Asian Australas. J. Biosci. Biotechnol. 2016, 1 (3), 564-571

Asian-Australasian Journal of Bioscience and Biotechnology
ISSN 2414-1283 (Print) 2414-6293 (Online)
www.ebupress.com/journal/aajbb

Article

Antibacterial activity of copper nanoprticles and Watercress (*Nasturtium officinale R. Br.*) extracts

Asma G. Oraibi[1*], Rana A. Mohsien[2] and Mallic Habeeb[3]

[1,3]College of Applied Biotechnology, Departement of Agricultural Biotechnology, Al-Nahrain University, Baghdad, Iraq
[2]College of Applied Biotechnology, Departement of Molecular and Medical Biotechnology, Al-Nahrain University, Baghdad, Iraq

*Corresponding author: Asma G. Oraibi, College of Applied Biotechnology, Departement of Agricultural Biotechnology, Al-Nahrain University, Baghdad, Iraq. Mobile: 00 964 7826443072; E-mail: asma_kata@yahoo.com

Received: 19 October 2016/Accepted: 27 November 2015/ Published: 28 December 2016

Abstract: This study was aimed to detect the antibacterial activity of *N. officinale* leaves water and ethanol extract alone or mixed with a specific concentrations of copper nanoparticles (800mg/L). Studied Bacterial samples consist of two gram-positive bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two gram-negative bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*). Results showed that there was a significant increase in the mean inhibition zone at 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and water leaves extract (J) and 1:1 (v/v) of 0.5mg/10ml mg/10ml copper nanoparticles and ethanol leaves extract (F) compared with 0.5mg/10ml leaves water extract (B) and 0.5mg/10ml leaves ethanol extract (C), but there was no significant differences obtained between the types of treatments and the standard antibiotic that used as control (250 mg/10ml Cefotaxime), the highest mean inhibition zone was recorded in *Staphylococcus aureus* bacteria compared with other bacterial species. The interaction revealed that the highest mean inhibition zone obtained in 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and water leaves extract (J) in *Staphylococcus aureus* bacteria and the lowest mean inhibition zone observed in 1.0mg/10ml copper nanoparticles (I) in *Pseudomonas aeruginosa* bacteria while mean inhibition zone significantly increased in *E. coli* bacteria at 1:1 (v/v) of 0.5mg/10ml mg/10ml copper nanoparticles and ethanol leaves extract (F), 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and water leaves extract (J), and 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and ethanol leaves extract (K). No significant differences recorded in the mean inhibition zone of *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria in various treatments.

Keywords: *Nasturtium officinale*; antibacterial activity; copper nanoparticles; Watercress

1. Introduction

Watercress is an aquatic plant species with the botanical name *Nasturtium officinale*. It is a rapidly growing, aquatic or semi-aquatic, perennial plant native to Europe and Asia; and one of the oldest known leaf vegetables consumed by humans. It is currently a member of the family Brassicaceae, botanically related to garden cress, mustard, radish and wasabi-all noteworthy for their piquant flavor. The hollow stems of watercress are floating, and the leaves are innately compound, small, white and green flowers are produced in clusters and are frequently visited by insects, especially hoverflies such as Eristalis flies (Van *et al.*, 2015). *N. officinale* contains a relatively large amount of vitamins B1, B2, C and pro-vitamin A, folic acid, glucosinolates, iodine, iron, protein, and especially calcium and sulphur compounds, which influence its characteristic odor, but also adds to its nutritional benefits (Palaniswamy *et al*., 2003). The active constituents of watercress extract may strengthen or stimulate the immune response by interacting with various parameters of the immune system,

