari, R. (2020). To determine the bacteriological profile of acute conjunctivitis in cases in NMCH. *European Journal of Molecular & Clinical Medicine* 7, 3345–3350.
39. Patel, D., Kapasi, M., Gohil, H., & Anshuman, K. (2022). Antimicrobial activity of routine spices against pathogenic microorganisms. *Current Trends in Biological Sciences*, 72-81
40. Patel, D., Kapasi, M., Gohil, H., & Anshuman, K. (2022). Antimicrobial activity of routine spices against pathogenic microorganisms. *Current Trends in Biological Sciences*, 72-81
41. Ramalivhana, J. N., C. L. Obi, A. Samie, B. C. Iweriebor, P. Uaboi-Egbenni, J. E. Idiaghe & M. N. B. Momba (2014). Antibacterial activity of honey and medicinal plant extracts against Gram negative microorganisms. *African Journal of Biotechnology*, 13(4), 616- 625.
42. Ramirez, D. A., Porco, T. C., Lietman, T. M., & Keenan, J. D. (2017). Epidemiology of Conjunctivitis in US Emergency Departments. . *Journal of American Medical Association Ophthalmology*, 135(10), 1119-1121.
43. Sharma, L., Mishra, J., & Sharma, D. (2023). Pharmacological role of *Ficus religiosa* a hidden sacred fig plant: A systematic literature review. *European Chemical Bulletin*, 2(S3), 3054 – 3080
44. Solanki, N., Khandla, H., Gohil, A., & Anshuman, K. (2022). Antimicrobial Activity of Citrus Lemon Juice on Different Microorganisms. *Thanuj International Publishers, Tamil Nadu, India*, 63.
45. Walia, A., Kumar, N., Singh, R., Kumar, H., Kumar, V., Kaushik, R., & Kumar, A. P. (2022). Bioactive Compounds in Ficus Fruits, Their Bioactivities, and Associated Health Benefits: A Review *Journal of Food Quality*, 19.
46. Yusuf, A., Almotairy, A. R. Z., Henidi, H., Alshehri, O. Y., & Aldughaim, M. S. (2023). Nanoparticles as drug delivery systems: A review of the implication of nanoparticles' physicochemical properties on responses in biological systems. *Polymers*, 15(7), 1596; <https://doi.org/10.3390/polym15071596>
47. Zriouli, E. R., Yacoubi, E. H., Imtara, H., Mesfioui, A., Hessni, E. A., Kamaly, A. O., Alshawwa, S. Z., Nasr, F. A., Ouaritini, Z. B. & Rochdi, A. (2023). Chemical composition, antioxidant and antibacterial activities and acute toxicity of *Cedrus atlantica*, *Chenopodium ambrosioides* and *Eucalyptus camaldulensis* essential oils. *Molecules*, 28(7), 2974. <https://doi.org/10.3390/molecules28072974>

Figures

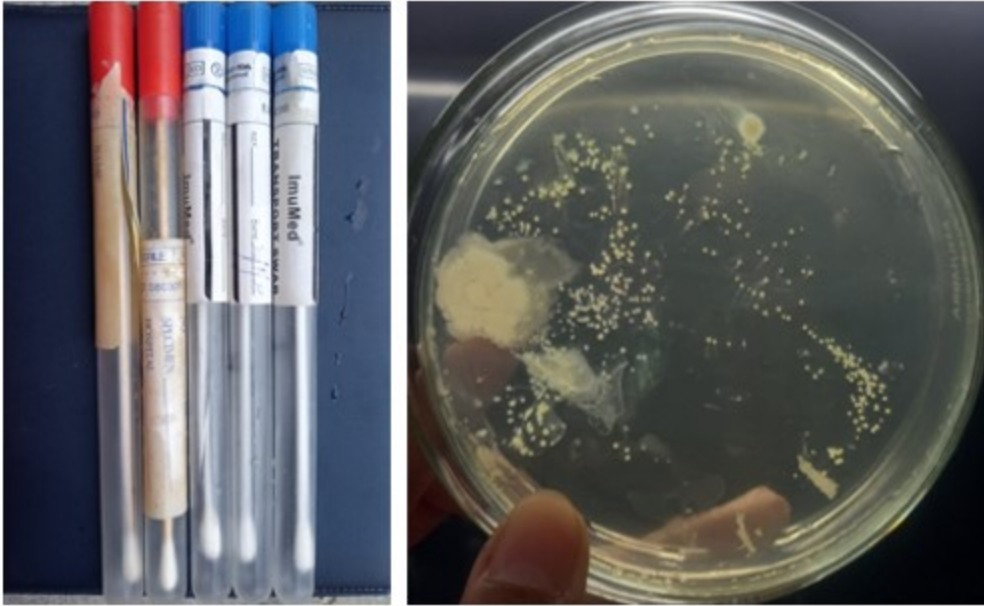


Figure 1

Bacterial sampling sticks (left), Spreading of samples (right)

