International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (4): 115-121
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved

Antibacterial Effect of Silver Nanoparticles from *Origanum majorana* Leaf Extract in First-Aid Bandage

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Received: 06 Jul 2019 / Accepted: 08 Aug 2019 / Published online: 1 Oct 2019
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Abstract

Green synthesis of silver nanoparticles has an edge over the other nanoparticles for its unique eco-friendly characteristics. Medicinal plants are phenomenally essential for the synthesis of these Nano-sized particles which has numerous advantages in the field of medicine and pharmacology. *Origanum majorana* leaves are used in the present study for the synthesis which is further extended to analyze its property as an antibacterial and antifungal agent. Both the tender and mature leaves of the plant were used in the study. The silver nanoparticles produced from the leaf of *O. majorana* has potential antibacterial effect against pathogens like *Staphylococcus aureus* and *Escherichia coli*. It proved to be a good antifungal agent also against pathogens like *Candida albicans* and *Aspergillus flavus* with this observation, the study was extended towards the application aspect where the nanoparticles were coated over the first aid bandage to analyse its effect. Levels of protection proved that the nanoparticles of *O. majorana* leaves, both mature and tender, can be used as antibacterial and antifungal protective agent.

Keywords
Antibacterial, antifungal, green synthesis, haemolytic activity, nanoparticle.

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INTRODUCTION:

Nanoparticle is about 1 to 100 nanometers in size and has application of extremely small things that can be used across all the other science fields, such as chemistry, biology, physics, materials science and engineering. Nanoparticle research is an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields [1]. Synthesis of nanoparticles is a crucial area of research, probing for a nature-friendly manner for current science. Countless methodologies are emerged to synthesize noble metal nanoparticles of specific shape and size depending upon requirement [2].

Nanoparticles can be classified into different types according to the size, morphology, physical and chemical properties. Some of them are carbon-based nanoparticles, ceramic nanoparticles, metal nanoparticles, semiconductor nanoparticles, polymeric nanoparticles and lipid-based nanoparticles. Nanoparticles possess unique electrical, optical as well as biological properties and

are thus applied in catalysis, bio sensing, imaging, drug detective, Nano device fabrication and in medicine [3].

Origanum Majorana: *M. hortensis* is a Mediterranean perineal herb of Lamiaceae family, commonly called sweet majoram and used to add flavour in culinary purpose. It has been proved to have high potential as an antioxidant [4]. It finds its place in religious ceremonies and has several uses in therapeutic remedies, insecticidal effect due to terpenes, and carries several traditional uses [5].

MATERIALS AND METHODS:

Collection of Plant Sample:

The plant was collected in and around Coimbatore. Identification of the plant specimens were done at Botanical Survey of India, TNAU campus, Coimbatore as *Origanum majorana* (Voucher Number: BSI/SRC/ 5/23/2018/Tech./2266). The fresh leaves were stored in refrigerator and used for Green synthesis of silver nanoparticles.

Synthesis of Silver Nanoparticles: 20 g of leaves were boiled with 100 ml of distilled water at 80˚C for 1 hour. After boiling, the brown coloured extract was separated by filtration and it was used for the reduction of silver nitrate to silver nanoparticles using silver nitrate (1mM) as precursor and incubated in water bath for 10 minutes at 60º C. The collected pellet was dried in hot air oven for 24 hours at 100ºC.

Hemolytic activity: The blood sample was collected and anticoagulant EDTA was added. The blood sample was centrifuged for 3 minutes. The supernatant is discarded and the pellet is collected and washed with PBS buffer and centrifuged. The process is repeated for three times. 0.5mL of cell suspension and silver nanoparticles of mature and tender leaves were added in different concentrations of 100µL, 300µL, and 500µL and made up to 0.5mL of PBS. It was incubated for 30 min at 37° C. The mixture was centrifuged at 1500 rpm for 10 minutes. Free haemoglobin was in the supernatant and is added to 96 micro titre plates and is checked for its value. The PBS and distilled water served as the maximum and the minimal value control. The haemolytic activity observed is calculated using the formula,

% Haemolysis = A t - A n / A c - A n × 100

Where A t is the absorbance of the test sample, a n is the absorbance of control (PBS), A c is the absorbance of control (distilled water).

