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Bioconjugated nano-bactericidal complex for potent activity against human and phytopathogens with concern of global drug resistant crisis

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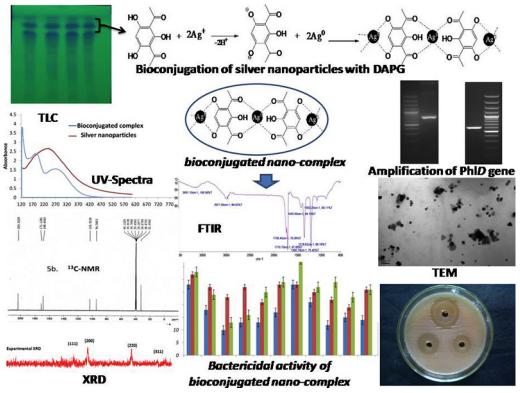
HIGHLIGHTS

- Novel strategies for antimicrobial resistance
- WHO: Antimicrobial resistant top priority
- Bioconjugated complex
- Human and phytopathogens

GRAPHICAL ABSTRACT



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ABSTRACT

The present study emphasizes the need for novel antimicrobial agents to combat the global drug resistant crisis. The development of novel nanomaterials is reported to be of the alternative tool to combat drug resistant pathogens. In present investigation, bioconjugated nano-complex was developed from secondary metabolite secreted from endosymbiont. The endosymbiont capable of secreting antimicrobial metabolite was subjected to fermentation and the culture supernatant was assessed for purification of antimicrobial metabolite *via* bio-assay guided fraction techniques such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and column chromatography. The metabolite was characterized as 2,4-Diacetylphloroglucinol (2,4 DAPG) which was used to develop bioconjugated nano-complex by treating with 1 mM silver nitrate under optimized conditions. The purified metabolite 2,4 DAPG reduced silver nitrate to form bioconjugated nano-complex to form association with silver nanoparticles. The oxidized form of DAPG consists of four hard ligands that can conjugate on to the surface of silver nanoparticles cluster. The bioconjugation was confirmed with UV–visible spectroscopy which displayed the shift and shoulder peak in the absorbance spectra. This biomolecular interaction was further determined by the Fourier-transform spectroscopy (FTIR) and nuclear magnetic resonance (NMR) analyses which displayed different signals ascertaining the molecular binding of 2,4,DAPG with silver nanoparticles. The transmission electron microscopy (TEM) analysis revealed the cluster formation due to bioconjugation. The XRD analysis revealed the crystalline nature of nano-complex with the characteristic peaks indexed to

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Bragg's reflection occurring at 2θ angle which indicated the (111), (200), (220) and (311) planes. The activity of bioconjugated nano-complex was tested against 12 significant human and phytopathogens. Among all the test pathogens, *Shigella flexneri* (MTCC 1457) was the most sensitive organisms with 38.33 ± 0.33 zone of inhibition. The results obtained in the present investigation attribute development of nano-complex as one of the effective tools against multi-drug resistant infections across the globe.