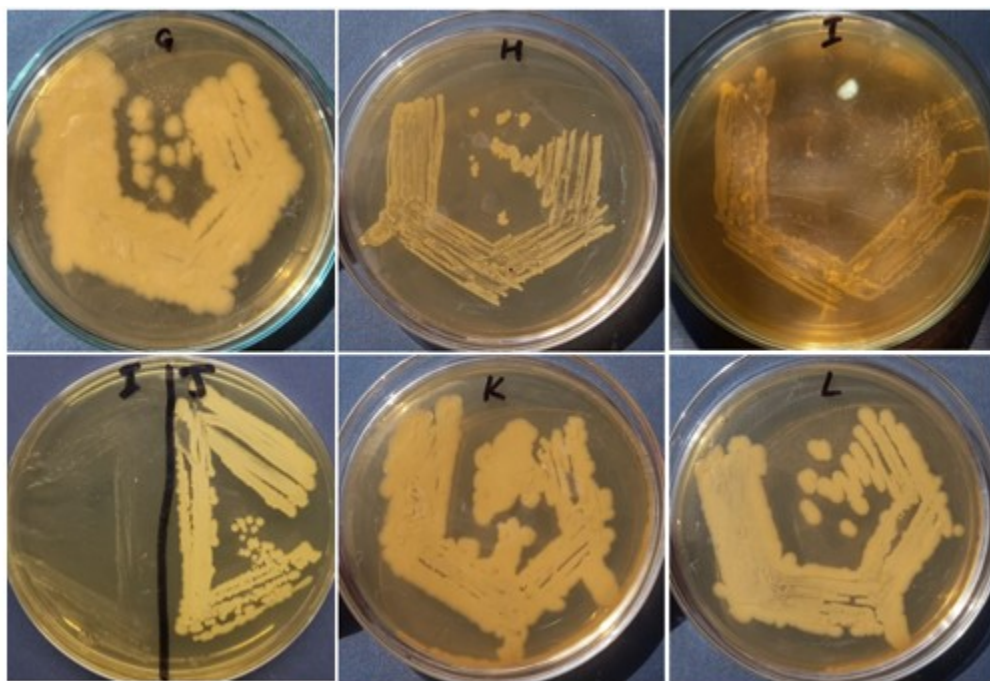


Figure 2

Quadrant method streaking of six isolated bacterial strains G, H, I, J, K, L



Figure 3

Pathogenicity test

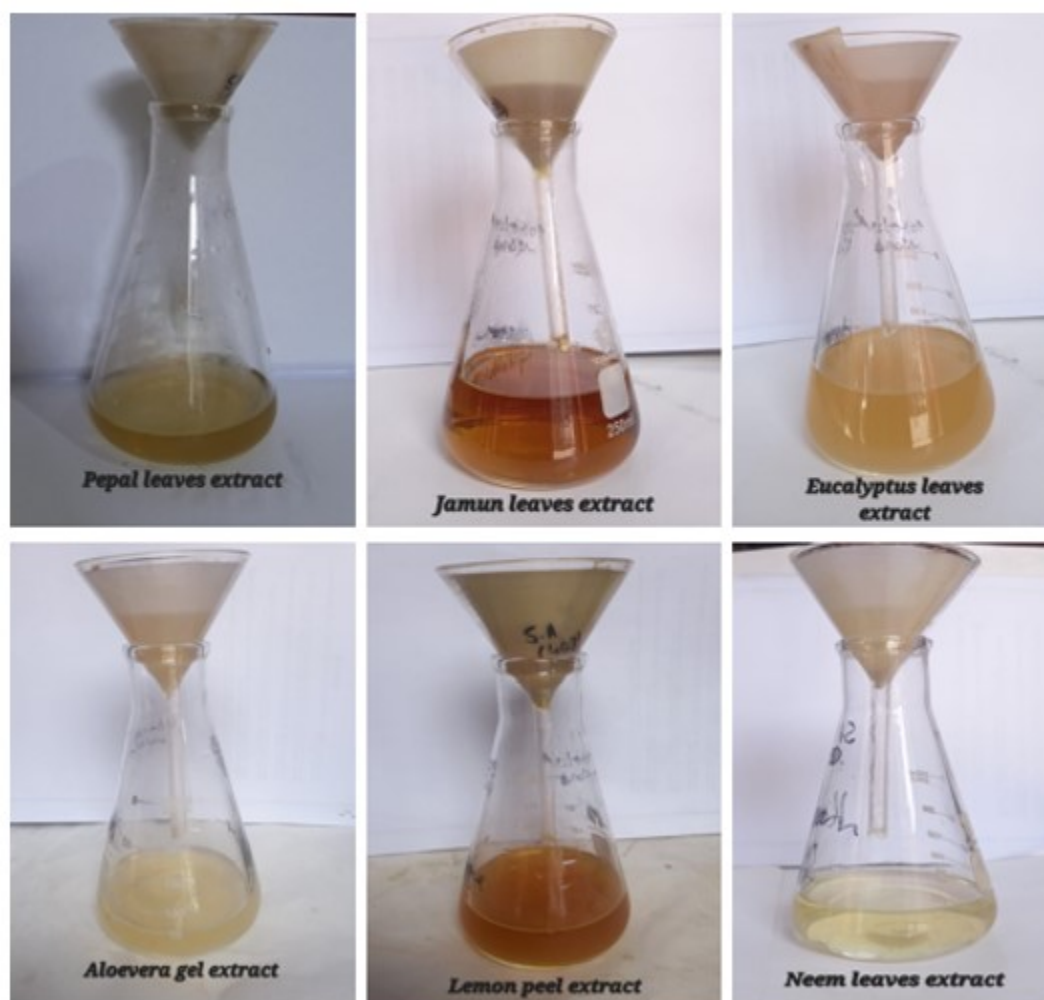