Antibacterial Activity of AgNPs: The antibacterial activity was done by using synthesized AgNPs and plant extract against two types of bacteria in Nutrient agar plates by Well diffusion method. And the antibiotic disc was placed in the plate for both organisms were Amoxillin antibiotic.

Materials:

- Plant extract
- Synthesized AgNPs
- Nutrient agar media
- Bacterial Culture
- Antibiotic

Procedure: The antibacterial activity of synthesized AgNPs and plant extract were evaluated against human pathogens such as gram negative (*Escherichia coli*) and gram positive bacteria (*Staphylococcus aureus*).

The strains were sub cultured in nutrient broth for 24 hours at 37ºC. Each strain was spread uniformly into the individual nutrient agar plates using sterile glass L-rod. 100µL of AgNPs (Mature & Tender), 100 µL of leaf extract (Mature & Tender) and 100µL of distilled water along with standard antibiotic Amoxicillin 1mg/mL discs were impregnated in the nutrient agar medium. After 24 hours incubation at 37ºC, the different level of zone of inhibition was measured.

Antifungal Activity of AgNPs: The antifungal activity was done by using synthesized AgNPs and plant extract against two types of fungi in Potato Dextrose Agar (PDA) plates by well diffusion method. And the fungicide was placed in the plate for both organisms as positive control. The two cultures of *Candida albicans* and *Aspergillus flavus* were grown in a PDA broth and stored.

Materials:

- Plant extract
- Synthesized AgNPs
- PDA media
- Fungal Culture (*C. albicans* & *A. flavus*)
- Fungicide

Procedure: The antifungal activity of synthesized AgNPs and plant extract were evaluated against common pathogens such as *C. albicans* and *A. flavus*. The sterile water was used as negative control. The strains were sub-cultured in PDA broth for 3 days in 37ºC. Each strain was spread uniformly into the individual PD Agar plates using sterile glass L-rod. After this well cut in four sides of PDA plates. And 100µL of AgNPs (Mature & Tender), 100 µL of leaf extract (Mature & Tender) and 100µL of distilled water along with 100 µL of Fungicide (1mg/mL) were impregnated in the PD agar medium. The plates were incubated for 3-4 days in incubator. After 3-4 days incubation at 37ºC, the different level of zone of inhibition was measured. It indicates the activity of silver nanoparticles against fungal pathogens.

Activity of first-aid bandage with AgNPs: The activity of AgNPs in first- aid bandage were done by

using Disc diffusion method. This activity was against the human pathogen such as *Staphylococcus aureus* and *Escherichia coli*. The normal first-aid bandage disc was used as a positive control to compare the first- aid bandage with AgNPs.

Materials:

- Plant extract
- Synthesized AgNPs
- Nutrient agar media
- Bacterial Culture (Gram +ve & Gram -ve)
- First- aid bandages

Procedure: The activity of first- aid bandage with AgNPs and plant extract were evaluated against human pathogens such as Gram negative (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*). The strains were sub cultured in nutrient broth for 24 hours at 37 ºC. Each strain was spread uniformly into the individual nutrient agar plates using sterile glass L-rod. The disc of first- aid bandages was dipped in 100µL of AgNPs (Mature & Tender), 100 µL of leaf extract (Mature & Tender) and 100 µL of distilled water along with normal first- aid bandage disc were placed in the nutrient agar medium. The plates were incubated for 24 hours at 37 ºC. After 24 hours incubation at 37 ºC, the different level of zone of inhibition was measured. The zones indicated the activity of first-aid bandage with AgNPs human pathogens.

RESULTS AND DISCUSSION:

Leaf extract preparation: The leaf extracts of both mature and tender leaves were prepared at 80 ºC in water bath and the brown colour extracts were obtained.

