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Effect of Photosynthesized Silver Nanoparticles Using *Lawsonia inermis, Pergularia daemia* and *Canna indica* against Wound Pathogens

Senthamarai Selvi Venugopalan*, Jayasakthi Mohanasundharam

Department of Biochemistry, Bharathidasan College of Arts and Science, Erode, Tamil Nadu, India

ABSTRACT

To present study is to isolate, identify bacterial cultures from wound samples (Proteus, Escherichia coli, Staphylococcus aureus, Klebsiella and Bacillus) and to assess the antibacterial efficiency of aqueous and solvent extracts and silver nanoparticles of Pergularia daemia, Canna indica and Lawsonia inermis against bacterial strains. The pathogens were isolated, identified on the basis of Gram's reaction and biochemical test. The aqueous and acetone extracts P. daemia, C. indica and L. inermis was used to test the antibacterial activity by agar well diffusion method. The silver nanoparticle was synthesized, characterized and confirmed using UV-Visible spectrum of each reaction mixtures at about 24 h of reaction using UV-Visible Spectrophotometer. The synergistic mixtures were prepared with different types of combination form of silver nanoparticles produced by P. daemia, L. inermis and C. indica. This study confirm the presence of E. coli, S. aureus, Bacillus species, Proteus species and Klebsiella species isolated from various wound infection samples. The L. Inermis showed maximum zone of inhibition at 25.2 ± 0.28 mm for E. coli and the minimum zone of inhibition of Proteus sp at 19.40 ± 0.56 mm. The antibacterial activity of water extract of C. indica. The maximum inhibition zone was observed against S. aureus. The silver nanoparticles were confirmed by color changes from dark green to brown color. The peak value was obtained at 454.5 nm for C. indica and 446 nm for L. inermis. The plants considered have found use in the field of nanotechnology.

Keywords: *Lawsonia inermis, Pergularia daemia, Canna indica*, Antibacterial activity, Silver nanoparticles

INTRODUCTION

Human beings are very commonly infected by microorganism in the living environment, which sometimes leads to illness and other health hazards. Microorganism harmful to human being is termed as pathogens. In the recent past, due to the emergence and increase of such pathogenic strains resistant to multiple antibiotics [1]. The basic source of phytochemical constituents is the only resource for the establishment of several pharmaceutical industries. Nano particles, generally considered as particles with a size of up to 100 nm, exhibit completely new or improved properties as compared to the larger particles of bulk material that they are made up of [2]. The plant *Pergularia daemia* (Asclepiadaceous) [3], *P. daemia* is used as anthelmintic, laxative, antipyretic and expectorant, diarrhoea and malarial intermittent fevers [4], antifertility [5], anti-diabetic [6], analgesic, antipyretic and anti-inflammatory [7]. *Canna indica* is a widely used as traditional medicine for the treatment of many complains. The pharmacological studies showed that this plant exerted antibacterial, antiviral anthelmintic, molluscicidal, anti-inflammatory, analgesic immunomodulatory, antioxidant, cytotoxic, hemostatic, hepatoprotective, antidiarrheal and other effects [8].

Lawsonia inermis, belong to the family Lythraceae [9]. The majority of phytochemical constituents of *L. inermis* are found to be possess significant anti-inflammatory, analgesic and antipyretic activities [10], antiviral [11], antibacterial [12], antifungal [13], antiparasitic [14], larvicidal activity [15] and anticancer [16] properties.

MATERIALS AND METHODS

Collection of plant material and processing of plant sample

They matured *P. daemia, C. indica* and *L. inermis* leaves were collected from Perundurai, Erode District, Tamil Nadu, India (Figure 1). The leaves of *P. daemia, C. indica* and *L. inermis* was washed with tap water and then rinsed with distilled water. The leaves were shade dried and powdered using electrical blender. The powder was stored in air tight glass containers, protected from Sunlight until required for analysis.

