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Original article

Nutritional assessment study and role of green silver nanoparticles in shelf-life of coconut endosperm to develop as functional food

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ABSTRACT

Tender coconut water is a pure and nutritious drink which play important role as nutraceuticals and pharmaceuticals contributes to the rapid growth of the functional food industry. In the mean-time the safety and shelf-life of the food is crucial for the both product as well as consumers. The intervention or application of nanotechnology gives immense a solution for the prolonged sustainability of the food products. This work reports on the nature of physiological changes of coconut liquid endosperm along with the interaction of its DNA with green route synthesized Ag nanoparticles (AgNPs) using *Garuga pinnata* leaf, an important ethnomedicinal plant. The physical and nutritional study of the coconut water were carried by UV–visible, XRD, NMR analysis whereas the synthesized Ag nanoparticles (AgNPs) were characterized by UV–Visible spectrophotometer, Raman Spectroscopy, DLS, AFM and FE-SEM analysis. The pH of the endosperm was found to decrease from 6.31 to 4.01, following an exponential decay trend and giving a decay constant of ~8.8 h. The broad absorption peak at ~310 nm gradually turns featureless with elapse of time. The proton nuclear magnetic resonance (H^1-NMR) spectrum essentially revealed the presence of esters or organic acids, confirming a sudden fall in the rate of intensity in the immature coconut endosperms as compared to the matured coconut cases. While the pentosyl methyl group (~1.4–1.5 ppm) concentration is observably lowered, free amino acid (~1 ppm) is apparently suppressed in the former specimen. Gel electrophoresis of 10 kb DNA with Ag nanoparticles (AgNPs) showed a gradual decrease of band intensity for a concentration varying between 3:1 and 1:1. The less intense band was due to the lack of migration of DNA into the micropores of the gel as a consequence of interaction of negatively charged DNA with negatively charged AgNPs. The study of DNA interaction with AgNPs could help identifying and addressing the nature of degradation process while considering prevention from microbial attack and make the coconut water as potential functional food entity.
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1. Introduction

Health and food quality have been the topics of intense research with realistic concern that appear in the way, such as degradation, preservation, and shelf-life enrichment. Amongst fruits, coconut occupies a special place in our day-to-day life as it offers not only ready-to-serve natural soft drink but also provides necessary elements for making several household and commercial items. Essential nutrients and natural minerals are the key components in the portion of liquid endosperm of the coconut, making the liquid part of the coconut a delicious and edible part of the coconut. The taste of the tender coconut water along with its white flesh is highly

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appreciated by the common people. In terms of quality and reliability; it is just the perfect gift of nature and thus cannot be compared with any of the man-made soft drink products in use. Bearing a net calorific value of ~0.17/g (Cutter and Wilson, 1954; Davies et al., 1995), the liquid portion of the coconut water forms the highly nutritious component of the coconut endosperm. The pH of the liquid endosperm lies in the range of 4.6–5.6. The coconut endosperm is believed to have formed through the nuclear mode of development (Wolter et al., 1991). The cellular endosperm is initially very lucid but later on it gets solidified upon complete development. In contrast to the endosperms of other plants (e.g., wheat and corn), the process of cellularization mechanism in the coconut fruit is unable to fill up the entire embryo sac cavity, leaving the cavity solution filled with nutrients of cytoplasmic origin (Knutzon et al., 1995). The inside portion of the endosperm is believed to be divided into two succulent portions *viz.* meat (fleshy stuff) and a fair mineral water, commonly known as coconut water (Abraham and Mathew, 1963). Due to the presence of high fat content, the pulp and the kernel are commonly called as the cash crop; whereas in the present situation, coconut is accepted far more than its oil seed capacity. The most selling part of coconut besides its edible region is the envelope, with the biological name mesocarp or husk. The husk portion is usually processed into marketable ropes, carpets and in cushion industries. The other part, called 'endocarp' is a huge repository of good quality activated charcoal.