Synthesis of Silver nanoparticles:

The presence of reddish brown colour in the reaction mixture indicated the formation of silver nanoparticles. Present findings showed resemblance to the results already reported in the case of extract of *Origanum vulgare* [6]. They reported that when the extract of their respective test plants was challenged with silver nitrate (1 mM) they turned reddish brown colour and it formed silver nanoparticles as shown in the figure 1 placed in the Eppendorf. Similar results and colour was observed for the tender plant leaves too.

Antibacterial activity of AgNPs:

Antibacterial activity of two different sample of silver nanoparticles and aqueous plant extracts were checked and the results were noted.

Two different aqueous plant extracts and the synthesized silver nanoparticles of *O. majorana* produced zone of clearance against both Gram positive and Gram negative such as *Escherichia coli* and *Staphylococcus aureus* (Figure 1). The diameter of zone of inhibition was measured to be as given in Table 1. The organisms showed better resistance towards the Silver Nanoparticles than the plant extracts. The previous works have also proved that the organisms such as *Staphylococcus aureus* and *Salmonella typhi* showed better resistance towards the Silver nanoparticles[1] (Indhumathy *et al*., 2014). The another study was proved that *S. aureus* (27mm), *C. albicans* and *S. pneumoniae* (25 mm), then 23 mm for Gram-negative Bacteria *E. coli, K. pneumonia, P. aeruginosa* and *P. volgaris* suggesting that synthesized nanoparticles have good antibacterial action against Gram-positive organism than Gram-negative organisms [7].

Table 1: Antibacterial activity of AgNPs and leaf extracts (Mature and tender)

	Zone of Inhibition	
Sample	***Staphylococcus aureus***	***Escherichia coli***
Standard (Antibiotic)	16mm	-
Mature leaf extract	18mm	12mm
Tender leaf extract	14mm	12mm
AgNPs (Mature leaf)	24mm	15mm
AgNPs (Tender leaf)	20mm	14mm

The table shows that the activity of the silver nanoparticles has higher activity than the leaf extracts against the human pathogens. The silver nanoparticles of mature and tender samples have better zone of inhibition against both *S. aureus* and *E. coli* of about 24mm, 15mm and 20mm, 14mm respectively. Similarly, the leaf extracts of mature and tender shown activity less than the Silver nanoparticles against both organisms, 18mm, 12mm and 14mm, 12mm respectively. Here it is concluded that the silver nanoparticles of both mature and tender samples have greater activity than the leaf extracts of mature and tender leaves against Gram positive and Gram negative bacteria.

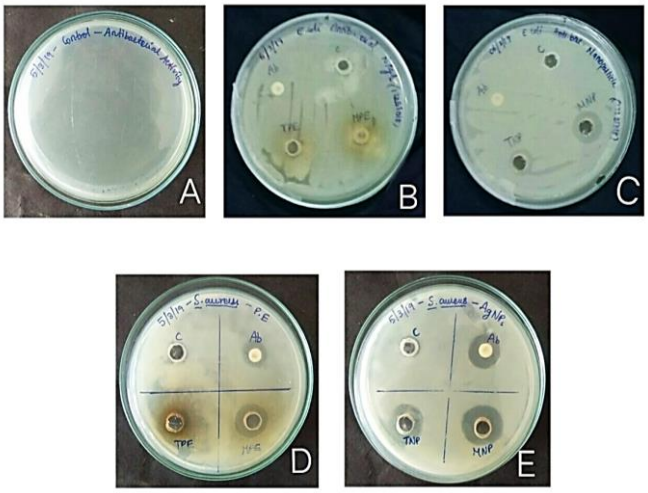

Figure 1: Antibacterial activity A) Control B) Activity of leaf extracts against *E. coli* C) Activity of AgNPs against *E. coli* D) Activity of leaf extracts against *S. aureus* E) Activity of AgNPs against *S. aureus*.

Antifungal activity of AgNPs:

Antifungal activity of two different sample of silver nanoparticles and aqueous leaf extracts were checked and the results were noted.