watercress is the richest source of glucosinolates, that can be hydrolyzed to produce phenethyl isothiocyanate (Abu-Zinadah, 2008). N. officinal is used to cure abdominal pain and is eaten as a vegetable and in salads in Iraq-Kurdistan region-Sulaimani City .This herbis used to treat diabetes, bronchitis and dieresis as antiulcerogenic, in treatment of scurvy, tuberculosis, influenza, asthma nutritional supplement and digestive aid (Carvalho, 2005). Hecht and Shiny, (2007) reported that extract of fresh leaves of *N. officinale* are used as a traditional herbal treatment of burns, soft tissue wounds and skin infections in developing countries, where it has been shown that the extract had an effect on the growth and proliferation of keratinocytes and fibroblasts in culture, they also showed that the extract stimulated the expression of many proteins on the adhesion complex and fibronectin by human keratinocytes. Nanoparticles present stronger antimicrobial effects than either microparticles or metal surfaces. Copper nanoparticle corrosion in distilled water is quite different as compared with microparticles (Karlsson *et al*., 2013). Cu2+ transformation ratio of microparticles increases slowly with the immersion time and levels off eventually, this transformation ratio increases sharply with the immersion time, reaching a peak rapidly, so new toxic mechanisms, depending on the cellular characteristics at the nano-scale, emerge by taking into account the role of the particle size itself (Gaetke *et al.*, 2003). The best example relate with the direct incorporation of nanoparticles into the cell via endocytotic mechanisms. Afterward the cellular uptake of ions increases as ionic species are subsequently released within the cells by nanoparticle dissolution, a process often referred as "the Trojan horse mechanism" (Cronlom *et al*., 2011). This high intracellular concentration gained after nanoparticle dissolution within the cell likely results in massive oxidative stress. Copper nanoparticles have a significant promise as bactericidal agent (Cieoffi, 2005). Other nanoparticles such as platinum, gold, iron oxide, silica and its oxides and nickel have not shown bactericidal effects in studies with *Escherichia coli* (Williams *et al*., 2006). Ivan and Branka (2004) concentrated the antibacterial effects of silver and copper nanoparticles using single representative strains of *Escherichia coli* and *Bacillus subtilis*, that copper nanoparticles demonstrated superior antibacterial activity compared to the silver nanoparticles, also silver and copper nanoparticles supported on various suitable materials, such as carbon, polyurethane foam, polymers and sepiolite have also been effectively used for bactericidal applications.

2. Materials and Methods

2.1. Collection and drying of plant materials

N. officinale plant leaves were collected from the local markets in Baghdad city in October 2015. *N. officinale* leaves were dehydrated by air-drying at ambient temperature of 23-25 0C in the dark in order to avoid the degradation of pigments and polyphenolic compounds. The plants were powdered to uniform particle size (Dalia *et al*., 2014).

2.2. Preparation of extracts

50g of each *N. officinale* leaves powder was extracted with 250ml of sterilized distilled water and 250ml of 70%Eethanol using magnetic stirrer for 24 hrs at room temperature. Extract were filtered through Whatman filter paper No.1 then it was concentrated in vacuum at 40°C using a rotary evaporator. The dried extracts was stored properly (Piyush *et al*., 2015). Then two different concentrations of leave extracts (water and ethanol extracts) were prepared (0.5 and 1.0mg/10ml) to be used alone or with copper nanoparticles at the same concentrations.

2.3. Sterilization of extracts

N. officinale leave extracts were sterilized using Millipore filter in a laminar air flow cabinet.

2.4. Preparation of media for bacterial cultures

Muller Hinton agar medium was prepared by Suspension 38g of Muller Hinton agar medium in 1000 ml sterilized D.W, heat till boiling to dissolve the medium completely, the final pH of the medium was adjusted to 6.8 using pH-meter. Sterilized by autoclaving at 15Ibs pressure and 121°C for 15 min, mix well before poring. Then the media was poured into a disposable Petri dishes in a depth of 3 to 4mm, After solidification, all prepared plates containing medium were kept at 4°C till use to provide a firm surface for wells making which were filled with 25microletter of prepared *N. officinale* leaves water and ethanol extracts alone or mixed with specific concentrations of copper nanoparticles.

2.5. Measurement of bacterial cultures concentration

Bacterial isolates were supplemented from bacterial isolates bank in Microbiology Lab., Post graduate laboratories, College of Applied Biotechnolgy, Al-Nahrain university, at which single colonies from cultures

grown on nutrient agar for 18-24 hrs were transferred to test tubes containing 5ml of normal saline and mixed well by vortex, then bacterial growth was compared with McFarland tube No.0.5 turbidity standard solution, which was equivalent to a bacterial inoculums concentration of 1.5*10^8 cell/ml.