The physiological assessment of liquid endosperm of coconut has begun since mid-forties. The same is consumed for its nutritious values and traditional beliefs (Giambelli, 1998; Gruca et al., 2014; Ohler, 1984; Satyanarayana et al., 1982). Besides its application in the intravenous hydration of patients in need in rural regions, it offers superb medical benefit e.g., in oral rehydration care owing to the presence of its high potassium content (Campbell-Falck et al., 2000). It also provides aid against dreaded diseases like myocardial infarction (Anurag and Rajamohan, 2003). Moreover, coconut water is used in plant tissue culture industry after being used successfully as a new component for callus cultures (Anurag and Rajamohan, 2003; Eeuwens, 1976). Studies have shown that, continuous consumption of fresh coconut water could help controlling hypertension and anxieties (Campbell-Falck et al., 2000). The coconut water has its cytokinin –type activity besides its nutritive functions(Hansen et al., 1984; Prades et al., 2012). It is worth mentioning here that, the cytokinin, such as; kinetin and trans-zeatin have potential to check anti-ageing, anti-carcinogenic as well as anti-thrombotic effects (Barciszewski et al., 2000; Mooi et al., 2015; Vishakh et al., 2014). The antioxidant systems of human body can be supported by rich amount of inorganic species, minerals and vitamins present in the coconut water (Loki and Rajamohan, 2003). In contrast, essential organic components commonly found are amino acids, nitrogenous compounds, lipids others(Campos et al., 1996; Matsui et al., 2007; Yong et al., 2009).

The preservation and commercialization of this nutritionally rich liquid could provide ample scope in the food and beverage industry at large. It has been observed that several nano-scaled materials like silver nanoparticles and gold nanoparticles have been reported to reduce and inhibit the microbial contamination (Mohanta et al., 2018, 2016; Mohanta and Behera, 2014), thereby increasing the shelf life of the food products. Though soft drinks with added coconut flavor are available in specific super markets of certain countries, the real coconut water loses its quality once extracted from the whole shell. Nanotechnology can be an asset to enhance the shelf-life of the product once we acquire complete information as regards interaction of its DNA with nano-inclusions.

The green route of AgNPs synthesis was carried by using *G. pinnata* (Burseraceae), is well known for its ethno-medicinal properties against gastro- intestinal diseases and cancer. Green synthesis of nanoparticles has gained much attention owing to its economic, eco-friendly and non-toxic preparation strategies. Nature has gifted us a huge plant diversity, which forms the source of potential traditional medicine as well as natural catalytic agents. *G. pinnata* is woody tree, found in deciduous forest of Similipal Biosphere Reserve, India. The leaf contains many active phytochemicals such as carbohydrates, flavonoids, saponins, steroids, triterpenoids and tannins (Ara et al., 2006). However, there are no reports on the production of AgNPs using *G. pinnata* leaf extract and this is first time report on the AgNPs synthesis using the water extract. The green synthesis is always beneficial and preferable over the chemical and physical synthesis to avoid the hazardous by-products and as current study is on the coconut water which is a cheap source in development of functional food reveals the positive implication of the green synthesized AgNPs for increase the safety and the shelf-life as well.

The present study is an attempt to understand the impact of physical degradation of tender coconut water on nutraceutical values as well as the interaction of its DNA extract with different sized green Ag nano-particles (AgNPs).

2. Experimental: Materials and methods

2.1. Sample collection and preparation

In the Bay of Bengal coastline, several tender coconuts in between the age groups of 6–10 months, were recognized and identified in the trees of and located at a distance of 30 kms away from Kolkata metro in the state of West Bengal. Based on the fruit's maturity, the fruits were collected and were thus grouped following careful examination as shown in Fig. 1. Using cold water, coconuts were washed and then subjected to further chlorinated conditions using 300 rpm of sodium hypochlorite for time duration of ~15 min in order to eliminate the microbial contamination if any on the fruit. The fruits were further washed for eliminating residual chlorine present in the sample and then subjected to air-drying (Rolle, 2007). Coconut water was then collected by cutting the nut with a sterilized knife. Contamination during this process was taken care of which is mostly of natural origin (Reddy et al., 2007). The entire coconut endosperm sample was utilized within 24 hrs. of collection and without special chemical processing. Procured from Merck Ltd., the chemicals used were of analytical grade and used without further purification.

2.2. Analytical measurements of coconut water

2.2.1. Filtration, pH and optical absorption measurement

The suspended particles of the two raw coconut water were removed using filtration means of Whatman paper of filter paper

Fig. 1. Image of ripened and fresh green coconut chosen for the experiment.

size 42. Different physical techniques were employed for the analyses of the filtered coconut water. Two different aged coconut were selected for the time dependent pH measurement. The coconuts were labeled as slightly tender (S_1) and tender coconut (S_2). The calculations were measured using digital pH meter at 90 min time interval first and then gradually the intervals of measuring the PH values were increased to 72 hrs. 3 ml glass cuvettes were used to evaluate the optical absorption in UV–Vis spectroscopy (Carry 100 spectrophotometer, Varian). In the two minutes interval up to 72 hrs. the measurements were taken.