Two different aqueous leaf extracts and the synthesized silver nanoparticles of *O. majorana* produced zone of clearance against both organisms such as *Candida albicans* and *Aspergillus flavus* (Figure 2). The diameter of zone of inhibition was measured to be as given in Table 2. The organisms showed better resistance towards the silver nanoparticles than the leaf extracts. The previous work proved the synthesised AgNPs prepared from *Aloe Vera* leaf extract showed antifungal activity against *Rhizopus sp*. and *Aspergillus sp* [8]. The study also proved that Ag-NPs has remarkable potential as an antifungal agent in treating fungal infectious diseases. To provide information on the mode of action of Ag-NPs, its ability to dissipate the membrane potential of *C. albicans* and *Saccharomyces cerevisiae* were investigated [9].

Table 2: Antifungal activity of silver nanoparticles and leaf extracts (Mature and tender)

	Zone of Inhibition	
Sample	***Candida albicans***	***Aspergillus flavus***
Standard (Antibiotic)	24mm	28mm
Mature leaf extract	14mm	16mm
Tender leaf extract	12mm	14mm
AgNPs (Mature leaf)	20mm	22mm
AgNPs (Tender leaf)	16mm	20mm

Table 2 shows that the activity of the silver nanoparticles has higher activity than the leaf extracts against the fungal pathogens, *C. albicans* and *A. flavus*. The silver nanoparticles of mature and tender samples have better zone of inhibition against both *C. albicans* and *A. flavus* of about 20mm, 22mm and 16mm, 20mm respectively. Similarly, the leaf extracts of mature and tender showed activity less than the silver nanoparticles against both organisms, 14mm, 16mm and 12mm, 14mm respectively. Here it is concluded that the silver nanoparticles of both mature and tender samples have greater activity than the leaf extracts of mature and tender leaves against fungal pathogens.

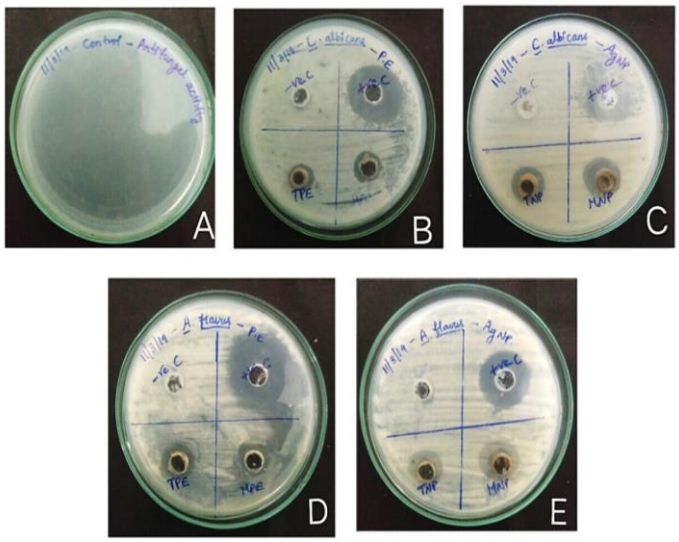

Figure 3: Antifungal activity A) Control B) Activity of leaf extracts against *C. albicans* C) Activity of AgNPs against *C. albicans* D) Activity of leaf extracts against *A. flavus* E) Activity of AgNPs against *A. flavus*.

Activity of First-aid bandage with AgNPs:

Activity of first-aid bandage with two different samples (mature and tender leaves) of silver nanoparticles and aqueous plant extracts were observed and the results were noted. The commercially available first-aid bandages (RBX-AID) were cut into small disc sizes and were dipped in the silver nanoparticles and aqueous leaf extracts (mature and tender leaves) for which the results are noted below.