2.6. Determination of inhibition zones of treatments

By using cotton swab, a touch of bacterial culture (broth) was transferred to Muller Hinton agar medium and streaked three times by rotating the plate approximately 60º between streaking to ensure even distribution of the inoculums, the inoculated plates were placed at room temperature for 10 min to allow absorption of excess moisture (Atlas *et al*., 1995). Then, using sterilized pauster pipette for making wells (the wells were arranged so as to avoid the development of overlapping of inhibition zones) which were then filled with 25µl of the sterilized plant extracts alone (0.5 and 1.0mg/10ml for water and ethanol extracts) or mixed with copper nanoparticles (0.5 and 1.0mg/10ml). The plates were incubated at 37ºC for 18-24 hrs. After incubation, inhibition zones were measured using ruler for determination their diameters (mm). The same procedure was made for antibiotic standard by using Cefotaxime 250mg/10ml D.w. Adding 25microletter of antibiotic to the wells in plates of Muller Hinton agar medium then the plates were incubated at 37ºC for 18-24hrs. After incubation, inhibition zones were measured by ruler also and the results were compared with the standards as in NCCLs (2007).

3. Results and Discussion

Results in Table 1 shown that there was a significant increase in the mean inhibition zones according to the type of treatment at 1:1 (v/v) of 1.0 mg/10 ml copper nanoparticles and water leaves extract (J) (31.2 mm) and 1:1 (v/v) of 0.5 mg/10 ml copper nanoparticles and ethanol leaves extract (F) (32.7 mm) compared with 0.5 mg/10 ml leaves water extract (B) (24.5 mm), 0.5 mg/10 ml leaves ethanol extract (C) (24.2 mm) and 0.5 mg/10 ml copper nanoparticles (D) (23.0 mm) treatment type and there was no significant differences recorded between all treatments and the control (30.2 mm), *Staphylococcus aureus* bacteria recorded the highest mean inhibition zones (39.0 mm) compared with other types of bacteria used in these experiments.

The interaction between the treatment type and the type of bacteria reveled that the highest mean inhibition zones were recorded at 1:1 (v/v) of 1.0 mg/10 ml copper nanoparticles and water leaves extract (J) and *Staphylococcus aureus bacteria* (39.0 mm) while the lowest mean was obtained at 1.0 mg/10 ml copper nanoparticles (I) in *Pseudomonas aeruginosa* bacteria (14.0 mm); eventually, there was no significant differences were investigated in *Pseudomonas aeruginosa and Staphylococcus aureus* in all treatment types but there was a significant increase in the mean inhibition zones in *E. coli* bacteria at 1:1 (v/v) of 0.5 mg/10 ml copper nanoparticles and ethanol leaves extract (F) (39.0 mm), 1:1 (v/v) of 1.0 mg/10 ml copper nanoparticles and water leaves extract (J) (35.0 mm) and 1:1 (v/v) of 1.0 mg/10 ml copper nanoparticles and ethanol leaves extract (K) (37.0mm) compared with control (28.0 mm); While in *Streptococcus pyogenes* there was a significant decrease in the mean inhibition zones at 0.5 mg/10 ml leaves water extract (B) (24.5 mm), 0.5 mg/10 ml leaves ethanol extract (C) (27.0 mm), 0.5 mg/10 ml copper nanoparticles (D) (25.0 mm), 1:1 (v/v) of 0.5 mg/10 ml copper nanoparticles and water leaves extract (E) (21.0 mm) , 1.0 mg/10 ml leaves water extract (G) (27.0 mm), 1.0 mg/10 ml leaves ethanol extract (H) (26.0 mm) , 1.0 mg/10 ml copper nanoparticles (I) (31.0 mm) and 1:1 (v/v) of 1.0 mg/10 ml copper nanoparticles and ethanol leaves extract (K) (29.0 mm) in comparison to the control (39.0 mm)*;* In spite of the lower concentrations of the *N. officinale* leaves extracts and copper nanoparticles (alone or mixed) but they provide a suitable bactericidal effects compared with the control in spite of its high concentration (Cefotaxime antibiotic at 250 mg/10 ml); and these results were agreement with those obtained by Theivasanthi and Alagar (2010) who reported that copper nanoparticles act as bactericidal against different types of Gram (-) and Gram (+) bacteria. Dalia *et al*. (2014) investigated the antibacterial activity of *N. officinale* leaves ethanol extracts against five types of Gram (+) bacteria. Figures 1, 2, 3 and 4 shown the inhibition zone of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes* respectively as a result of using *N. officinale* leaves water and ethanol extracts alone or mixed with copper nanparticles.