2.2.2. Measurements using proton NMR

By employing a segregating funnel, the topical organic layer of the coconut extract has been separated from the liquid portion at the bottom. After collecting the organic fraction, the aqueous part was mixed with 20 ml of hexane and the process was repeated several times. The non-aqueous part was then scooped out and then transferred to a 300 ml oval shaped flask comprising of ~10 g of anhydrous sodium sulfate (Na_2SO_3), required for further drying. Resulting solution was further concentrated to a 2 ml in a spinning vacuum evaporator (EYELA, Model CCA-1110, Japan) set at a temperature of 40 °C. Samples of coconut water of amount 5 μl have been taken in the NMR tube and were further dissolved in 0.65 ml of $CDCl_3$ (deuterated chloroform, Chloroform-d). Using H^1-NMR (Jeol, 400 MHz) the selected samples were subjected to detect respective bands (in ppm) which employs trimethyl silane (TMS) as the basic reference for H^1 chemical shifts. Using the standard procedure, further the peaks were quantified and integrated for further examinations (Purkayastha et al., 2012).

2.2.3. X-ray diffraction measurements

The powder, extracted from the endosperm of sample *S2* has been considered before and after sonication (Ultrasonic Power Corporation, Freeport Illinois) and powder XRD (Bruker D8 Advance X-ray diffractometer using λ = 1.54 Å, CuK_α) has been used for structural analysis.

2.3. Synthesis and characterization of AgNPs using G. pinnata

2.3.1. Synthesis of silver nanoparticles

For the synthesis of AgNPs, the reaction mixture was made by mixing aqueous leaf extract of *G. pinnata* (1 ml) and 1 mM $AgNO_3$ (9 ml) in a clean Erlenmeyer flask and kept in constant stirring for overnight. The reaction parameters are maintained of temperature 25 °C and pH 6.7. As a control, same experimental set up was maintained without $AgNO_3$ solution. Post reaction time, the AgNPs were purified by continuous centrifugation (10,000 rpm; 30 min; 10 °C) using miliQ water by removing the other reaction by-products. The dried AgNPs were kept at 4 °C for further study.

2.3.2. Characterization of silver nanoparticles

The synthesis of the AgNPs (reduction of the Ag^+ ions by *G. pinnata* extract) was observed in UV–Vis spectrophotometer (Lambda 35^R PerkinElmer, USA) ranges of 400–600 nm at a resolution of 1 nm in RT. The average size and surface charge of AgNPs were analyzed by Zetasizer (ZS 90, Malvern, UK). For measurement of the intensity of synthesized silver nanoparticles, the as-prepared AgNPs were subjected to Raman spectroscopy using Lab Ram HR 800 Micro-Raman Spectroscope (Horiba Jobin –Yvon, France) with an excitation wavelength of 514 nm Ar+ ion laser (Dieringer et al., 2006). The nano-scale morphology of AgNPs were confirmed by field emission scanning electron microscope (Zeiss, Germany) performed at acceleration voltage of 15KV. The detail procedures of all these characterizations were done according to the previous published articles from our research (Mohanta et al., 2015, 2018, 2017). The surface morphology of silver nanoparticles was also examined using an Atomic Force Microscope (AFM) and the as-prepared AgNPs solution was drop wise casted over the silicon wafer followed by drying and were subjected to AFM studies using the AFM device (Bruker AXS Pte Ltd., Innova) (Nayak et al., 2015; Nayak et al., 2016a).

2.4. DNA extraction, isolation and treatment with Ag nanoparticles (AgNPs)

In order to increase the shelf-life of coconut water, we intended to interact DNA with Ag nanoparticles, latter being regarded as a powerful antibacterial agent. At first, DNA was extracted from the coconut water, followed by isolation and then, subjected to run in the gel electrophoresis unit (BioRad, USA). The DNA isolation was carried out by following a standard protocol with certain modifications(Angeles et al., 2005). It is worth mentioning here that, since the mature endosperm has a high content of lipids and galactomannan contaminants, young leaves were chosen for DNA isolation by using a higher salt concentration (2 M) in the extraction buffer and polyvinyl poly pyrrolidone. The extracted DNA is then allowed to interact with different concentrations of green synthesized silver nano-particles (AgNPs). The concentration of AgNPs was varied in the range of 200–1000 ng/ml. Using Gel Electrophoresis, DNA-Ag NPs conjugates, native coconut DNA and control AgNPs were run simultaneously in different lanes for nearly 30 min at a potential difference of 120 mV.

After 30 min. of gel-run, it was placed in the non-UV based Gel-Doc instrument (BioRad, USA) for taking the image of the bands for analyses.