Figure 1: (A) *Lawsonia inermis,* (B) *Pergularia daemia,* (C) *Canna indica*

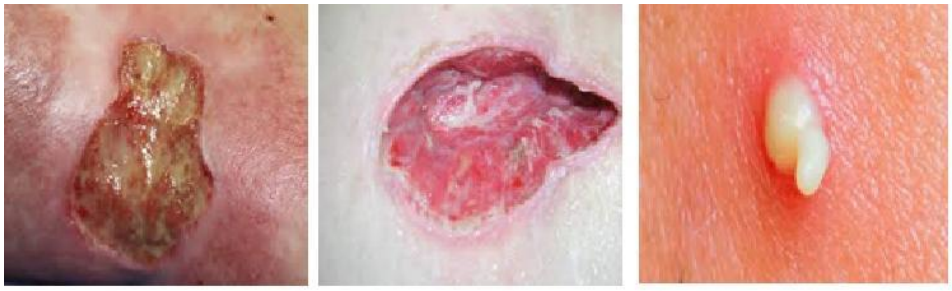


Figure 2: Collection of wound sample

Collection of clinical pathogens

A total of 5 *E. coli, Proteus* sp, *Bacillus* sp, *Klebsiella* sp and *S. aureus* isolates were screened from significant symptomatic wound sample (Figure 2). The isolates were obtained from Medical College and Hospital, Perundurai, Erode, Tamil Nadu, India.

Processing of wound sample

Isolation of pathogen

An aseptically collected wound sample was inoculated, with the help of standard inoculating loop, on the Mannitol salt agar, Mac Conkey Agar plates and was incubated at 24 h at 37°C. Following incubation, the growth of bacterial colonies was observed and results were recorded.

Identification of pathogens

The pathogens were isolated, identified on the basis of Gram's reaction and biochemical characteristics [17]. The results were confirmed with the help of Bergey's Manual of systematic bacteriology.

Biochemical characterization [18]

Gram staining, EMB agar, Macconkey agar, Mannitol agar, Indole test, Voges Proskauer (VP) test, Citrate utilization test, Catalase test, Nitrate reduction test, Triple sugar iron agar test and Methyl red (MR) test.

Preparation of plant extraction

10 g of powdered sample was soaked in 100 ml of aqueous and acetone for 3-4 days in dark at room temperature. The extracts were filtered using what man filter paper. The filtrate was concentrated to dryness under reduced pressure at 40°C using water both and stored at 4°C for further use.

Antibacterial activity of plant extract

Agar well diffusion technique [19]

The antibacterial activity of *P. daemia, C. indica* and *L. inermis* extracts. All media plates (9 cm in diameter) were prepared. After agar gets solidify, the well (7 mm in diameter) was cut to produce a total of 3 wells per each agar plate. Bacterial suspension was swabbed on agar plates using sterile cotton buds. The inoculums were allowed to dry for 5 min. Then, 100 µl of each extract was added individually to each well of agar plate and allowed to distribute at room temperature for 15-20 min. After the incubation at 30°C for 24 h, all the plates were examined for any zones were measured.

Synthesis of silver nanoparticles [20]

The cold water extract of *P. daemia, C. indica* and *L. inermis* (0.05 gm) was added to distilled deionized water (10 ml) with vigorous stirring for 4 h. The assess the effect of temperature on silver nanoparticles was studied by the reaction in water bath at different temperature (30°C-80°C). The set up was incubated in dark to avoid the minimization of photo activation of silver nitrate at 37°C. Silver nanoparticle was gradually synthesized during the incubation period. Throughout the reduction process, the solution was set aside at a room temperature in the dark to avoid phytochemical reactions. The synthesis of silver nanoparticles were characterized and confirmed by UV-Visible spectrophotometer.

UV-visible spectra analysis [21]

The reduction of silver ions was measured using UV-visible spectrum of each reaction mixtures at about 24 h of reaction. A small aliquot was drawn from the reaction mixed and a spectrophotometer (ELICO SL 159) UV-visible spectrophotometer.

Preparation of synergistic mixture for antibacterial activity [22]

The synergistic mixtures were prepared with different types of combination form of silver nanoparticles synthesized by *P. daemia, L. inermis* and *C. indica.* It was prepared with a combination of different plant synthesized silver nanoparticles such as 1:1 ratio of *L. inermis* and *P. daemia*, *P. daemia* and *C. indica, C. indica* and *L. inermis* and 1:1:1 ratio of *L. inermis* and *P. daemia* and *C. indica* and the antibacterial activity of synergistic mixture was performed by agar well diffusion method and Minimum Inhibitory Concentration (MIC).

