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Antibacterial Activity of *Thespesia populnea* Mediated Nanoparticles

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A B S T R A C T

Many medicinal plants have been used for centuries in daily life to treat microbial diseases all over the world. In this study, the *in vitro* antibacterial activity of *Thespesia populnea* methanolic extract (TPE), *Thespesia populnea* methanolic extract mediated nanosilver particles (TPNS) and *Thespesia populnea* methanolic extract mediated nano zinc oxide particles (TPNZ) were investigated. The results of *in vitro* antibacterial studies indicated highest antibacterial activity for TPNS as evidenced by lowest MIC value (10.62 µg/ml) when compared to TPNZ (25 µg/ml) and TPE (125 µg/ml) against *Staphylococcus aureus* isolated from milk sample of clinical bovine mastitis case.

Introduction

Nanotechnology is one of the most fascinating research areas in modern material science. Nanoparticles are gaining importance in the fields of biology, medicine and electronics owing to their unique physical and biological properties (Morones *et al.*, 2005). Recent studies are focused towards synthesis of nanoparticles using plant materials like, iron, copper, calcium, gold, palladium, zinc and silver. Silver and zinc oxide has been recognised of its importance in chemistry, physics and biology due to its unique properties. Hence, among the metal nanoparticles, silver nanoparticles (AgNPs) and Zinc oxide nanoparticles (ZnONPs) synthesised from medicinal plants have received much attention for their various biological properties such as anthelmintic (Seema and Amrish 2012), antilarvicidic (Sundaravadivelan *et al.*, 2013), antioxidant (Kumara *et al.*, 2014), anticancer (Vasanth *et al.*, 2014), anti-inflammatory (Rafie and

Hamed 2014), hepatoprotective (Bhuvaneswari *et al.,* 2014), wound healing (Seema *et al.,* 2014) and antimicrobial (Marutikesava *et al.,* 2014). Nano silver and nano ZnO particles are proven to have antibacterial (John, 2016; Aparna *et al.,* 2018 and Panaceck *et al.,* 2006) activity against a wide range of gram positive and gram negative organisms including multidrug resistant bacteria. In recent times, herbal mediated synthesis of nano particles compared to other methods of synthesis is drawing much attention due to its less toxicity, low cost, eco-friendly nature, controlled particle size and stability (Li *et al.,* 2011). *Thespesia populnea* commonly known as Indian tulip tree, is an evergreen tree that belongs to family, Malvaceae. The leaves of *T. populnea* contain a number of bioactive constituents like flavonoids, alkaloids, phenolic compounds, saponins and steroids (Sharma *et al.,* 2011). Further, it was reported to possess antibacterial (Krishnamoorthy *et al.,* 2014; Shekshavali and Hugar, 2012 and Archana *et al.,* 2010), anti-inflammatory (Ilavarasan*et al.,* 2011 and Vasudevan *et al.,* 2007) and antioxidant activities (Vadlapudi and Naidu, 2009; Raju *et al.,* 2003). Both the herbal extract and nano particles together has been proved beneficial in exerting bactericidal action, reducing damage to tissues, because of their ability to penetrate deep into the cells. Hence, in this study an attempt has been made to evaluate the antimicrobial activity of TPE, TPNS and TPNZ.

Materials and Methods

Collection and identification of plant material

The leaves of *Thespesia populnea* were collected from in and around Tirupati in Chittor district of Andhra Pradesh. The plant was identified and authenticated by a taxonomist in the Department of Botany, University College of Science and Arts, S.V University, Tirupati.

Preparation of methanolic leaf extract

Thespesia populnea methanolic leaf extract (TPE) was prepared by using cold maceration method. The leaves of *T. populnea* were shade dried and ground to a coarse powder. About 100 g of leaf powder was soaked in 500 ml of 95% methanol (v/v) for 72 h with intermittent mixing using a glass rod and then filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated by rotary evaporator and then air dried. Extract was weighed and the percentage yield was calculated with reference to the air-dried material.

Synthesis of TPE mediated nano silver particles

Thespesia populnea solution (2%) was prepared by dissolving *T. populnea* methanolic leaf extract in the distilled water. Silver nitrate solution (0.1M) was prepared and to 10 ml of 2 % TPE, 90 ml of 0.1 M silver nitrate solution was added at 95^0 c with vigorous stirring. Then change in colour of the solution was observed from pale yellow to brown which indicates the formation of TPE mediated silver nanoparticles. The prepared solution was cooled to room temperature and particles were allowed to settle for 24 h. The solution was then changed to a plastic container for further characterization.