Table 1. Inhibition zones diameters (mm) as a results of treatment four types of bacteria with *N. officinale* leaves water and ethanol extract alone or mixed with copper nanoparticles.

Bacteria / Treatment	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus pyogenes*	Mean
A	28.0	19.0	35.0	39.0	30.2
B	26.0	17.0	31.0	24.0	24.5
C	21.0	17.0	32.0	27.0	24.2
D	19.0	15.0	33.0	25.0	23.0
E	33.0	19.0	31.0	21.0	26.0
F	39.0	20.0	37.0	35.0	32.7
G	23.0	14.0	31.0	27.0	23.7
H	34.0	19.0	35.0	26.0	28.5
I	31.0	14.0	33.0	31.0	27.2
J	35.0	18.0	39.0	33.0	31.2
K	37.0	19.0	37.0	29.0	30.5
Mean	29.6	17.3	34.1	28.8	
L.S.D 0.05	**Type of treatment = 6.1; Type of bacteria= 13.5; Interaction=6.1**				

A) *Escherichia coli*

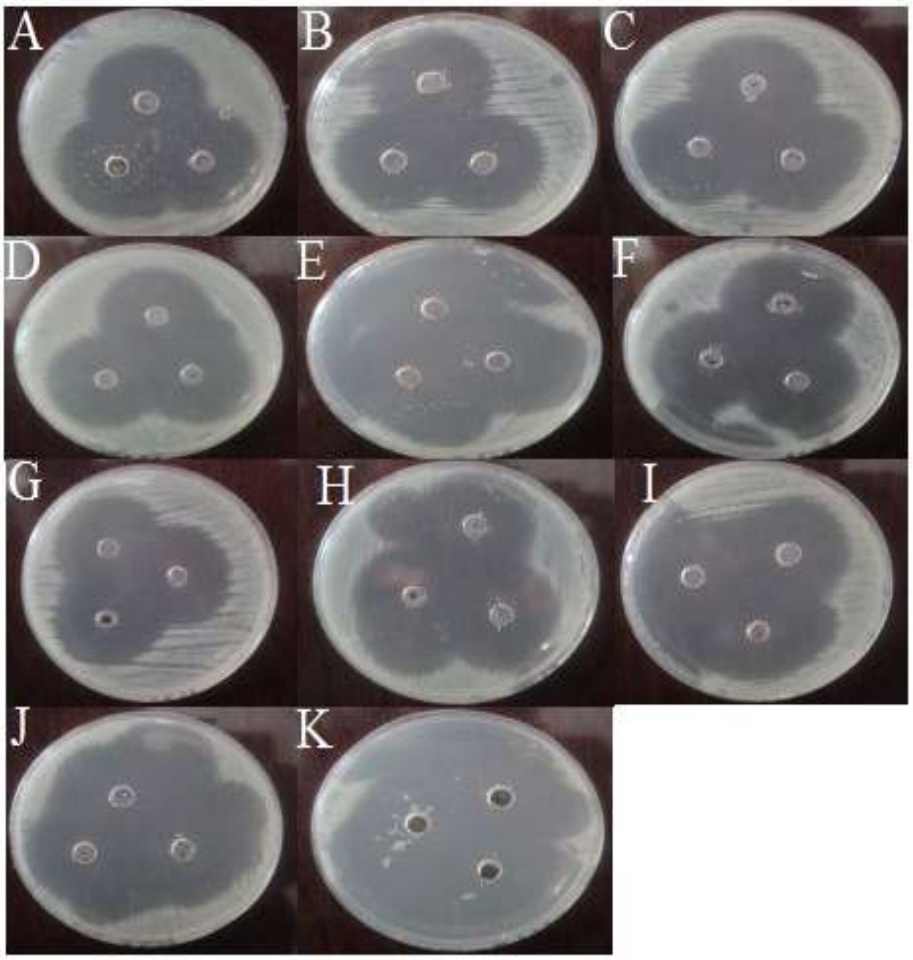

Figure 1. Antibacterial activity of *N. officinale* leaves water and ethanol extract alone or mixed with copper nanoparticles against *Escherichia coli*. (A) control, (B) 0.5mg/10ml leaves water extract, (C) 0.5mg/10ml leaves ethanol extract, (D) 0.5mg/10ml copper nanoparticles, (E) 1:1 (v/v) of 0.5mg/10ml copper nanoparticles and water leaves extract, (F) 1:1 (v/v) of 0.5mg/10ml mg/10ml copper nanoparticles and ethanol leaves extract, (G)1.0mg/10ml leaves water extract, (H) 1.0mg/10ml leaves ethanol extract, (I) 1.0mg/10ml copper nanoparticles, (J) 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and water leaves extract, (K) 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and ethanol leaves extract.