RESULTS AND DISCUSSION

In the present study the *in vitro* antibacterial activity of *L. inermis, P. daemia* and *C. indica* used by Indian peoples was proved to have therapeutic properties. The antibacterial activity was expressed at changeable degrees with the activity being strains and does dependent. Five bacteria's were used for antibacterial studies. Table 1, showed the isolation and identification of 5 bacterial pathogens (*E. coli, S. aureus, Bacillus* sp, *Proteus* sp and *Klebsiella* sp) from various wound infection samples.

Table 1: Antibacterial activity of synthesized silver nanoparticles by *Lawsonia inermis, Pergularia daemia* and *Canna indica*

Isolates from wound pathogens	Zone of inhibition (mm)		
	Lawsonia inermis	***Pergularia daemia***	***Canna indica***
Bacillus sp	8.35 ± 0.49	8.15 ± 0.21	9.40 ± 0.56
Proteus sp	7.25 ± 0.35	9.05 ± 0.07	7.30 ± 0.42
S. aureus	9.30 ± 0.42	7.10 ± 0.14	14.35 ± 0.49
E. coli	8.20 ± 0.28	14.45 ± 0.63	15.25 ± 0. 35
Klebsiella sp	7.40 ± 0.56	19.20 ± 0.28	20.45 ± 0.63

The aqueous extract of *Lawsonia inermis* showed minimum zone of inhibition at 20.15 ± 0.21 mm for *Klebsiella* sp and 20.45 ± 0.63 mm for *Proteus* sp, the maximum inhibition zone was observed against *E. coli* at 23.25 ± 0.35 mm. The acetone extract of *L. inermis* showed maximum zone of inhibition at 25.2 ± 0.28 mm for *E. coli* and the minimum zone of inhibition of *Proteus* sp at 19.40 ± 0.56 mm. Similar results were reported by Anonymous, 1996 he reported ethanol and methanol extract showed lowest activity (16.82 mm, 10.25 mm).

In the *In vitro* antibacterial activity with various solvent extracts of leaves of *L. inermis* effective against *S. aureus, B. subtilis, E. coli.* In addition, some other researchers also indicated that n-hexane, chloroform and methanol extracts of the leaves of the plant displayed antibacterial activity against *S. aureus, B. subtilis* and *E. coli* [23]. Taking into consideration it as a potential antibacterial agent, we under took antibacterial activity study using n-hexane, chloroform, ethyl acetate, benzene, acetone and acetonitrile extracts of leaves of *L. inermis* (Figures 3 and 4).

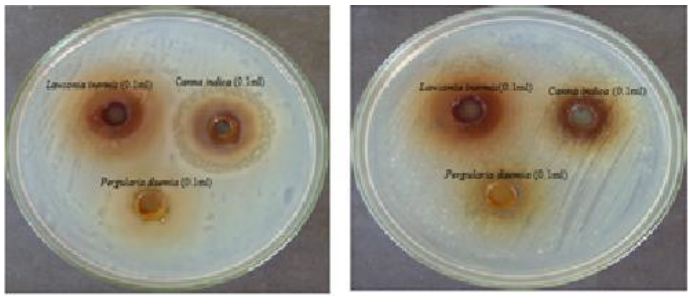
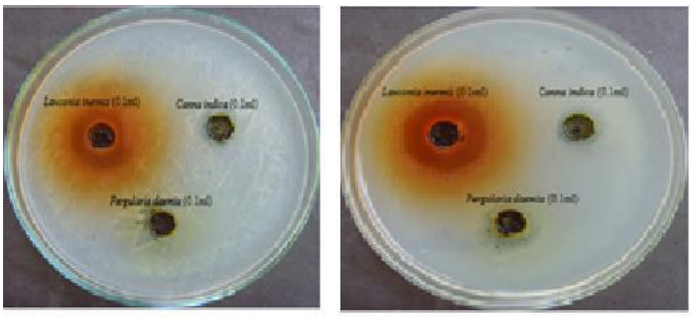


