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Effect of Silver Nanoparticles Synthesised from Whey Protein in Combination with Hibiscus sabdariffa Flower Oil Extract on Bacterial Biofilm

Sekar Aashruthaa and Sathish Sankar*
Department of Microbiology, Centre for Infectious Diseases, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077
***Corresponding author**
Dr. Sathish Sankar,
Department of Microbiology, Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Science, Saveetha University,
Chennai-77, Tamil Nadu,India.
Email:sathishsankar.sdc@saveetha.com

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Abstract

Introduction Silver nanoparticles have unique properties that are of great interest in current research. They are good antiviral agents and photosensitisers. To further enhance our knowledge,silver nanoparticles were synthesised using the help of whey protein.Whey is a protein complex derived from milk. It has antioxidant properties and is a good chelating agent.*Hibiscus sabdariffa*, commonly called roselle,is a plant from the malvaceae family. It's added in food,herbal drinks and also used as a flavouring agent. Roselle has has excellent antibacterial,antioxidant properties and is also a good hepatoprotective agent.Few other studies have attempted the biosynthesis of silver nanoparticles using *Hibiscus sabdariffa* leaf extracts

Aim To assess the effect of silver nanoparticles synthesised from whey protein in combination with *Hibiscus sabdariffa* flower oil extract and antibiotics on biofilms.

Materials and methods Silver nanoparticles were synthesised using whey protein. Oil extract of *Hibiscus sabdariffa* was taken using the hydrodistillation method. Hydrodistillation is a conventional method which uses water or steam for the extraction of bioactive compounds, mostly essential oils. This technique is regularly performed via a setup recognized as the Clevenger apparatus or simple steam distillation. In the Clevenger apparatus, the hydrated sample is heated to vaporise volatile constituents. 2

layers (aqueous and oil-rich) are achieved and oil can be further separated by separating funnels.

Results The results suggested that the essential oil didn't have any significant anti microbial activity on its own. But on mixing with antibiotic(amoxicillin), it had a synergistic combination of inhibitory activity, which was higher than antibiotic treatment alone. Hence,it can be used for treatment in combination with antibiotics. Further studies can be done to check its efficacy on various other microbes.In vivo studies can be taken up to check the effects on human beings

Conclusion The silver nanoparticles in combination with *Hibiscus sabdariffa* flower oil extract+amoxicillin had good antimicrobial properties. Further studies can explore its potential for usage in human beings.

Key words: Silver nanoparticles, *Hibiscus sabdariffa*, hydrodistillation, whey protein, antimicrobial activity, Universal Health, Diseases, Well-being,Health, International Health policy

Introduction

Silver nanoparticles, also known as AgNPs, are nanoscale silver particles that normally have sizes between one and one hundred nanometers. Their distinct physical, chemical, and biological characteristics have attracted a lot of attention from a variety of industries, such as electronics, medicine, and catalysis. Silver nanoparticles' ability to operate as effective agents against bacteria, fungus, and viruses is one area in which they have demonstrated promise (1,2).

An intriguing application is the creation of silver nanoparticles utilising whey protein as a stabilising and reducing agent. Rich in amino acids, whey protein is a byproduct of making cheese and has been discovered to work well as a capping and reducing agent in the green synthesis of nanoparticles (3). Green synthesis is the employment of safe, non-hazardous chemicals in combination with ecologically friendly materials and circumstances.

Because of their high surface area and compact size, which allow them to interact with virus particles, the resultant silver nanoparticles, when stabilised by whey protein, may show increased antiviral effects(4).

Furthermore, when exposed to light, silver nanoparticles can function as photosensitizers and produce reactive oxygen species. Photodynamic therapy applications can benefit from this characteristic.

The temperature, reaction duration, and reactant concentrations are some of the synthesis parameters that might affect the characteristics of the produced silver nanoparticles. Green synthesis techniques, such as the one utilising whey protein, are becoming more and more well-liked because they don't use any potentially dangerous chemicals and are environmentally friendly (5).

Hibiscus sabdariffa, commonly known as roselle, is a plant that belongs to the Malvaceae family. It is widely cultivated in tropical and subtropical regions for its vibrant red calyces,

which are used in various culinary and medicinal applications(6).The fleshy, red sepals, the leaves, and sometimes the seeds are commonly used.Anthocyanins, flavonoids, and polyphenols are abundant in roselle, which adds to its antioxidant qualities(7)(8).Few studies have found that roselle extracts may have antibacterial qualities. This explains why it has been used traditionally in some cultures to treat infections.There is evidence to suggest that roselle may have hepatoprotective properties, meaning it could help protect the liver from damage (9).Few other studies have attempted the biosynthesis of silver nanoparticles using *Hibiscus sabdariffa* leaf extracts. This study has used flower petals for oil extraction.