3. Results and discussion

3.1. Physical degradation mechanism of tender coconut endosperm

The coconut water degradation was analyzed by several physical and chemical characterization techniques. The variation in the time dependent pH were studied over a period of 72 hrs. Reduction in the pH value from 6.31 to 4.03 has been observed. Apparently, the specimen under study becomes more acidic upon exposure to the ambient environment. In other words, H^+ concentration would increase with prolonged time. With the sudden fall in the pH value, the trend represents physical degradation feature owing to absorption of CO_2 to form carbonic acid. Coconut water degradation with time has been calculated using UV–Vis spectroscopy. Fig. 2(b) shows the time-dependent optical series of the tender coconut water. With increasing time, the intensity of absorption peak located at ~260 nm is found to get reduced. Secondly, the broad absorption peakat 310 nm gradually turns featureless with elapse of time. We speculate that, the functional groups present tend to degraderapidly the specimen being susceptible to ambient environment.

The chemical behavior of the coconut water has been elucidated using proton NMR analyses. In the ^{1}H NMR spectra, signature due to obtaining of different types of metabolites, such as, amino acids, organic acids (0–2.5 ppm), carbohydrates (3–5 ppm), aromatic compounds comprising phenyl propanoids and nucleotides (6.5–10 ppm) can be clearly witnessed (Lee et al., 2009). Earlier organic acids, sugar alcohols and free sugars were invariably shown to be prevalent in coconut water (Yong et al., 2009). As seen from Fig. 3(a & b), NMR spectra of tender (S_2) and S_1 (mature) is exhibited. At ~3.6 ppm value mark, the comparative plot of S_1 and S_2, the peak of sugar, alcohol exhibited a gradual decrease in the peak intensity. Also, could be noticed that the peaks corresponding to esters and organic acids at ~2.4–2.5 ppm, there is an observation of decrease in the peak intensity in case of the tender (S2) coconut

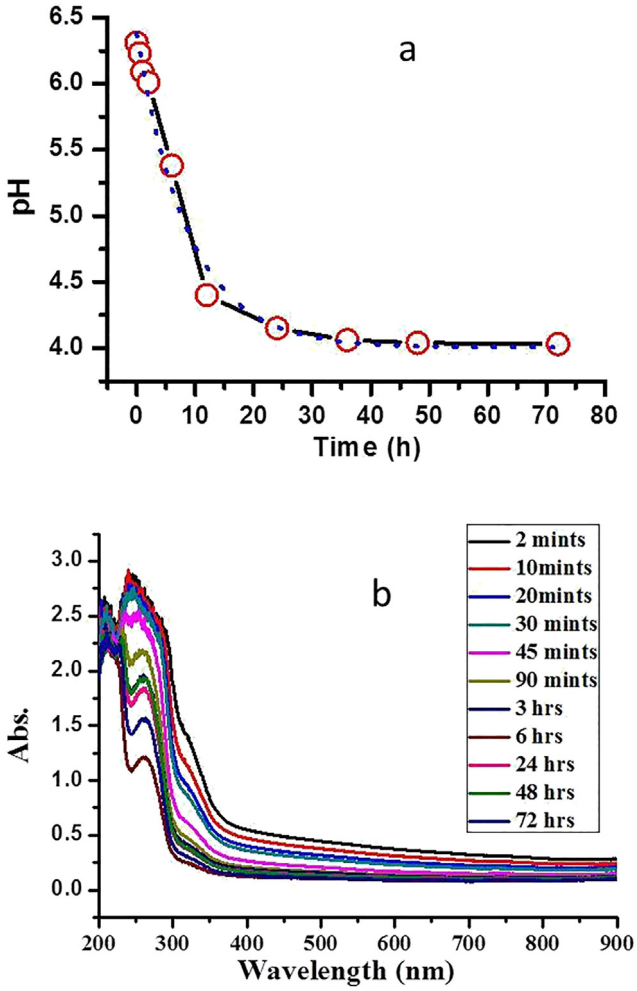

Fig. 2. (a) The change in pH of the coconut water with time, (b) UV–Visible spectra of coconut water at different time of interval.

as when compared with the mature coconut water (**S1**). It could be noticed that the concentration of the pentosyl methyl group (~1.4–1.5 ppm) seems to be minimized and the intensity of peaks for the free amino acids (~1 ppm), observed to be reduced in case of S2 than S1. Net decrease in the functional groups' positions in case of S2. characterizes under-developed endosperm product with freely growing amino acid content prior to maturation. Not surprisingly, the NMR signals due to free amino acids and organic acids shoot up with maturation and thus could attain maxima for a fully developed coconut endosperm.