Figure 3: Antibacterial activity of aqueous extract by plate method



Staphylococcus aureus ***Klebsiella* sp.,**

Figure 4: Antibacterial activity of acetone extract by plate method

The antibacterial activity of *Pergularia Daemia* water extract against selected pathogens. The highest efficacy of water extract against *Bacillus* sp, at 16.3 ± 0.42 mm of zones and other species produced less than 10 mm. The acetone extract of *P. daemia* produced maximum efficiency against *E. coli* at 14.15 ± 0.21 mm of zones, and the other species of *S. aureus, Proteus, Bacillus* and *Klebsiella* produced maximum 9.20 ± 0.28. This is in accordance with an earlier investigation of [24] which showed the *P. daemia* acetone extracts showed higher activity 21 mm of zone of inhibition for *S. aureus* sp and aqueous extracts in low activity such as 14 mm zone of inhibition. The antibacterial activity was observed in ethyl acetate extracts and ethanol extracts of *P. daemia* against *S. aureus, P. aeruginosa, A. hydrophila, E. coli and S. typhi* [25]. Similarly, [26] reported that the ethanol extract of *P. daemia* exhibited antibacterial activity of *P. daemia* leaf extract was tested against *B. subtilis, S. aureus, E. coli* and *P. vulgaris.* Their experiment was determined by disc diffusion method and their results showed that ethyl acetate extract of *Pergularia*

daemia was found to be effective. It was also isolated a new bioactive compound, 6-(4,7-dihydroxy-heptyl) quinine, a novel agent which is proved to be responsible for antibacterial activity [27]. Perusal of literature reveals the presence of certain bioactive substance in *P. daemia* such as glucoside, terpenoids, sterols, alpha-amyrin acetate, beta-amyrin, beta-sitosterol, lupeol acetate, hentriacontane, betaine and different polypeptides [28].

Results showed the antibacterial activity of water extract of *Canna indica*. The maximum inhibition zone was observed against *S. aureus* (32.3 ± 0.42 mm) and other species were produced 10 mm zone. Except *Staphylococcus* sp. (14.25 ± 0.35 mm) all the other bacterial species produced resistant to acetone extract of *C. indica.* [29] reported that the methanolic extract and water extract of *C. indica* leaves and flowers proved antibacterial activity against *B. subtilis.*

To prove the synthesis of Silver nanoparticles after 20 min incubation, a peak specific for the synthesized silver nanoparticles (1 mM) at 420 nm for *P. daemia*, 400 nm for *C. indica* and *L. inermis.* It was confirmed primarily by the change in color after mixing leaf extract and 1 mM of silver nanoparticles and kept in boiling water bath. The color changes from dark green to shades brown color. The peak value of the silver nanoparticles was obtained at 454.5 nm for *C. indica* and 446 nm for *L. inermis*.

The antibacterial activity of silver nanoparticles of *C. indica, P. daemia* and *L. inermis*. The maximum zone of inhibition of *C. indica* and *P. daemia* was observed at 20.45 ± 0.62 mm and 19.20 ± 0.28 respective for *Klebsiella* sp. and the minimum zone of inhibition of *C. indica* was observed at 7.30 ± 0.42 mm for *Proteus* sp. and *P. daemia* produced zones at 7.10 ± 0.14 mm for *S. aureus*. *L. inermis* silver nanoparticles have no ability to kill the pathogens (Table 2), it was observed less than 10 mm of zone (Figures 5-7).

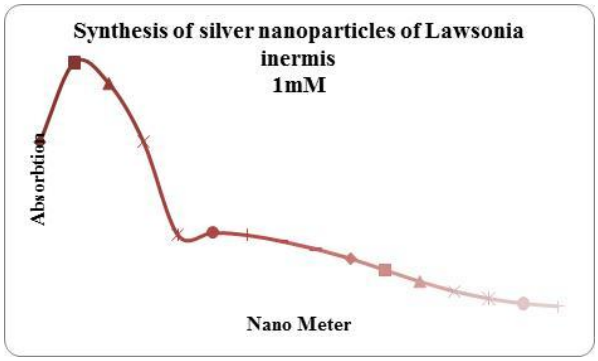


Figure 5: Synthesis of silver nanoparticles using *Lawsonia inermis* under UV-visible spectroscopy at the range of 300-600 nm

Figure 6: Synthesis of silver nanoparticles using *Pergularia daemia* under UV-visible spectroscopy at the range of 300-600 nm