Materials and Methods

Preparation of whey liquid

Cow milk was obtained from the local vendor in Chennai. The milk was heated and allowed to boil. The milk was coagulated by adding a few drops of lemon juice to it. The coagulated contents were removed by filtering, and the remaining solution was used for the nanoparticle preparation.

Formulation of silver nanoparticles

Silver nitrate solution (10 mM) was placed in a beaker covered with aluminium foil. The solution was heated for 15 min at 60 °C. Then 5 mL of the prepared whey liquid was added and the reaction was allowed to proceed for 1 h (4).

Characterization of silver nanoparticles

The preliminary confirmation of the formation of silver nanoparticles stabilised with whey protein (w-AgNPs) using a UV-visible spectrophotometer (JASCO 760D). The prepared w-AgNPs were examined using a field emission scanning electron microscope (JEOL, JSM-IT800) attached with an energy dispersive X-ray spectroscopy (EDX, Oxford) to determine the morphology and elemental composition.

Figure 1 Synthesis of nanoparticles using whey protein

Figure 2 AgNP formulation using whey protein

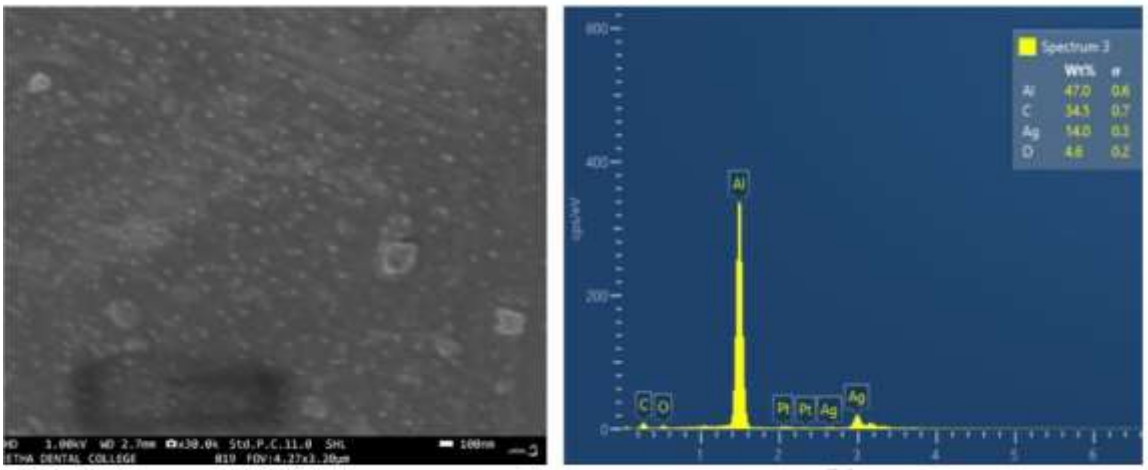

Figure 3 Characterisation assays of silver nanoparticles
Fig 3(a) SEM image of AgNPs
Fig 3(b) EDX results

Hydrodistillation of *Hibiscus sabdariffa*

Oil extract of *Hibiscus sabdariffa* was taken using hydrodistillation method.Hydrodistillation is a conventional method which uses water or steam for the extraction of bioactive compounds, mostly essential oils.This technique is regularly performed via a setup recognized as the Clevenger apparatus or simple steam distillation. In the Clevenger apparatus, the hydrated sample is heated to vaporise volatile constituents.2 layers (aqueous and oil-rich) are achieved and oil can be further separated by separating funnels (10).

Figure 3 Clevenger apparatus set up

The Clevenger apparatus consists of a round-bottom flask, a water-cooled condenser, and a collection tube. The plant material is typically placed in the round-bottom flask along with water. As the mixture is heated, the essential oil evaporates and carries through the condenser, where it condenses back into a liquid form. The condensed essential oil then accumulates in a graduated collection tube.

RESULTS

Figure 4 Oil extracted from *Hibiscus sabdariffa* flowers

In the Clevenger apparatus, the hydrated sample is heated to vaporise volatile constituents.The oil settled as a separate layer on top of water. It was separated and extracted by using a separating funnel.