B) *Pseudomonas aeruginosa*

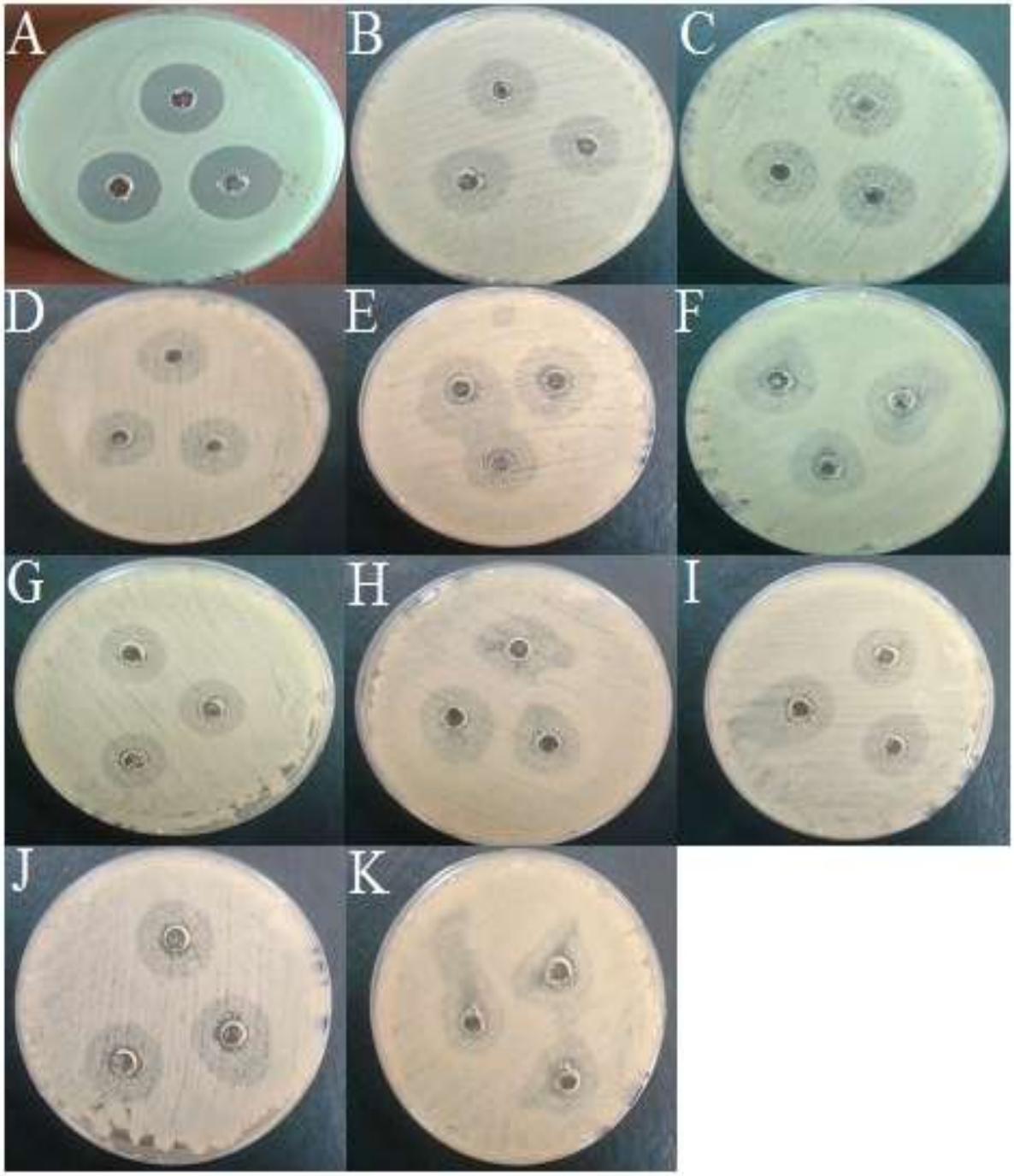

Figure 2. Antibacterial activity of *N. officinale* leaves water and ethanol extract alone or mixed with copper nanoparticles against *Pseudomonas aeruginosa*. (A) control, (B) 0.5mg/10ml leaves water extract, (C) 0.5mg/10ml leaves ethanol extract, (D) 0.5mg/10ml copper nanoparticles, (E) 1:1 (v/v) of 0.5mg/10ml copper nanoparticles and water leaves extract, (F) 1:1 (v/v) of 0.5mg/10ml mg/10ml copper nanoparticles and ethanol leaves extract, (G)1.0mg/10ml leaves water extract, (H) 1.0mg/10ml leaves ethanol extract, (I) 1.0mg/10ml copper nanoparticles, (J) 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and water leaves extract, (K) 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and ethanol leaves extract.