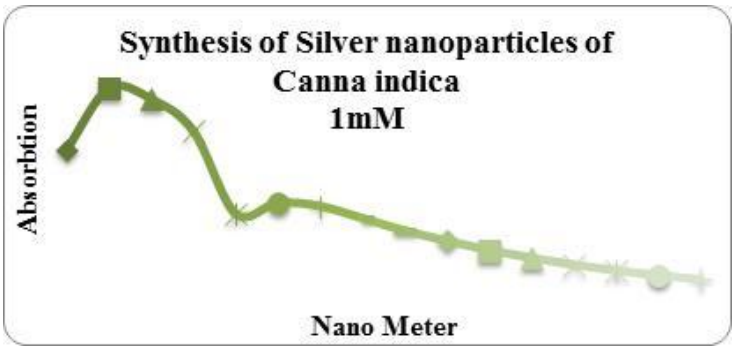


Figure 7: Synthesis of silver nanoparticles using *Canna indica* under UV-visible spectroscopy at the range of 300-600 nm

Table 2: Antibacterial activity of silver nanoparticles synthesized by *Lawsonia inermis, Pergularia daemia* and *Canna indica*

Isolates from wound pathogens	Zone of inhibition (mm)		
	Lawsonia inermis	***Pergularia daemia***	***Canna indica***
Bacillus sp,	8.35 ± 0.49	8.15 ± 0.21	9.40 ± 0.56
Proteus sp,	7.25 ± 0.35	9.05 ± 0.07	7.30 ± 0.42

S. aureus	9.30 ± 0.42	7.10 ± 0.14	14.35 ± 0.49
E. coli	8.20 ± 0.28	14.45 ± 0.63	15.25 ± 0. 35
Klebsiella sp,	7.40 ± 0.56	19.20 ± 0.28	20.45 ± 0.63

The above result explained the synergistic effect of silver nanoparticles synthesized by selected plants. Synergistic effect of *P. daemia* and *C. indica* silver nanoparticle produced highest zone at 19.25 ± 0.35 (*Proteus* sp). Compared to *L. inermis*+*P. daemia* AgNPs and *C. indica*+*L. inermis* AgNPs. Mixing of *L. inermis, P. daemia* and *C. indica* produced maximum zone at 15.05 ± 0.07 (*Klebsiella* sp) (Figures 8 and 9).

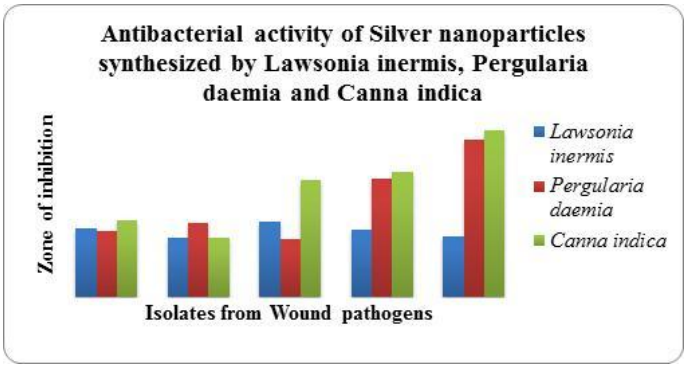


Figure 8: Antibacterial activity of silver nanoparticles synthesized by *Lawsonia inermis, Pergularia daemia* and *Canna indica*

