Fermented Rice Water for Biosynthesis of Silver Nanoparticles (AgNPs) and its Antimicrobial Activities against Microorganisms Associated with Skin Infection

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Abstract: As a skin treatment, rice water is becoming increasingly popular. It is said to aid in the treatment of a variety of skin ailments. Despite the fact that rice water has some genuine benefits, science has yet to fully validate many of its claims. The aim of this study is to biosynthesize nanoparticles using fermented rice water and to carry out the antimicrobial activity of the synthesized nanoparticles. To synthesize the silver nanoparticles, rice was subjected to a 48-hour fermentation process to obtain fermented rice water (FRW), which served as a bio-reductant and stabilizer for the nanoparticle synthesis and was used as the antimicrobial agents. UV-Visible spectroscopy was used to characterize the particles. The antimicrobial potential of FRW-AgNPs was assessed against common clinical bacterial and fungal isolates associated with skin diseases and infections (*Staphylococcus aureus*, *Candida albicans* and *Trichophyton rubrum*). The inhibitory effects of FRW-AgNPs were compared to those of FRW alone. The colloidal AgNPs were brownish in color and had a maximum absorption wavelength of 380nm, indicating that nanoparticles had formed. FRW-AgNPs demonstrated antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* strains, inhibiting their growth with inhibitory zones measuring 21.3 mm and 22.0 mm, respectively. In contrast, FRW displayed lower inhibitory effects, with inhibitory zones of 13.3 mm and 13.0 mm against the respective strains highlighting the enhanced antibacterial and antifungal activity of FRW-AgNPs compared to FRW alone. Additionally, FRW-AgNPs completely inhibited the growth of *Trichophyton rubrum* at a concentration of 100 µg/ml. This study successfully biosynthesized silver nanoparticles using fermented rice water and demonstrated their promising antimicrobial properties against clinically relevant fungal strains associated with skin disease and infection.

Key Words: Fermented Rice Water, Nanotechnology, Nanoparticle, Silver Nanoparicles, Green synthesis.

INTRODUCTION

Rice (Oryza sativa) is a staple food for nearly half of the world's population, providing nearly all of their daily calories in Asia. Rice water is commonly consumed after soaking or boiling rice, but rice water is generally discarded in many food preparation procedures around the world (Marto *et al.* 2018). According to Chakraborty (2022), rice water has been traditionally believed to enhance hair thickness and beauty, with its usage dating back over 1,000 years in Japan. Furthermore, rice water has gained increasing popularity as a skin treatment due to its reported benefits in addressing various skin issues, making it an attractive option as it is easily prepared at home and cost-effective (Chakraborty, 2022). Notably, rice water contains certain components that have demonstrated skin protective and reparative properties (Chakraborty, 2022). However, despite some acknowledged advantages, several claims regarding rice water's effects on hair and skin await comprehensive scientific validation (Marto *et al.*, 2018; Morse, 2019).

In recent years, the pursuit of novel bioactive compounds to combat skin aging has gained momentum, accompanied by a growing concern within the cosmetics industry for natural products, preferably sourced from organic farming (Marto *et al.,* 2018). Rice water, a natural ingredient, offers a promising solution in skincare formulations due to its inherent advantages of being cost-effective and user-friendly (Marto *et al.,* 2018). It can be derived from various types of rice commonly consumed in human diets, as well as from rice residues generated by the rice industry, thereby transforming it into a valuable resource with diverse applications (Marto *et al.,* 2018).

Fermented rice water, renowned for its sour flavor and transparent appearance, not only serves as a nutrient-rich elixir abundant in vital vitamins, minerals, and amino acids but also acts as a carrier of probiotic bacteria (Kumaran *et al.,* 2021). Within the fermentation process, lactic acid bacteria play a pivotal role by breaking down anti-nutritional components present in the rice, thereby enhancing the bioavailability of micronutrients and minerals such as iron, potassium, and calcium (Martins *et al.,* 2011; Thilagavathi *et al.,* 2019). Additionally, the phenolic compounds encompassed in fermented rice, encompassing derivatives like p-hydroxybenzoic acid, syringic acids, and hydroxycinnamic acid, further augment these beneficial activities (Martins *et al.,* 2011; Thilagavathi *et al.,* 2019).