Figure 5 Test for antimicrobial potential of the essential oil

The essential oil didn't have any significant anti microbial activity on its own. But on combining with antibiotic(amoxicillin), it showed synergistic combination inhibitory activity, which was higher than results of usual antibiotic treatment. A clear zone of inhibition was seen,indicating the efficacy of the treatment.

Discussion

Nanotechnology has witnessed a surge in interest due to the unique properties exhibited by nanoparticles. Silver nanoparticles, in particular, have attracted attention for their antimicrobial and catalytic properties. However, traditional methods of synthesis involve the use of toxic chemicals, raising environmental concerns. The green synthesis approach, utilising plant extracts, addresses these concerns by providing a sustainable and environmentally friendly alternative.(23)

Green synthesis of nanoparticles using plant extract bases provides a sustainable and environmentally friendly alternative to traditional methods (27). The use of plant extracts not only serves as a reducing agent but also imparts stability to the nanoparticles. This approach has the potential for applications in various fields, including medicine and catalysis, while minimising the environmental impact associated with nanoparticle synthesis (11)(24). Previous studies synthesised silver nanoparticles using various plant extracts and evaluated their properties. This study took on the novel idea of formulating the silver nanoparticles using whey protein. Whey protein was used in this study to ensure a clean approach to formulation of nanoparticles. Using plant extracts is the easier method but over exploitation of those may lead to extinction and endanger the plant species (1,12).

The synthesised silver nanoparticles were both stable and of a consistent size.Few other studies highlighted that silver nanoparticles that were green synthesised using *Hibiscus sabdariffa* had a spherical irregular shape and good size (4,11)(13).

The Clevenger apparatus is a simple yet effective device used for the extraction of essential oils from plant materials, especially aromatic plants. The apparatus is commonly employed in laboratories and industries involved in essential oil production, as well as in research settings.This apparatus is particularly suitable for the extraction of essential oils from plant materials that contain volatile compounds. The Clevenger apparatus allows for the separation of essential oils from non-volatile components, providing a concentrated and purified product (14).

The Clevenger apparatus allows for the extraction of essential oils using a gentle and non-destructive method. The steam distillation process helps prevent the degradation of delicate compounds present in the plant material.The extraction process in the Clevenger apparatus involves the use of steam, which minimises the exposure of the plant material to high temperatures. This is important for preserving the integrity of heat-sensitive compounds in the plant.The distillation process in the Clevenger apparatus typically results in a more pure extract compared to other extraction methods. This is because the essential oil is separated from other plant components during the distillation process. This device was used to optimise all the benefits for improved study outcomes (15).

Hibiscus sabdariffa is grown in tropical and subtropical regions around the world.It is cultivated for its culinary and medicinal uses, and the plant is relatively easy to grow.Hibiscus sabdariffa has been used in traditional medicine in various cultures for its potential health-promoting properties.In some regions, the plant is used to make traditional beverages, sauces, and syrups.It is a natural colouring agent and flavouring component (16).

Some research suggests that *Hibiscus sabdariffa* may have antiviral and anti hypertensive effects (17).The plant contains polyphenols that have been studied for their ability to inhibit the replication of certain viruses and was also reported to have anti diabetic activity(21)(22).There is also evidence to suggest that *Hibiscus sabdariffa* may have antiparasitic activity,anti inflammatory activity and antineoplastic activity (18,19)(20).This study explored the antimicrobial potential of silver nanoparticles synthesised using *Hibiscus sabdariffa* and results suggest that the oil extract did not have any effect on it own. In combination with amoxicillin, it showed synergistic activity. Further research can explore and evaluate the efficacy of this combination. Causing synergism between 2 materials enables us to reach higher infection control (18).

The zone of inhibition assay is widely used in clinical microbiology to determine the effectiveness of antibiotics against specific bacterial strains (26,27).The size of the zone of

inhibition can be measured and is indicative of the susceptibility of the microorganisms to the antimicrobial substance. Larger zones generally suggest greater susceptibility,as seen in this study.

Conclusion

The silver nanoparticles in combination with *Hibiscus sabdariffa* flower oil extract+amoxicillin had good antimicrobial properties. Further studies can explore its potential for usage in human beings.Further studies can be done to check its efficacy on various other microbes.In vivo studies can be taken up to check the effects on human beings

Conflict of Interests

The authors would like to declare no conflict of interest in the present study.

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