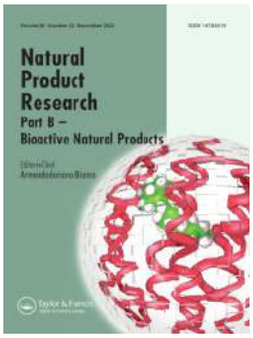

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SHORT COMMUNICATION

Naringenin biosynthesis and fabrication of naringenin mediated nano silver conjugate for antimicrobial potential

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ABSTRACT

The development of resistance, instability and high doses are some drawbacks of biologically active natural products. Modification of natural compounds to make it broad spectrum is the standard approach in drug design. This paper sets to modify the naringenin by silver nanoparticle conjugation to enhance its already reported pharmacological activities. The naringenin-nano silver conjugate was synthesized by one-step green synthesis, that is, sunlight exposure confirmed by UV spectroscopy. The bio-synthesized naringenin-nanosilver conjugate was tested for antia-canthamoebal and antimicrobial potential. The antibacterial potential was increased by 5.8–6.14 fold against Gram positive bacteria, that is, *S. aureus* and *Bacillus subtilis* and 4.5–16.5 fold against Gram negative bacteria, that is, *Escherichia coli* and *Pseudomonas aeruginosa*. The standard naringenin-nanosilver con-jugate significantly reduced the LC50 values against the Acanthamoeba cells, by, 66% and 36%, as compared to substrate naringin and standard naringenin respectively while biotrans-formed naringinin-nanosilver conjugate reduced LC50 by 50.56%, compared with biotransformed naringinin. Hence modification of natural product as nanoconjugate is the best practice for improvement as an effective drug.

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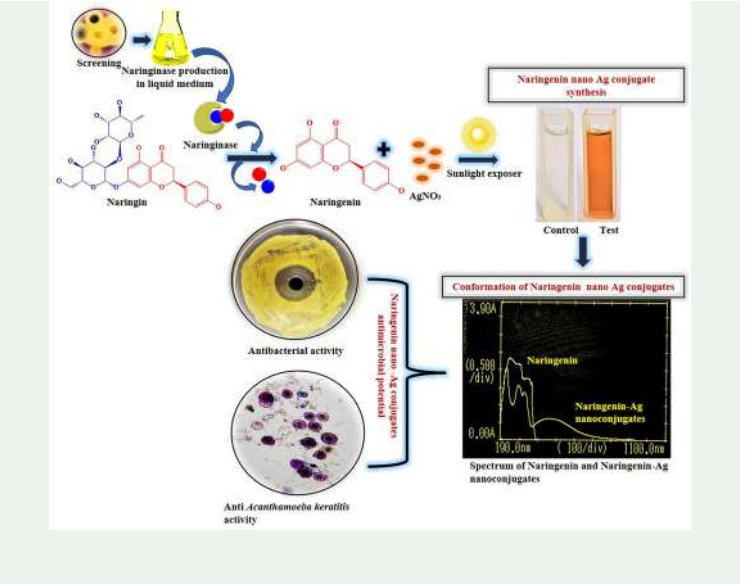

1. Introduction

Naringinase (EC 3.2. 1.40) hydrolyses naringin, the main bittering component in citrus fruit, into tasteless naringenin (Puri and Banerjee 2000). Bacteria, yeast, and fungi produce naringinase enzyme. Among these, fungi have been widely explored, and *Aspergillus niger* is reported as the best producer of naringinase (Kishi 1955). Naringenin (4′, 5, 7-trihydroxy flavanone) is a heterocyclic flavonoid produced by plants and fungus. It has two benzene rings linked with a heterocyclic pyrole ring (Tripoli et al. 2007). Naringenin has been reported for various pharmacological activities such as antioxidant, antibacterial and anticancer activities (Song et al. 2015; Salehi et al. 2019). Although naringenin possesses a notable antibacterial activity against different antibiotic resistant bacterial pathogens (Ng'uni et al. 2015), the success rate at clinical translation is held up due to their instability, easy oxidation, pH intolerance, poor solubility, high intestinal metabolism, and low bioavailability (Kumar and Abraham 2016). The improvement of naringenin has been attempted to avoid these limitations and for superior antimicrobial efficiency (Ji et al. 2016). Silver nanoparticles are currently much explored due to its versatile nature, low toxicity, biocompatibility. Though green biosynthesis with plants and microorganisms was reported but requires longer duration and controlled conditions.