Nanoparticles are known to have a wide range of applications in human endeavors. AgNPs have been used extensively as antimicrobial agents in biomedicine, particularly in the control of pathogens, due to their multiple routes of killing bacteria and fungi (Koduru *et al.*, 2018; Badmus *et al.*, 2020). In their actions, AgNPs resemble a Trojan horse, debilitating multidrug resistant strains through a combination of protein denaturation, cell wall destruction, generation of toxic reactive oxygen species, DNA damage, disruption of ribosome assembly, enzyme inactivation, and cellular leakage (Lateef *et al.*, 2016; Martin-Trasanco *et al.*, 2019; Adeyemi *et al.*, 2020; Pareek *et al.*, 2021). AgNPs have found wide application in the production of consumer products with unprecedented antimicrobial properties as a value-addition as a result of these activities.

Nanoparticles are obtainable via physical, chemical, and biological means. However, several physical and chemical modes of synthesis of nanoparticles are application of resultant particles in biomedicine. Physical, chemical, and biological methods can all be used to create nanoparticles. However, the resultant nanoparticles are used in biomedicine in a variety of physical and chemical modes of synthesis. As a result, green chemistry, also known as biological synthesis, has gotten unprecedented attention in the last two decades due to its favorable characteristics, which include eco friendliness, low production costs, simple synthesis, and biocompatibility (Malik *et al.,* 2017). Several biomolecules derived from plants, animals, and microbes have been optimized for the production of metal nanoparticles in this regard (Adelere & Lateef, 2016, Gahlawat & Choudhury, 2019; Adelere & Lateef, 2021; Elegbede & Lateef, 2021). A variety of plant wastes have been studied in order to successfully synthesize metal nanoparticles or use them as nanoparticle carriers in a variety of applications (Adelere & Lateef, 2016; Bashir *et al.*, 2021).

While several studies have investigated the properties of fermented rice water, their findings have primarily focused on its benefits in promoting plant growth and inhibiting the proliferation of hepatocellular carcinoma cells (Thilagavathi *et al.,* 2019; Nabayi *et al.,* 2021). In the field of dermatology, limited scientific research supports the claimed skin benefits of rice water touted by cosmetic companies. Consequently, the empirical use of rice water as a bath component has become a traditional practice, particularly among Asian women. Although a formulation incorporating starch-based nanocapsules and an anti-inflammatory agent showed promising anti-inflammatory activity in a mouse model of cutaneous inflammation (Marto *et al.*, 2018), further scientific exploration is necessary to establish the dermatological effects of rice water and its potential as a skincare ingredient.

The primary goal of this study was to create silver nanoparticles from fermented rice water and test their antimicrobial activity on clinical bacterial and fungal isolates associated with skin disease and infection (*Staphylococcus aureus, Candida albicans* and *Trichophyton rubrum*).

MATERIALS AND METHODS

Preparation of sample, biosynthesis and Characterization of AgNPs

Parboiled rice was gotten from Ipata market, Ilorin, Kwara State and transported to the laboratory. One hundred grams of rice was washed and soaked for two days in 200 mls of distilled water. The rice was then strained through a sieve, and the filtrate was stored in the refrigerator at 4 ℃. The content is known as fermented rice water (FRW). As previously described by Oladipo et al. (2017) the FRW was used to synthesize AgNPs. 1 ml of the FRW was added to a reaction vessel containing 40 ml of a 1 mM AgNO3 solution. The reaction was carried out at room temperature (30 ± 2 ℃) with visual color development monitoring for 24 h, followed by the absorbance spectrum measurement using UV–visible spectrophotometer (Specord® 200 Plus) conducted over a wavelength range of 250–700 nm.

Antimicrobial Activities of FRW and FRW-AgNPs

Clinical bacterial and fungal isolates associated with skin diseases and infections, including *Staphylococcus aureus*, *Candida albicans*, and *Trichophyton rubrum*, were obtained from the Microbiology Laboratory of the University of Ilorin Teaching Hospital. These isolates were collected from patients presenting with skin-related conditions and were previously identified and characterized. The obtained clinical isolates were maintained in culture to ensure their viability and purity throughout the study.

The antimicrobial activity of FRW and FRW-AgNPs against the clinical isolates *Staphylococcus aureus* and *Candida albicans* was assessed using the agar well diffusion method on Mueller Hinton agar plates. To create wells on the agar plates, a cork borer with a diameter of 7 mm was used. Subsequently, 100 µl of FRW-AgNPs and FRW, both prepared at a concentration of 100 µg/ml, were added to their respective wells. The experiment was performed in duplicate to ensure accuracy and reproducibility. Following incubation, the plates were examined for the presence of inhibitory zones, which indicate the effectiveness of the tested samples against the microbial isolates. The sizes of the inhibitory zones were measured and recorded as an indicator of the antimicrobial activity.