Table 3: Antibacterial activity of AgNPs and leaf extracts (Mature and tender)

	Zone of Inhibition	
Sample	***Staphylococcus aureus***	***Escherichia coli***
Standard (First-aid bandage)	14mm	12mm
Mature leaf extract	12mm	10mm
Tender leaf extract	14mm	8mm
AgNPs (Mature leaf)	26mm	24mm
AgNPs (Tender leaf)	20mm	18mm

Two different aqueous leaf extracts (mature and tender) and the synthesized silver nanoparticles of *O. majorana* produced zone of clearance against both Gram positive and Gram negative organisms such as *Escherichia coli* and *Staphylococcus aureus* (Figure 3). The diameter of zone of inhibition was measured and the values are tabulated in Table 3. The table shows that the activity of the silver nanoparticles has higher activity than the leaf extracts against the human pathogens (*S. aureus* and *E. coli*). The silver nanoparticles of mature samples showed better zone of inhibition against both *S. aureus* and *E. coli* of about 26 mm, 24 mm respectively. Similarly, the silver nanoparticles of mature samples showed better zone of inhibition against both *S. aureus* and *E. coli* of about 20 mm, 18 mm respectively. The leaf extracts of mature and tender showed activity less than the silver nanoparticles against both organisms, 12 mm, 10 mm and 14 mm, 8 mm respectively.

When compared with the standard first-aid bandage values it was noted that silver nanoparticles samples (mature and tender leaves) showed significantly higher values on comparison of 26 mm (mature leaves), 20 mm (tender leave) against *S. aureus* showed 14 mm. Similarly, when compared with the standard first-aid bandage values it was noted that silver nanoparticles samples (mature and tender leaves) showed significantly higher values on comparison of 24 mm (mature leaves), 18 mm (tender leaves) against *E. coli* showed 12 mm. Here it is concluded that the silver nanoparticles of both mature and tender leaves have greater activity than the leaf extracts of mature and tender leaves against Gram positive and Gram negative bacteria. The organisms showed better resistance towards the

silver nanoparticles than the leaf extracts. The previous study was proved that the silver nanoparticles showed the antimicrobial activity against Gram positive and Gram negative bacteria. *Vitex negundo* L. was found and displayed strong potential for the synthesis of silver nanoparticles as antimicrobial agents [10]. In another study, it was proved that silver nanoparticles of *Origanum heracleoticum* leaf extract showed clear inhibition after incubation of 24hours against *E. coli* (21 mm), *P. aeruginosa* (27 mm), *K. pneumoniae* (23 mm), *S. aureus* (29 mm), *S. pneumoniae* (27 mm) [11].

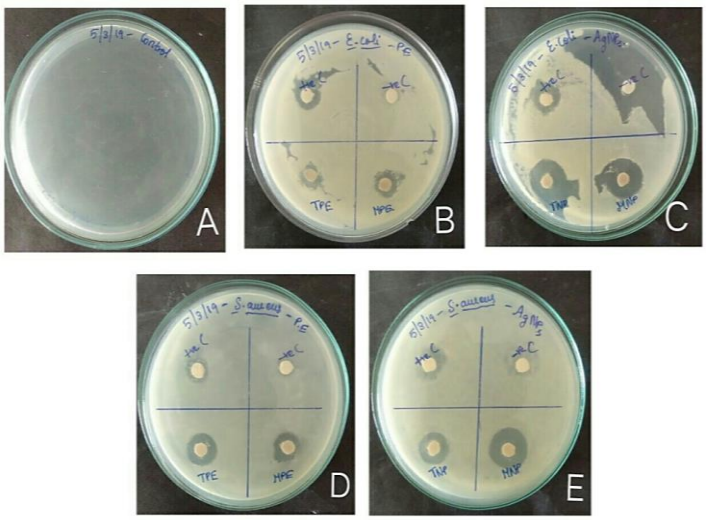

Figure 3: Activity of first-aid bandage A) Control B) Activity of first-aid bandage with leaf extracts against *E. coli* C) Activity of first-aid bandage with AgNPs against *E. coli* D) Activity of first-aid bandage with leaf extracts against *S. aureus* E) Activity of first-aid bandage with AgNPs against *S. aureus*.

CONCLUSION:

The nanoparticles synthesized from both mature and tender leaves of *O. majorana* showed remarkable antibacterial and antifungal activity. Also, hemolytic activity was exhibited when subjected to human erythrocytes which clearly indicates that this sample has potential to be used as a good antibacterial and antifungal agent. It has paved way for an excellent application in the preparation of first-aid bandage which can have an effective use in the commercial preparation of bandages.

CONFLICT OF INTEREST:

Conflict of interest declared none.

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