Figure 4

Aqueous plant extracts

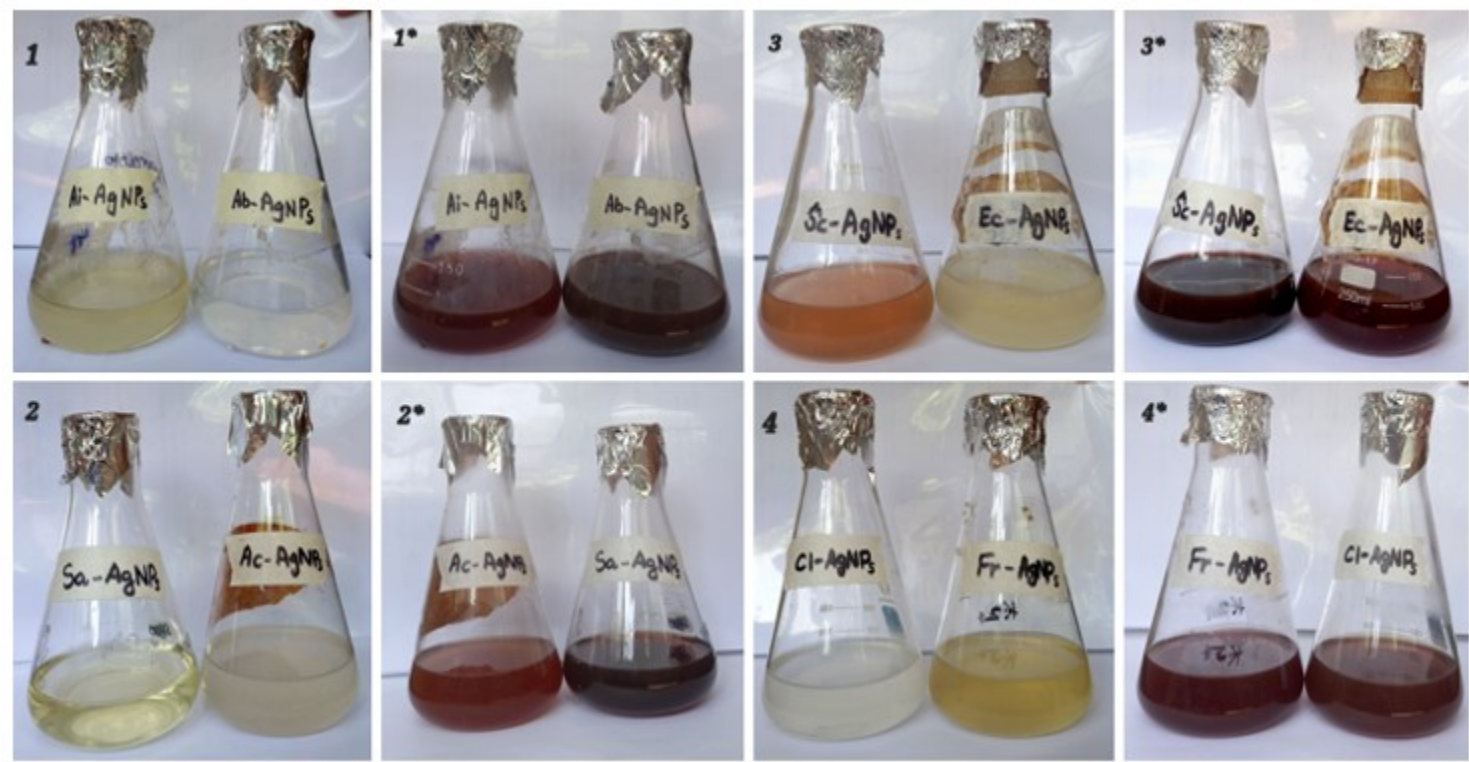


Figure 5

Synthesis of silver nanoparticles, (1, 2, 3, 4 before sunlight exposure and 1*, 2*, 3*, 4* after sunlight exposure)