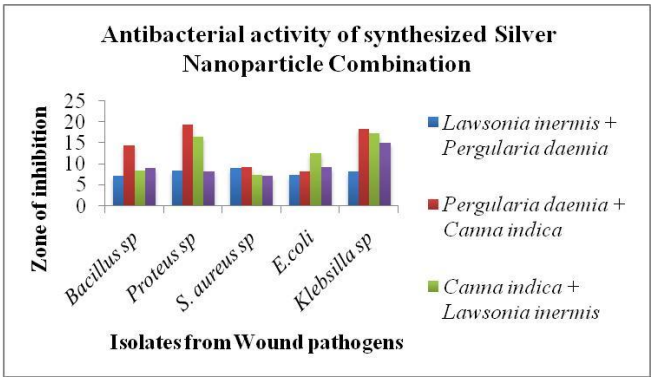


Figure 9: Antibacterial activity of synthesized silver nanoparticle combination

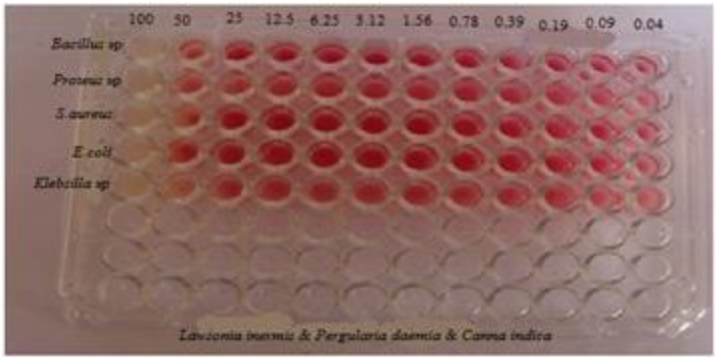


Figure 10: Synergistic effect of MIC using synthesized silver nanoparticles combination with *Lawsonia inermis, Pergularia daemia, Canna indica* by plate method

Result showed the MIC results for synergistic AgNPs of *P. daemia, C. indica* and *L. inermis*. The Minimum bactericidal concentration was obtained at 50 µl concentration of AgNPs solution. The antibacterial activity exhibited by silver nano particles depends on silver nitrate concentration. It is inversely proportional to the concentration of the activity and vice versa. This is due to the smaller particles have larger surface area available for interaction and will give more bactericidal effect than the larger particles [30]. Nano particles exhibit new or improved properties based on specific characteristics such a size, distribution and morphology. The cell membrane of microorganisms is negatively charged and silver nano particles are positively charged and positively charged silver nano particles accumulate on negatively charged cell membrane which leads to loses permeability and cell death [31]. Synergistic effect of MIC using synthesized silver nanoparticles in combination with *L. inermis, P. daemia* and *C. indica* by plate method (Figure 10). According to [32,33] metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by progression of ribosomal subunits proteins as well as some other cellular proteins and enzymes essential to ATP production becomes inactivated.

The other mechanism proposed by the formation of free radicals which subsequently induces membrane damage leading to efficient antimicrobial, interaction with biological macromolecules such as enzymes and DNA. Their interaction may cause damage to DNA and proteins resulting in cell death. Ag+ binds to functional groups of proteins, resulting in proteins denaturation [34,35].

The silver nano-particles however a efficient antimicrobial property due to their extremely large surface area, which provides better contact with microorganisms. It is reasonable to state that the binding of the nano particles to the bacteria depends on the interaction of the surface area available. Smaller particles having a larger surface area available for interaction will have a stronger bactericidal effect than larger particles [36].

CONCLUSION

The isolation and identification of five different strains from wound sample, and also collected *P. daemia, C. indica* and *L. inermis* leaves for searching of bio-active compound. Acetone extract of *L. inermis* produced highest zone of inhibition at 25.2 ± 0.28, *P. daemia* and *C. indica* showed maximum zone of inhibition at 14.15 ± 0.21 and 14.25 ± 0.35 respectively. The water extract of *L. inermis* produced highest zone of inhibition at 23.25 ± 0.35, *Canna indica* showed maximum zone of inhibition at 32.3 ± 0.42 and *P. daemia* produced maximum zone of inhibition at 16.30 ± 0.42. Silver Nanoparticles were synthesized at 1 mM and characterized by UV-Visible spectrophotometer. The *L. inermis* showed the peak absorbance around 400 nm. *P. daemia* at 420 nm and *C. indica* at 400 nm.

The antibacterial activity of *C. indica*, *P. daemia* silver nanoparticles demonstrated highest zone of inhibition against *Klebsiella* sp. (20.45 ± 0.63), (19.20 ± 0.28) respectively. *L. inermis* produced maximum zone of inhibition at 9.30 ± 0.42 against *S. aureus*. Antibacterial activity of synthesized silver nanoparticle combination showed good results against selected all pathogens. The plants considered have found use in the field of nanotechnology. These findings could be useful in searching new clinically important antibacterial activity and helpful in the treatment of wound and in replacing synthetic drugs as well. The synthesis is cheaper and faster than the conventional treatment.

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