In the present study, we proposed fungal naringinase mediated biotransformation of naringin into naringenin and further naringenin-nanosilver conjugate synthesis by green sunlight energy with enhanced antimicrobial potential against protozoa, bacteria and fungi.

2. Result and discussion

2.1. Naringenin biosynthesis

Naringin bio transforming fungus *Aspergillus oryzae* was screened by plate assay and further identified by 18S rRNA sequencing (Figure S1a). Among the microbial

producers of naringinase, fungi are widely reported. Among which *Aspergillus oryzae* has stated as a potential naringinase producer (Kishi 1955). Biosynthesized naringinase was applied for the biotransformation of standard naringin as a substrate. The extracted naringenin was further checked for nanoconjugate formulation with silver as a biotransformed derivative product of naringin.

2.2. Confirmation of naringenin biosynthesis

The biotransformed product, naringenin, was confirmed by thin layer chromatography (TLC) by comparing the Rf value with standard naringin and standard naringenin (Figure S11b). UV spectrum also confirmed the biotransformation of naringin (Figure S1c). The spectrum of flavonoids presents two wavelength ranges with distinct absorptions, a band II in the 240–310 nm range and a band I in the 300–400 nm range, referring to rings A and B, respectively (Farajtabar and Ghari 2013). The maximum absorption confirmed for naringin was 375 nm and for naringenin 310 nm (Habelt and Pittner 1983, Zhang et al. 2015). These absorptions are ascribed to the absorbance of ring A in its molecular structures (Harborne and Williams 2000; Pereira et al. 2007). These absorption peaks with high intensity and weak shoulder peaks can be attributed to 5, 7-dihydroxy-2, 3-dihydro-4*H*-chromen-4-one, and phenolic moiety present in the naringin structure (Yousuf and Enoch 2013).

2.3. Naringenin mediated nano silver conjugate fabrication

When naringenin solution was mixed with silver solution and exposed to sunlight, the colourless solutions turned transparent reddish-brown within 5 min exposure (Figure S2a). This primarily indicated the formation of naringenin-nano silver conjugate, further proved by UV- Visible spectrophotometric studies. The shift in the naringenin peak with an additional peak in the visible range at 420 nm (Figure S2b) was previously reported as nano silver (Anandalakshmi et al. 2016). Field emission scanning electron microscopy (FE-SEM) analysis revealed that naringenin mediated Ag nanoconjugate was predominantly nanorods with average particle size of 37 nm (Figure S2c). Nanorods naringenin Ag nanoconjugate indicated clear and uniform lattice fringes, which explained the crystalline nature of naringenin Ag nanoconjugate. The Zeta potential value of naringenin Ag nanoconjugate was found to be −5 mV. The negative Zeta potential value indicating the stability of naringenin Ag nanoconjugate (Figure S2d).

As various previous reports proved that the silver and gold nanoconjugates of natural products enhance the biological activity of the original compounds (Brahmkhatri et al. 2015; Chowdhuri et al. 2015; Muniyan et al. 2017; Shahbandeh et al. 2021), the synthesized conjugate was evaluated for its anti acanthamoebal and anti-microbial potential.

2.4. Possible mechanism of naringenin-nano silver conjugate formation

Naringenin structure revealed that 4-oxo substituent at the C ring contribute to the chelation of compounds such as heavy metals (Hernández-Aquino and Muriel 2018). Hence possibly naringenin-nano silver conjugate is formed by binding silver ions at the 4 keto

position of the C ring (Figure S4a). The replacement of silver at the 4 keto position has also been confirmed by sodium bisulphate reaction (Funiss et al. 1989) The coordination of metal ion at the A and C ring promoted the increase of some biological activities of free flavonoid (Pereira et al. 2007). The FT-IR spectrum of standard naringenin (Sigma-Aldrich N5893) and naringenin Ag nanoconjugate was evaluated (Figure S4b). The FT-IR spectrum of standard naringenin showed the characteristic signals at 1639.12 cm^{-1} (C = O stretching), 3056.75 cm^{-1} (O–H stretching); whereas the Naringenin Ag nanoconjugate indicated characteristic peaks at 2705.54 cm^{-1} (O–H stretching), 2624.67 (O–H stretching) 1630.30 cm^{-1} (C = O stretching). Changes to the stretching frequency of the phenolic O–H of naringenin from 3 056.75 to 2705.54 cm^{-1} in the Naringenin Ag nanoconjugate suggested the weak broad intermolecular interaction.