1. Introduction

Bioconjugation is an exciting scientific domains with innumerable potential to enhance the bioactive properties of the participating components (Huo, 2007). The bioconjugation of nanoparticles has gained tremendous interest owing to the multi-applicative properties such as biosensing, targeted drug delivery, bio-catalyst, bio-imaging and other value-added therapeutics (Petersen and Barcikowski, 2009). The bioconjugation chemistry between two moieties is based on different variants such as functional groups and physicochemical properties (Sperling and Parak, 2010). The complex molecule facilitates the desired application without altering the applicative properties. However, there are also possibilities of conjugating the secondary metabolites secreted from plants and microorganisms to address various health implications (Baker and Sreedharamurthy, 2012). One such area includes antimicrobial resistance, which has rapidly expanded recently with limited choice of available antibiotics (Syed et al., 2016b). The expansion of drug-resistant, pathogenic microorganisms has posed a severe threat to all live stock (Sosa, 2009). The development of safe antimicrobial agents is being considered to be one of the top priorities in scientific research, due in part to facts from the WHO. The antimicrobial resistance is categorized as an emerging global threat (Baker and Sreedharamurthy, 2012). Hence, in order to combat these pathogenic microorganisms, there has been extensive scientific research carried out by implementing different techniques. The bioconjugation of bioactive metabolites bearing antimicrobial potential with nanomaterials can open new scientific domains to develop potent antimicrobial agents which can facilitate multiple-modes of antimicrobial action with minimal adversity (Baker et al., 2017). The developed antimicrobial bioconjugates can offer greater advantages compared to conventional therapies used to control antimicrobial resistance (Rajchakit and Sarojini, 2017). For instance, it can target pathogenic microorganisms, control the release of drugs and target different sites such as DNA, cellular proteins, and enzymes (Baker et al., 2013). Based on these facts and consideration, the present study was designed and executed to develop bioconjugated nano-complex. The bioconjugation of nanoparticles was carried out with secondary metabolite 2,4 DAPG secreted from the endosymbiont. These endosymbionts are termed as endophytes, and are ubiquitous in all plant species (Alvin et al., 2014). Scientific studies highlight the potential roles of endosymbionts inhabiting host plants that are capable to mimic the host chemistry and secrete similar metabolites with profound activity (Golinska et al., 2015). Among the diverse class of endosymbionts, *Pseudomonas* is reported to be one of the dominant species (Ulrich et al., 2008). The association of plant and *Pseudomonas* suggest that during the course of interaction, *Pseudomonas* invade plant roots in the rhizosphere, occupy unique niches, and reside in plants to play important roles in maintaining plant health by secreting extracellular secondary metabolites (Gupta et al., 2013). The fluorescent *Pseudomonas* species are considered to be one of the merited microbial plethoras in secreting siderophores, phenazine-1-carboxamide, pyrrolnitrin, hydrogen cyanide, phloroglucinol and its derivatives (Shanmugaiah et al., 2010). The phloroglucinols are phenolic metabolites bearing broad-spectrum antimicrobial, antihelminthic and phytotoxic properties (Singh and Bharate, 2006). A survey of studies have reported antagonistic property of 2,4 DAPG against phytopathogens (Yang and Cao, 2011). In the present study, the bioconjugated complex was assessed against human and phytopathogens. The results were compared and validated with gentamicin as standard antibiotic. The obtained results are promising enough to open new avenues in the treatment of drug-resistant microbial pathogens.

2. Experimental procedures

2.1. Isolation of endosymbiont and screening for antimicrobial potential

Endosymbionts were isolated from healthy leaves and stems of *Annona squamosa* L. which have been reported in our previous publications. In brief, plant materials were rinsed with running tap water to remove adhering soil debris and processed for surface sterilization. The samples were immersed in 3.5% of sodium hypochlorite for 2 min followed by 70% of ethanol for 1 min, samples were washed three times with sterile distilled water. The samples were allowed to dry on sterilized filter paper and finally excised into 0.5 × 0.5 cm pieces using scalpel and placed on nutrient agar media. The plates were incubated at 37 °C and monitored daily for emergence of colonies from sterilized plant segments. The isolates were sub-cultured and assigned alpha-numeric code for screening antimicrobial activity *via* agar overlay assay which permits the rapid and quantitative selection of bioactive

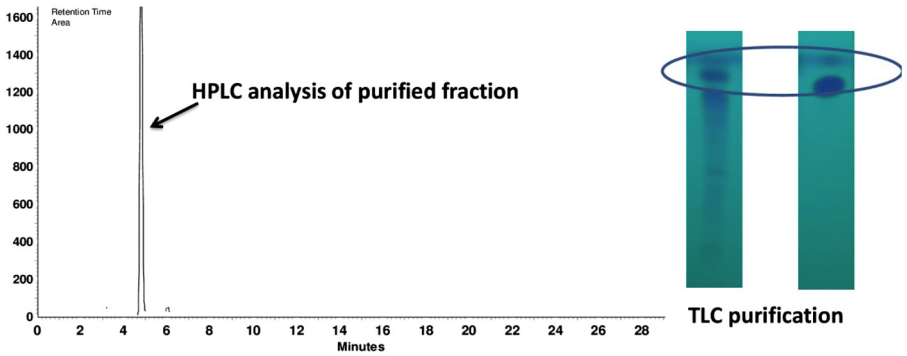

Fig. 1. a. HPLC purification of bioactive fraction b. TLC profile of crude extract and purified metabolite bearing activity.