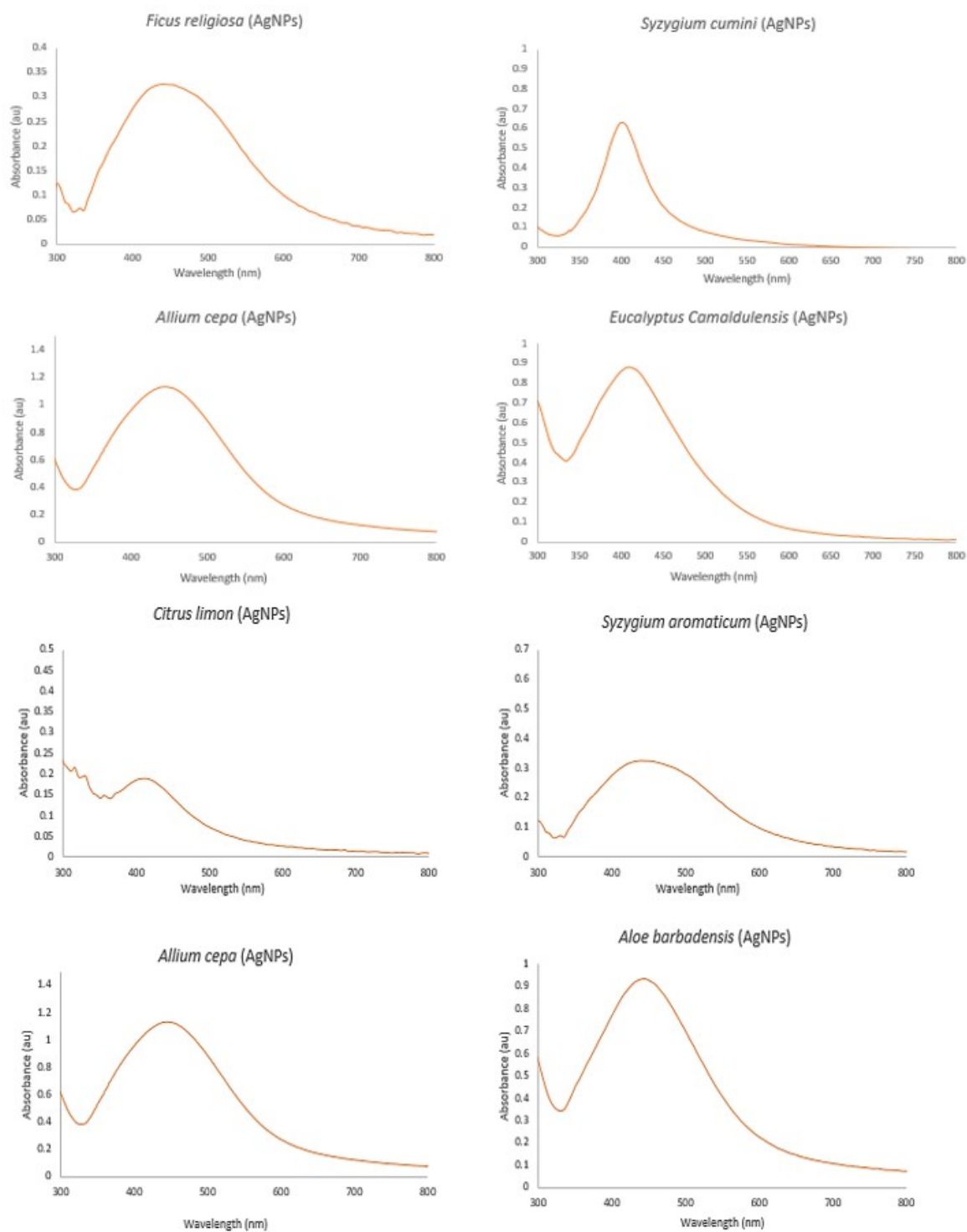


Figure 6

UV visible spectrophotometry of green synthesized AgNPs

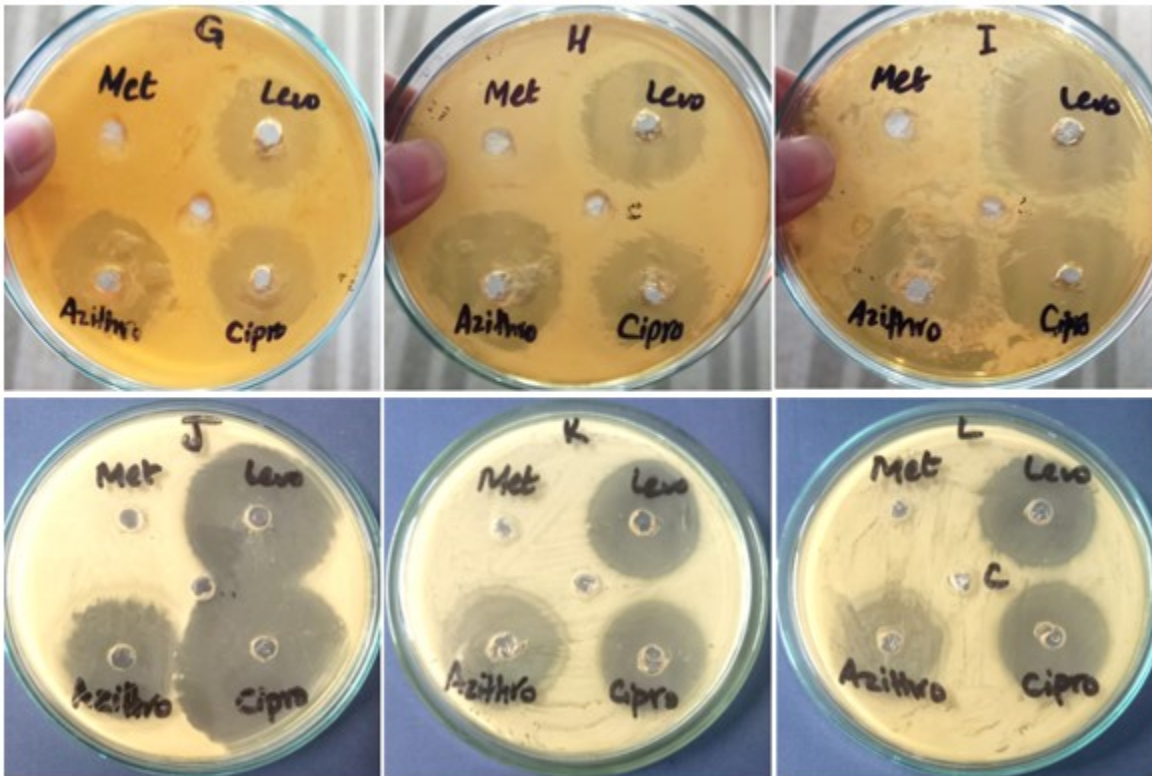


Figure 7