Synthesis of TPE mediated nano ZnO (TPNZ) particles

Zinc acetate 0.25 g was dissolved in 50 ml of distilled water and 4 ml of TPE was added drop wise and the resulting mixture was stirred for 10 minutes using magnetic stirrer, Finally the PH of the solution was adjusted to12, using 2 M NaOH. A white crystalline

precipitate of zinc oxide was obtained, which is washed repeatedly with water, filtered and dried in an oven at 60^0C to obtain zinc oxide nanoparticles.

Evaluation of Antibacterial activity of TPE mediated nanoparticles

To evaluate the antibacterial activity of *Thespesia populnea* mediated nanoparticles, the Minimum Inhibitory Concentration was estimated using broth tube dilution method as described by Geert Huys (2002). The turbidity standard was prepared by addition of 99.5 ml of H_2SO_4 (0.18 mol/l) to 0.5 ml of $BaCl_2$, $2H_2O$ (1.175%)with constant stirring to maintain a suspension. The correct density of the turbidity standard was checked by measuring the absorbance using Spectrophotometer. The absorbance at 625 nm should be 0.08 to 0.10 for 0.5 McFarland standard.

Further, inoculum was prepared from *S. aureus* culture in nutrient broth followed by incubation at 37° C for 18 h and the count was standardized to 0.5 McFarland unit. This led to the formation of a suspension containing approximately 1 to 2 x 10^8CFU / ml of *S. aureus.*

Thespesia populnea extract was diluted to get a series of concentrations from 500 µg/ml to 31.25 µg/ml, TPNS from 42.5 µg/ml to 2.65 µg/ml and TPNZ from 100 µg/ml to 6.25 µg/ml. followed by addition of 5 ml of sterile nutrient broth to all the test tubes. Around 50 µl of standardized broth culture was added to all the tubes and were incubated for 18 h at 37° C. The end point was defined as the lowest concentration of the test compound at which there was no visible growth. The growth in the tubes was compared to that with positive and negative controls. Ceftriaxone was used as an antibiotic control. The lowest concentration of the test compound inhibiting the growth of the organisms is recorded as MIC.

Results and Discussion

The *in vitro* antibacterial activity of the test compounds was evaluated by tube dilution method against *S. aureus* isolated from bovine clinical mastitis case.

In the present study, MIC of *T. populnea* extract (TPE), TPNS and TPNZ was found to be 125 µg/ml, 10.62 µg/ml and 25 µg/ml respectively depicting higher anti-bacterial activity of TPNS followed by TPNZ when compared to TPE alone. SreeVani *et al.,* (2016), Chaitanya *et al.,* (2013) and Muralidhar *et al.,* (2017) also reported the higher MIC values of phytogenic AgNPs against *S. aureus* when compared to AgNPs or herbal extract alone. Krishnamoorthy *et al.,* (2014) observed MIC of 125 and 250 µg/ml against *S. aureus* MTCC 737 and 7443 strains with TPE. Das and Chakraborty *et al.,* (2018) reported that silver nanoparticles were more potent antibacterial agents because of their lower MIC value (3.56 µg/ml) with respect to MIC value of ZnO nano particles (400 µg/ml) against *S. aureus*. Jones *et al.,* (2008) reported MIC of nano ZnO as 80 µg/ml while Elumalai and Velmurugan (2015) reported MIC value of *Azadirachta indica* leaf extract mediated nano ZnO as 6.25 µg/ml against *S. aureus.*

A number of possible mechanisms for antibacterial actions of nano silver havebeen proposed like penetration into bacterial cell membrane by altering the permeability of cell membrane (Rai, 2009 and Lok *et al.,* 2006), release of silver ions from AgNPs which further interact and inhibit function of sulfur containing proteins present in the bacterial membranes, bacterial DNA (Matsumura *et al.,* 2003) and affecting the mitochondrial respiratory chain resulting in cellular death (Sondi and Salopek-Sondi, 2004) and also

free radical induced oxidative stress due to sustained release of Ag+ ions inside bacterial cells (Kim *et al.,* 2007). Antibacterial activity of nano ZnO is due to direct contact of nanoparticles with cell walls of bacteria causing disintegration of bacterial cell integrity (Adams *et al.,* 2006 and Zhang *et al.,* 2007) and production of ROS which causes harm to bacteria and damage the lipids, DNA and proteins (Hirota *et al.,* 2010; Kirkinezos, 2001 and Raghupathi *et al.,* 2011).

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