2.5. Anti-Acanthamoeba activity

Currently available multipurpose contact lens disinfection systems are not fully effective against *Acanthamoeba* trophozoites and cysts. There is an urgent need to increase the disinfecting activity of these systems to prevent *Acanthamoeba keratitis* infections. Niyyati et al. (2018) also advocated use of gold and silver nanoparticles as lens disinfectant to avoid Acanthamoebal infections.

In the present study, we have used naringin derivative product, naringenin, against the *Acanthamoeba keratitis* (Figure S3a). Naringenin has been reported to be more potent because the sugar moiety in the naringin causes steric hindrance of the scavenging group (Alam et al. 2014) and acts as a more active chelator of metallic ions (Cavia Saiz et al. 2010). It was observed that substrate, that is, naringin, has less anti Acanthamoebal activity than biotransformed and standard naringenin.

There was a significant decrease in LC50 value of standard naringenin-nanosilver conjugate that is 66% and 36% as compared to naringin (substrate) and standard naringenin respectively. While biotransformed naringenin nano-silver conjugate reduced LC50 by 50.56% compared with biotransformed naringenin (Table S1). Hype in anti-*Acanthamoebal* activity of naringenin-nano silver conjugate compared to basic compounds is similar to our previous reports (Borase et al. 2013), suggesting that sugar moiety removed from naringin increases its activity (Alam et al. 2014). Similarly, Padzik et al. (2018) reported that tannic acid silver conjugates (AgTANPs) enhance their Acanthamoebal potential due to high absorption. Aqeel et al. (2016) reported that the gold nanomaterial conjugate of biguanides, that is, chlorhexidine digluconate, and various therapeutic agents enhance the anti-*Acanthamoebal* potential. As silver nanoparticles previously reported for nontoxic effect in ocular drug preparations (Santoro et al. 2007) the reported conjugate will be useful as safe future ocular drug in future.,

2.6. Antimicrobial activity

Biosynthesized nanoparticles (NPs) have been recently studied and proposed as a new generation anti-microbial agents (Hendiger et al. 2020). The naringenin-nano silver conjugate involves a significant part of naringenin and least part of silver; hence safe

and less toxic. The silver nanoconjugate biosynthesis was carried out in the presence of sunlight energy which triggers the electron transfer to metal ions and causes photochemical reduction (Ravi et al. 2013). The less time required for formation without additional energy input makes this method more efficient. The naringenin-nano silver conjugate was more effective against the tested pathogenic microorganisms (Figure S3b). The tested naringenin showed limited activity against both Gram positive bacteria but the tested naringenin-nano silver conjugate showed maximum increased activity by 6.0 fold for Gram positive bacteria and 13 fold increases against the Gram negative bacteria (Table S2). Naringenin has antimicrobial activity, especially against Gram positive bacteria (Celiz et al. 2011), but nano silver conjugate also shows antimicrobial potential against Gram negative bacteria. Gakiya-Teruya et al. (2020) reported the enhanced antibacterial activity of silver nanoparticle-peptide conjugate than the peptide; it was observed that the silver peptide conjugate was found more effective against *Escherichia coli*. Similarly, the silver conjugates of Cephradine and Vildagliptin were found more effective against *Klebsiella pneumoniae*, *Escherichia coli* K1, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Bacillus cereus* (Masri et al. 2018; Halawani et al. 2020). The Ag-nisin conjugate is also currently reported for more effective inhibition of biofilm formations and enhancing small to broad spectrum, that is by inhibiting *S. aureus* and *E. coli*, than the nisin (Zimet et al. 2021).

The chances of resistance for this naringenin-nano silver conjugate might be less due to multiple modes of action. Silver nanoparticles (AgNPs) are given particular attention over other metal nanoparticles due to their versatility and unique characteristics like low toxicity, biocompatibility, biocidal activity and electrochemical properties.

3. Experimental

Experimental details relating to this paper are given in the supplementary material.

4. Conclusion

This study proved that naringenin-nano silver conjugate enhanced activity and lowered the dose by 50–60% against Acanthamoeba cells. Similarly, in the case of antibacterial activity, the naringenin has limited activity, but its silver nanoconjugate showed a significant increase in antibacterial potential by 1.3–1.9 fold and broad spectrum nature. Hence, in conclusion, the synthesized naringenin-nanosilver conjugate synthesized by the green way proved promising enhanced properties in antimicrobial and anti Acanthamoebal applications.

Disclosure statement

The authors declare no competing financial interest.

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