C) *Staphylococcus aureus*

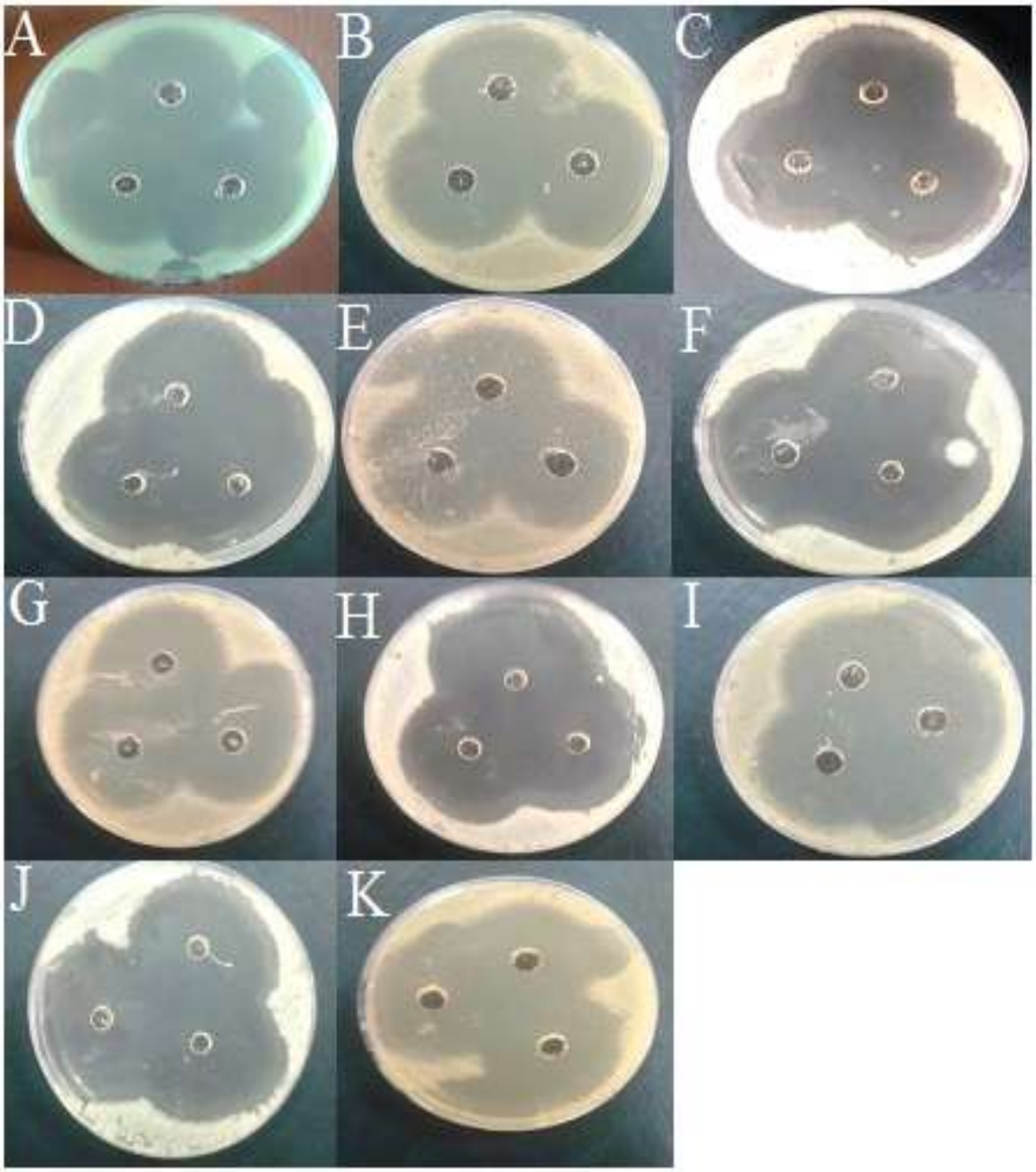

Figure 3. Antibacterial activity of *N. officinale* leaves water and ethanol extract alone or mixed with copper nanoparticles against *Staphylococcus aureus*. (A) control, (B) 0.5mg/10ml leaves water extract, (C) 0.5mg/10ml leaves ethanol extract, (D) 0.5mg/10ml copper nanoparticles, (E) 1:1 (v/v) of 0.5mg/10ml copper nanoparticles and water leaves extract, (F) 1:1 (v/v) of 0.5mg/10ml mg/10ml copper nanoparticles and ethanol leaves extract, (G)1.0mg/10ml leaves water extract, (H) 1.0mg/10ml leaves ethanol extract, (I) 1.0mg/10ml copper nanoparticles, (J) 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and water leaves extract, (K) 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and ethanol leaves extract.