A mycelial growth inhibition test was used to determine the antifungal activity of the against *Trichophyton rubrum* (Elegbede *et al.*, 2018). The FRW and FRW-AgNPs were infused with potato dextrose (PDA) and then inoculated with 7 mm agar plugs of *Trichophyton rubrum* cultures 48 hours old. All of the plates were incubated at 28 ± 2 ℃ and observed after 72 and 168 hours. The growth inhibition was determined by measuring the radial diameter (D):

$$\frac{\text{Dcontrol} - \text{Dtest}}{\text{Dcontrol}} \times 100\%$$

RESULTS AND DISCUSSIONS

Biosynthesis and characterization of FRW-AgNPs

Fermented rice water was found to be effective in mediating AgNP synthesis. The color of the FRW-AgNPs colloidal solution varied from light brown at zero minutes to dark brown after 24 hours, as shown in Fig. 1. Several researchers have described similar color formation of biosynthesized AgNPs (Lateef *et al.,* 2015a; Lateef *et al.,* 2015b; Lateef *et al.,* 2016; Aguda and Lateef, 2021), which they attribute to the presence of macromolecules such as proteins, polysaccharides, and other organic compounds in the biomaterial used. Aguda and Lateef (2021), for example, synthesized dark brown AgNPs from *Parkia biglobosa* Waste Water. Lateef et al. (2016) also used cobweb extract to make dark-brown AgNPs.

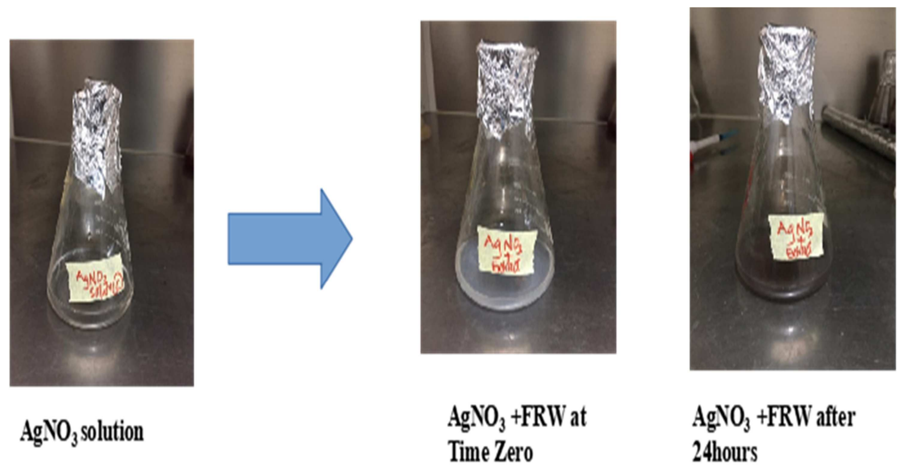

Fig 1: Progression of synthesis of FRW-$AgNO_3$

Analysis with UV–visible spectroscopy revealed that the colloidal FRW-AgNPs had maximum absorbance at wavelength of 380 nm as shown in Fig 2, indicating the presence of silver nanoparticles. Aslan et al. (2005) reported that the presence of silver nanoparticles can be indicated with an absorption spectrum ranging from 350 nm to 500 nm. The FRW, on the other hand, absorbed at 260 nm, which could be due to the presence of phenols in the fermented rice water (Thilagavathi *et al.*, 2019). The absorption of FRW-AgNPs is comparable to that of AgNPs previously reported by Aguda & Lateef, 2021; Hu *et al.*, 2019; Saravanakumar & Wang, 2018; Wang *et al.*, 2013.