Antibacterial activity of antibiotics by well diffusion method

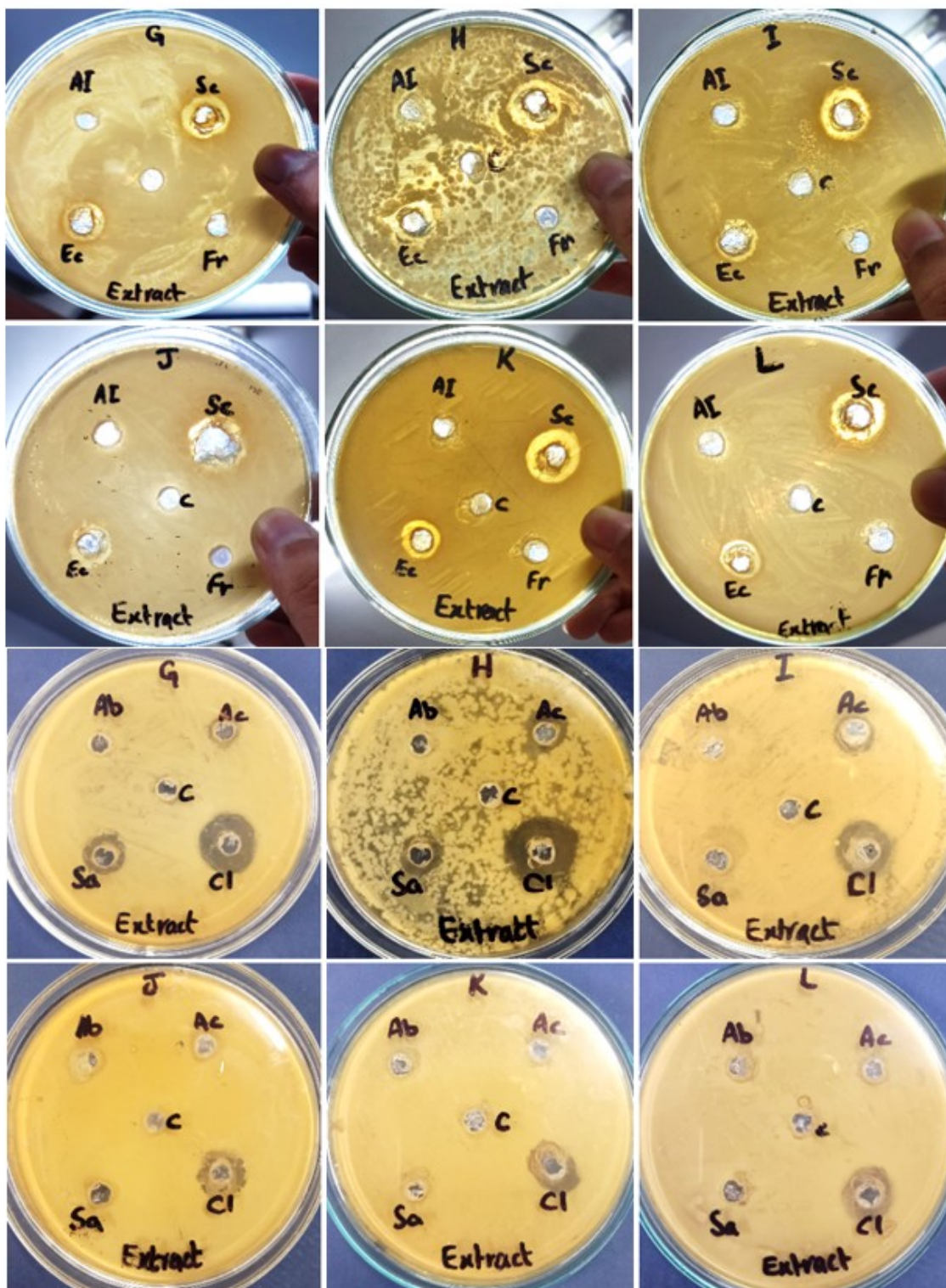


Figure 8

Antibacterial activity of aqueous plant extracts by well diffusion method