D) *Streptococcus pyogenes*

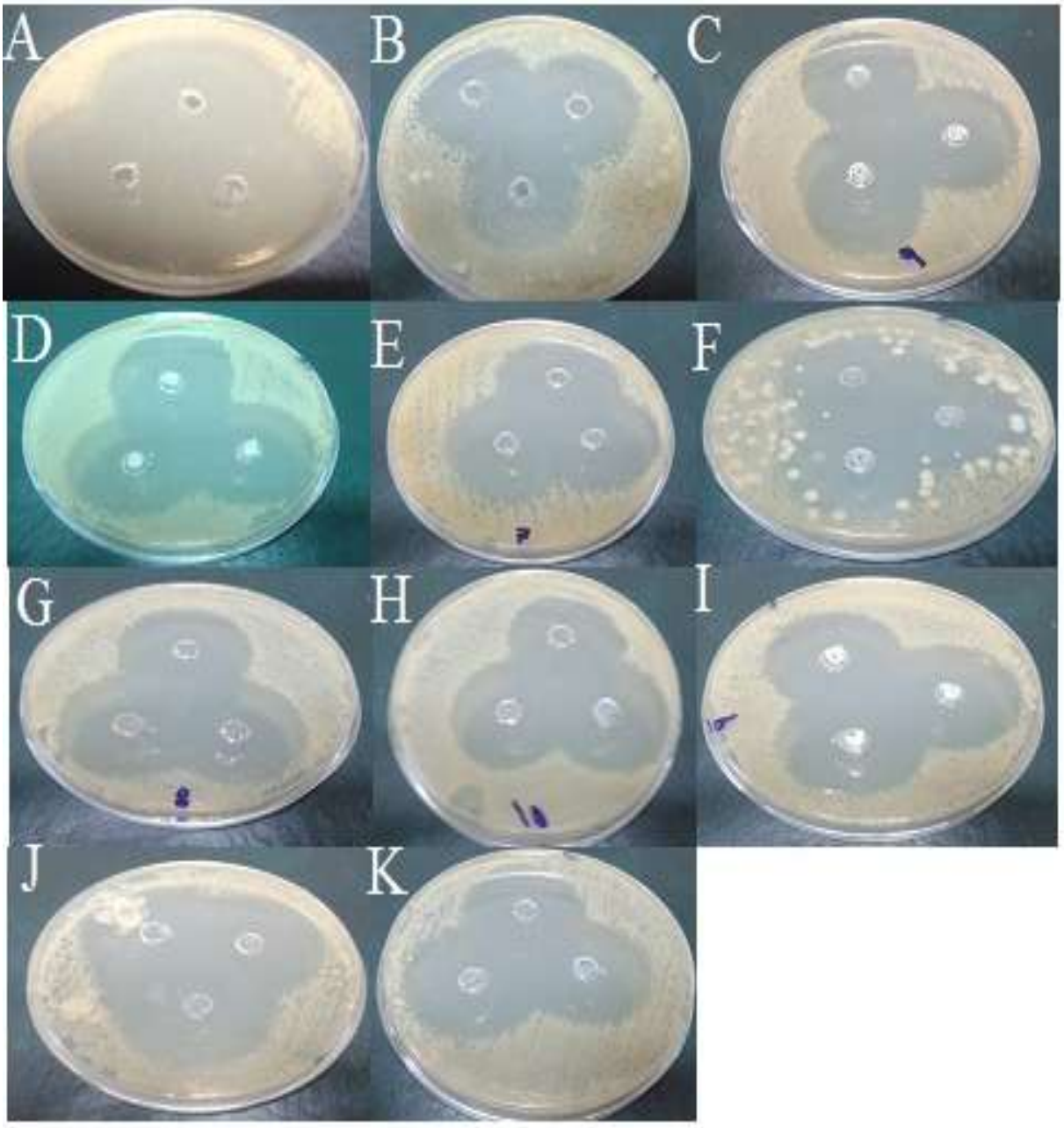

Figure 4. Antibacterial activity of *N. officinale* leaves water and ethanol extract alone or mixed with copper nanoparticles against *Streptococcus pyogenes*. (A) control, (B) 0.5mg/10ml leaves water extract, (C) 0.5mg/10ml leaves ethanol extract, (D) 0.5mg/10ml copper nanoparticles, (E) 1:1 (v/v) of 0.5mg/10ml copper nanoparticles and water leaves extract, (F) 1:1 (v/v) of 0.5mg/10ml mg/10ml copper nanoparticles and ethanol leaves extract, (G)1.0mg/10ml leaves water extract, (H) 1.0mg/10ml leaves ethanol extract, (I) 1.0mg/10ml copper nanoparticles, (J) 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and water leaves extract, (K) 1:1 (v/v) of 1.0mg/10ml copper nanoparticles and ethanol leaves extract.

4. Conclusions

In spite of the lower concentrations of the plant extracts and copper nanoparticles (0.5 and 1.0 mg/10 ml) compared with the standard antibiotic used, but it has proved highly efficiency as an anti bacterial agent that appeared as growth inhibition zone against different types of gram positive and gram negative bacteria used in these experiments.

Conflict of interest
None to declare.

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