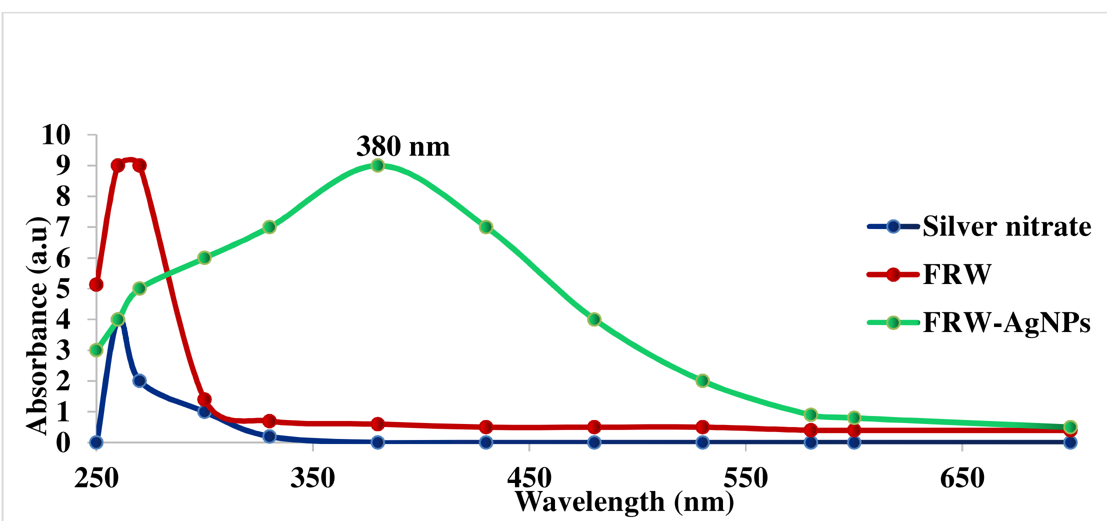

Fig. 2: UV–Vis absorption spectra of the Silver nitrate, FRW and biosynthesized FRW-AgNPs

Antimicrobial activities of the FRW and FRW-AgNPs

Plates 1 and 2 show the antimicrobial activity of the FRW and biosynthesized FRW-AgNPs against *S. aureus* and *C. albicans*, respectively. When compared to the FRW, FRW-AgNPs had significantly higher antimicrobial activity in terms of zone of inhibition against *S. aureus* and *C. albicans*. The maximum zone of inhibition of FRW-AgNPs was 21.3 mm and 22 mm against FRW which was 13.3 mm and 13 mm *S. aureus* and *C. albicans*, respectively. These results differ slightly from those reported by Hemlata et al., (2020) who reported maximum zone of inhibition at the highest concentration of Cp-AgNPs was 18 and 20 mm against *S. aureus* and *S. typhi* using disk diffusion method.

The antifungal activity against *Trichophyton rubrum* was evaluated by measuring the rate of radial mycelial growth inhibition. Control plates containing *T. rubrum* exhibited radial fungal growth, indicating the absence of any inhibitory effect. In contrast, the FRW-AgNPs demonstrated 100% antifungal potency against *T. rubrum* after 3 days of incubation (Figure 3), and this high level of inhibition was maintained even after 7 days. On the other hand, the FRW alone exhibited 100% antifungal potency against *T. rubrum* after 3 days (Figure 3), but the effectiveness decreased to 36% after 7 days (Table 2). These results indicate that the presence of silver nanoparticles, synthesized using the fermented rice water, significantly enhances the antifungal activity against *T. rubrum* compared to the use of fermented rice water alone. The sustained efficacy of FRW-AgNPs over the 7-day period suggests their potential as a long-lasting antifungal agent for the treatment of *T. rubrum* infections.

Thilagavathi et al. (2019) found that FRW-AgNPs can stop *S. aureus* from growing, which is similar to this study. Also, Rohaeti et al., (2016) described the synthesis of Bacterial cellulose (C) and its composites from rice waste water deposited silver nanoparticles, as well as their antimicrobial activity against *S. aureus*, *E. coli*, and the yeast *C. albicans*. AgNPs have previously been shown to inhibit *S. aureus* growth (Lateef *et al.,* 2016; Thilagavathi *et al.,* 2019; Aguda & Lateef, 2021; Gad El-Rab *et al.,* 2021), and this study adds to the growing list of such potent AgNPs. The large surface areas of small particles make them more likely to penetrate into bacterial cells, where they interact with enzymes, proteins, and nucleic acids to denature them and generate reactive oxygen species, resulting in cytocidal action (Aguda and Lateef, 2021). Because *C. albicans* and *T. rubrum* play a role in skin infection, the biosynthesized FRW-AgNPs are a valuable antifungal agent that can be used in biomedical applications.