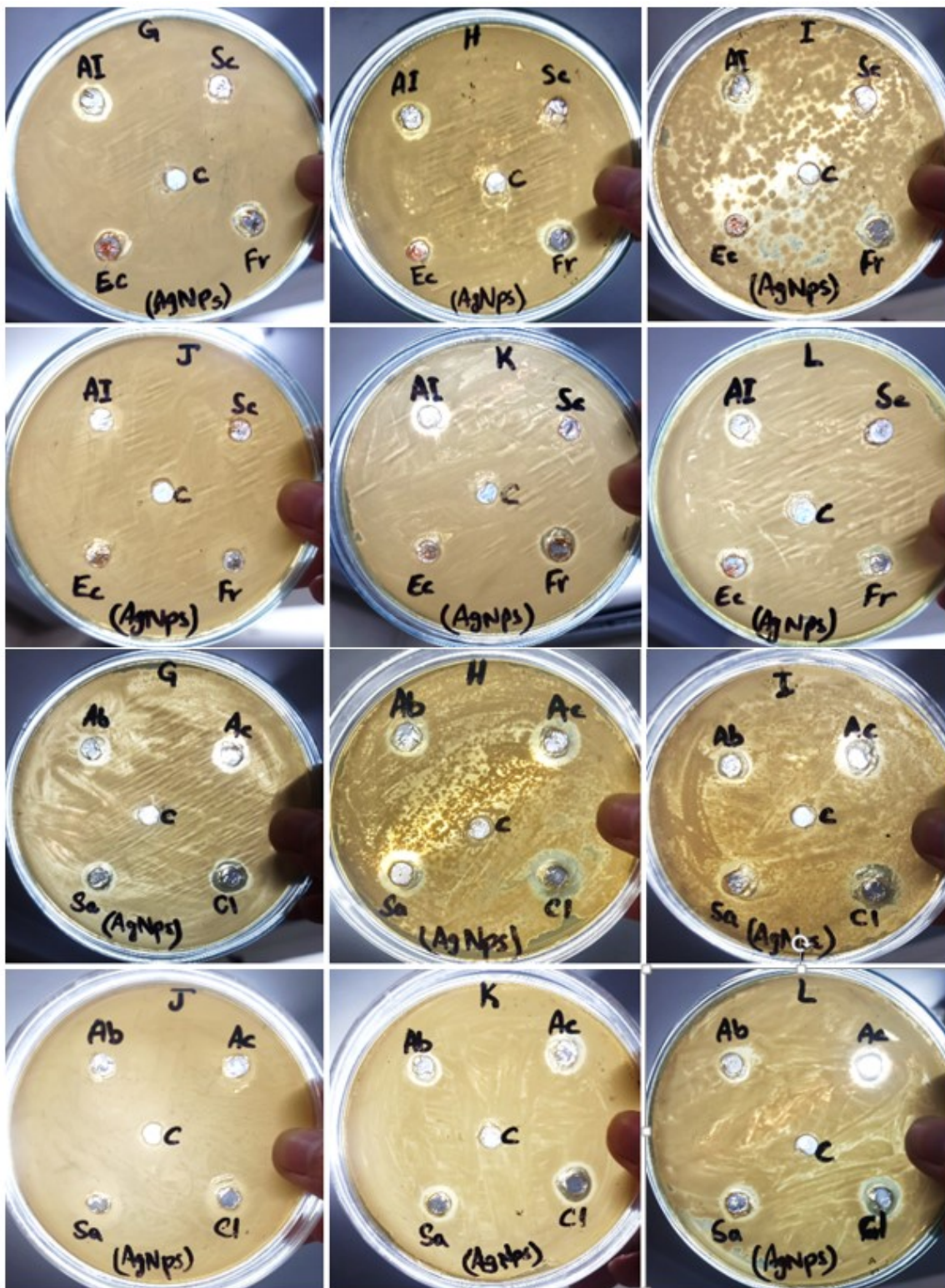


Figure 9

Antibacterial activity of green synthesized AgNPs by well diffusion method

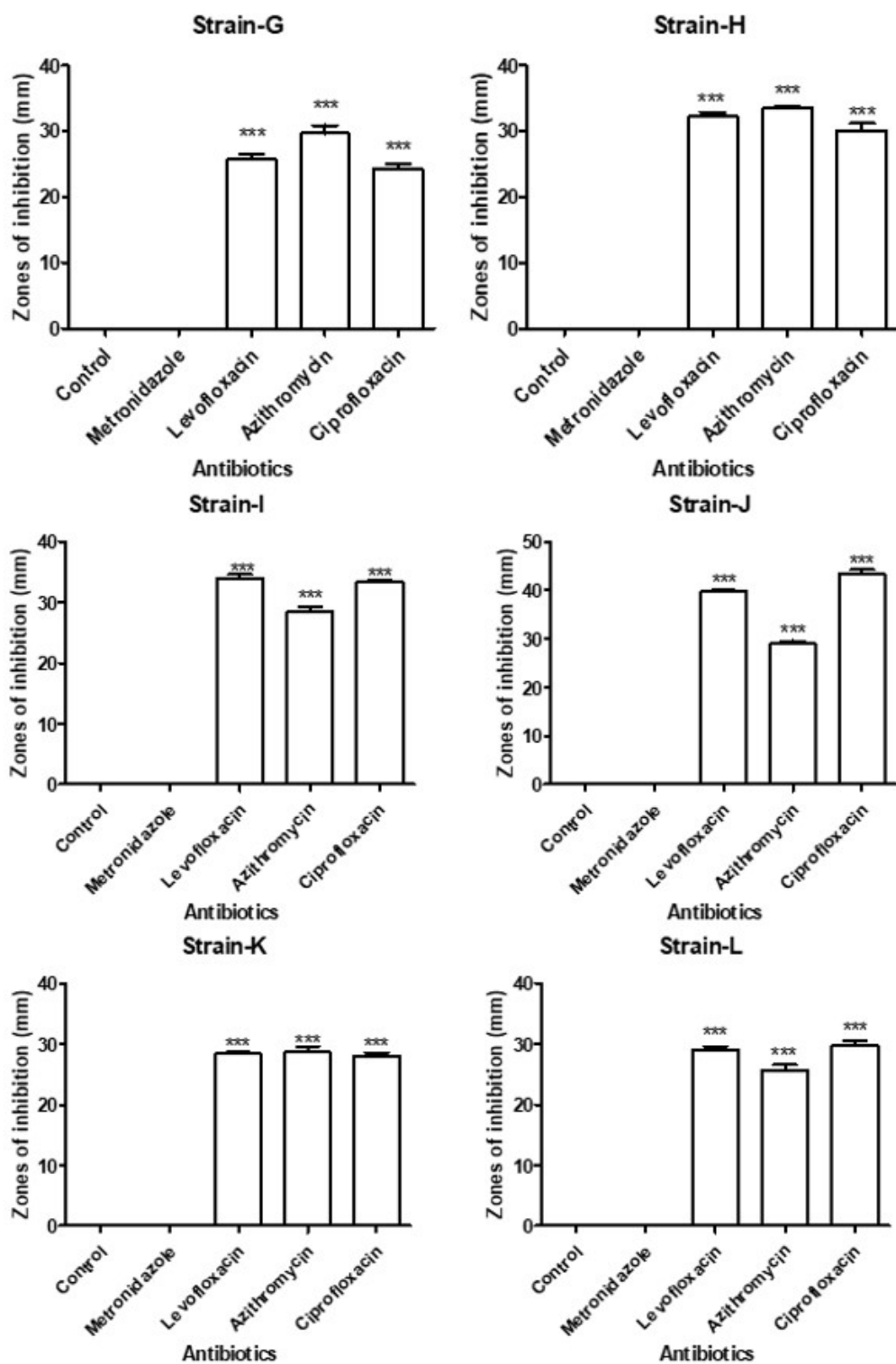


Figure 10

Antibacterial activity of antibiotics against bacterial strains, *** shows significant statistical difference between control and other groups.