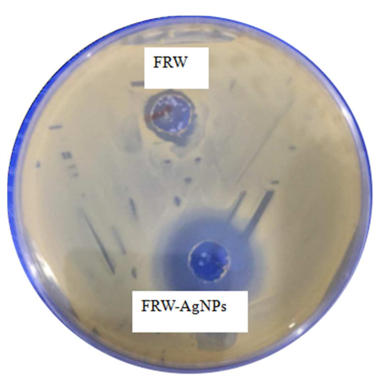

Plate 1: Antibacterial activity of FRW and FRW-AgNPs on *Staphylococcus aureus*

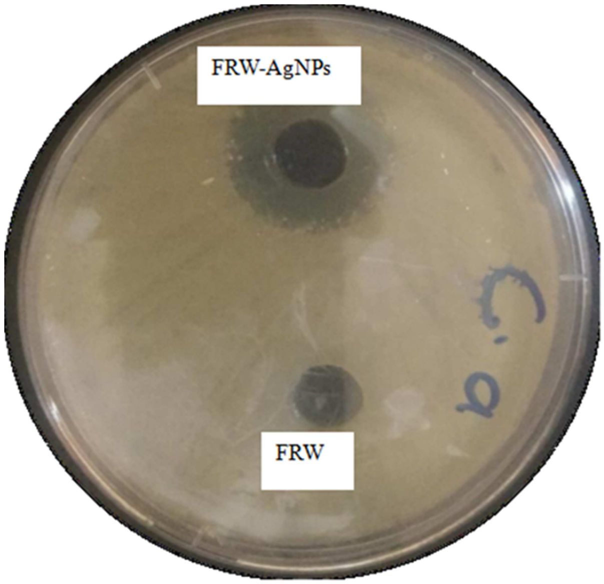

Plate 2: antifungal activities of FRW and FRW-AgNPs on *Candida albicans*.

Table 1: Zone of inhibition for *S. aureus* and *C. albicans*

Test samples	Zone of inhibition *Staphylococcus aureus* (mm)	Zone of inhibition *Candida albicans* (mm)
FRW-AgNPs	**21.3 ± 0.5**	**22 ± 0.5**
FRW	**13.3 ± 0.5**	**13 ± 0.5**

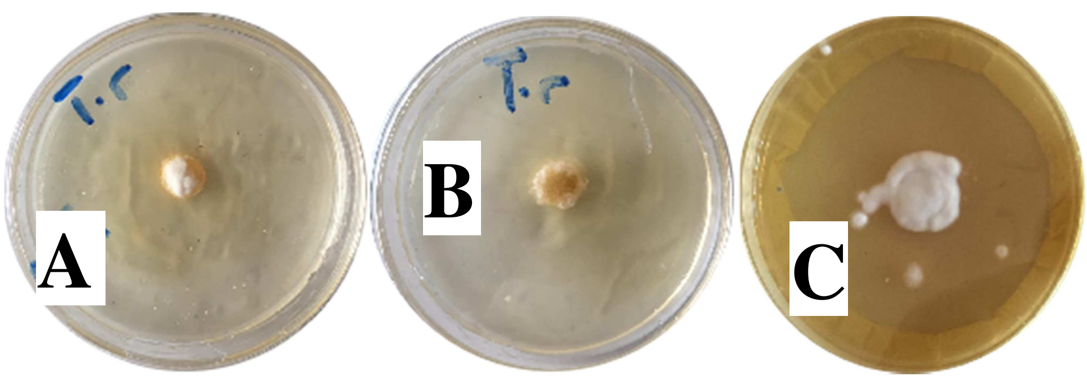

Fig 3: Antifungal activities of FRW and FRW-AgNPs on *Trichophyton rubrum* after 3 days A) FRW B) FRW-AgNPs C) Control.

Table 2: Radial growth measurement for *T. rubrum*

Test samples	Day 3	Day 7
FRW-AgNPs (%)	**100**	**100**
FRW (%)	**100**	**36**

CONCLUSION

This study demonstrates the significant antimicrobial potential of fermented rice water (FRW) and fermented rice water silver nanoparticles (FRW-AgNPs) against clinical isolates of *Staphylococcus aureus*, *Candida albicans*, and *Trichophyton rubrum*. FRW-AgNPs exhibit enhanced antimicrobial effects compared to FRW alone. Additionally, FRW-AgNPs display potent antifungal activity against *Trichophyton rubrum*. These findings highlight the potential of FRW and FRW-AgNPs as effective natural agents for skincare applications. Further research is needed to explore their mechanisms and optimize formulations for therapeutic use in dermatology.

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