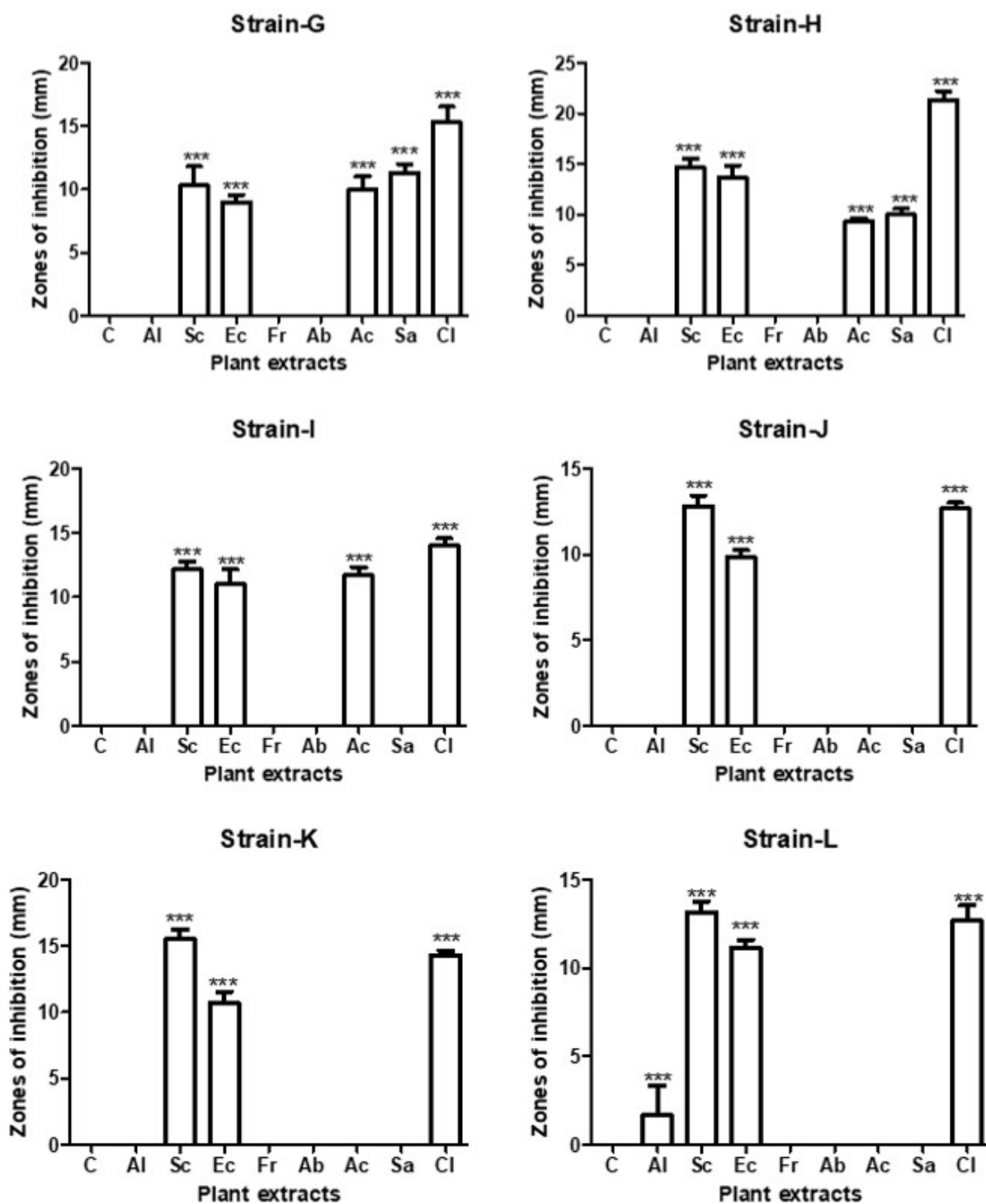


Figure 11

Antibacterial activity of aqueous plant extracts against 6 bacterial strains, *** shows significant statistical difference between control and other groups.

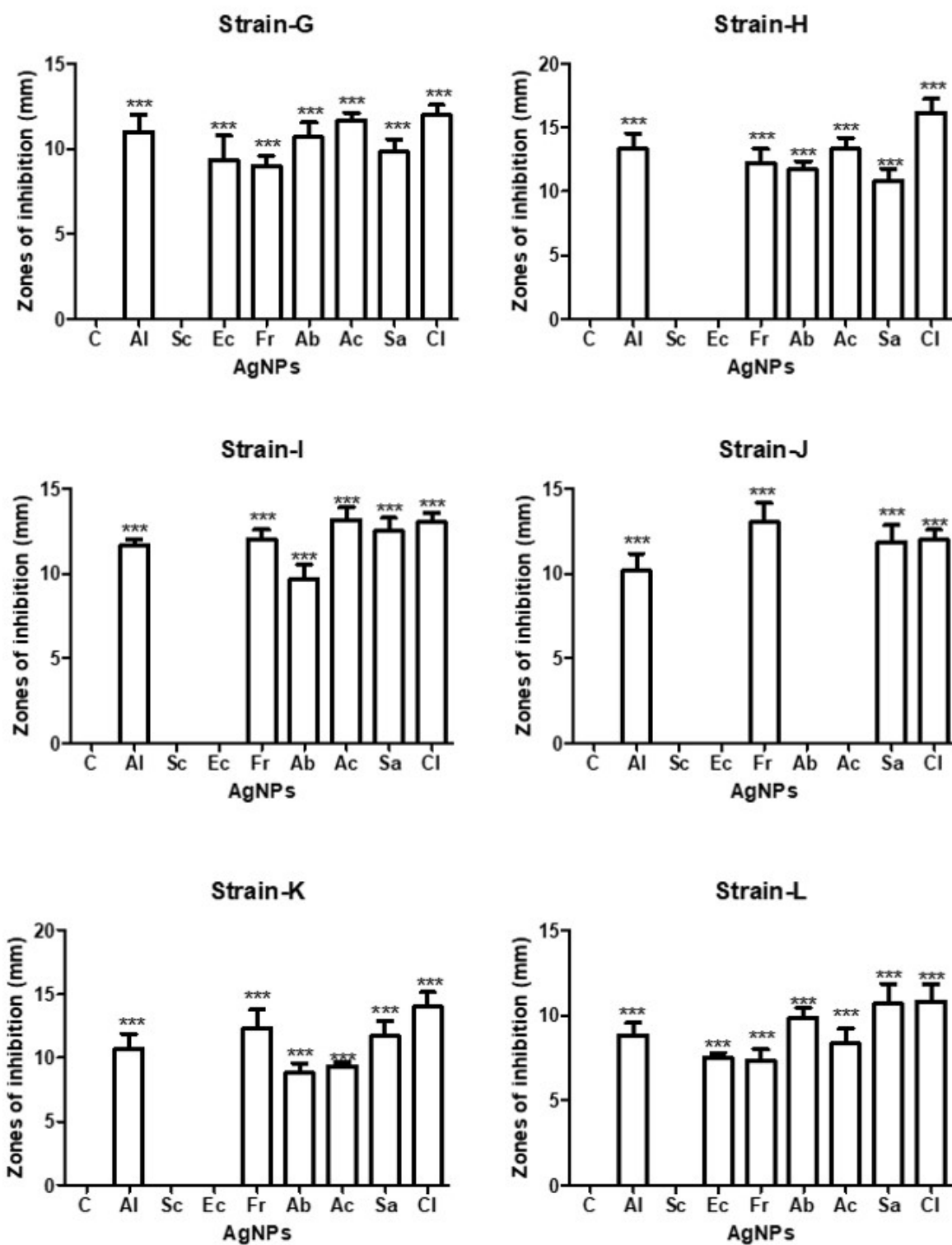


Figure 12

Antibacterial activity of green synthesized AgNPs against 6 isolated bacterial strains, *** shows significant statistical difference between control and other groups