3.2. X-ray powder diffraction (XRD) analysis of the endosperm

The tender coconut precipitate (*S2*) has been used for the X-ray diffraction measurements. Fig. 4(a) showed diffraction patterns acquired for unsonicated and sonicated samples. A number of weak diffraction peaks located at $2\theta \sim 20.6$, 25.6, 28.5, 40.5, 66.5, and 72.9° can be witnessed over abroad range diffraction angles. The small peaks can be assigned to diffraction signal which arise from the lattice planes of trace elements and minerals available within the sample under study. The diffraction peak40.5° is more intense for the sonicated sample than the unsonicated one. Fig. 4 (b) depicts a magnified view of the diffraction angle in the range 65–70° which high lights up-pression of 66.5° peak for the specimen processed after adequate sonication. The decrease in the peak intensity is attributed to the decline of degree of crystallinity that would have otherwise caused due to the presence of minerals and trace elements. Consequently, the sample becomes more amorphous as compared to the un-sonicated case. Intensity of amorphous nature in the samples is anticipated to be the result of net degradation in the bio-macromolecules existed in the coconut samples upon subjection to ultra-sonication treatment.

3.3. Synthesis and characterization of silver nanoparticles

The green synthesis of silver nanoparticles using *G. pinnata* leaf extract was confirmed through visual color change detection from pale yellow to deep brown color solution and the absorption peak in between 420 and 450 nm by UV–visible spectrophometer. The UV–vis spectrophotometer is the most convenient and simple method to preliminary study of the AgNPs synthesis by following the peaks at particular wavelength's absorption peak for the present AgNPs was found at 434 nm (Fig. 5) which is the characteristic peak for nano silver (reduction of Ag^+ ions to Ag^0). This particular characteristics peak is observed due to the surface plasmon resonance phenomenon where the polarized light hits silver ion at the interface of media or solution with different refractive indices by virtue of which the absorption peak is produced (Buszewski et al., 2017; Railean-Plugaru et al., 2016).

Dynamic light scattering (DLS) analysis was engaged to determine the particle size and surface zeta potential of the green synthesized AgNPs by measuring the arbitrary alternation in the intensity of light scattered from a colloidal suspension or solution. After the complete reduction of Ag^+ ions to Ag^0, the dispersed AgNPs exhibited Brownian motion that measured by fluctuations in the intensity of scattered light in the colloidal system. During the particle's movement, the translational diffusion co-efficient is calculated by employing the Stokes-Einstein equation gives the particle hydrodynamic size. In the present study, the polydisporsed AgNPs were formed and the diverse average particle sizes were found to be 84.12, 116.8 and 127.6 nm (Fig. 6A). Simultaneously the surface charges of the particles were found −25.6, −21.6 and −19.3 mV respectively (Fig. 6B). The size and charge of the particles highly impart during their different bioactivities as well as applications in other fields (Nayak et al., 2016b). The DLS gives a prominent confirmatory characteristic of a particle before its application in the cellular nanoplatform. As present investigation aims to study the role of AgNPs in the interaction with DNA, the size and charge matters a lot for the efficient AgNPs-DNA conjugation study.

Raman spectroscopy is usually performed for the measurement in the intensity of dis-orderedness and proportion of defect states in a particle or material (Dieringer et al., 2006). It could be seen from the Fig. 7 that the defect ratio (I_D/I_G) of the as-synthesized AgNPs exhibited the ratio value to be around 1.25 which indicates the defect proportions in the nanoparticles to be significant enough for the intrinsic disordered value (Paul et al., 2009). The proportions of the defect site in the as-synthesized AgNPs indicates that there are several gaps and voids available in the basic material structure, which is reflected in the marked I_D/I_G ratio. Such defect proportions in the nanoparticles signifies the rate of vacancies and the dangling bond existing in the nanomaterial. Vacancies of interstitial nature would result into the entrapment of oxygen molecules into their interplanar structure, which resulted into the materials slight oxidized nature.

The distinct surface morphology image of the green synthesized AgNPs by Field emission scanning electron microscopy (Fe-SEM) is depicted in Fig. 8. The average particle sizes along with the rough surface of the spherical AgNPs were clearly illuminated in the Fe-SEM images. It could be clearly seen from the Fig. 9, that the surface irregularity is evidenced from the 3D morphology from the AFM images. Such irregularity is attributed due to presence of several intermediate reductants and oxidants during green route

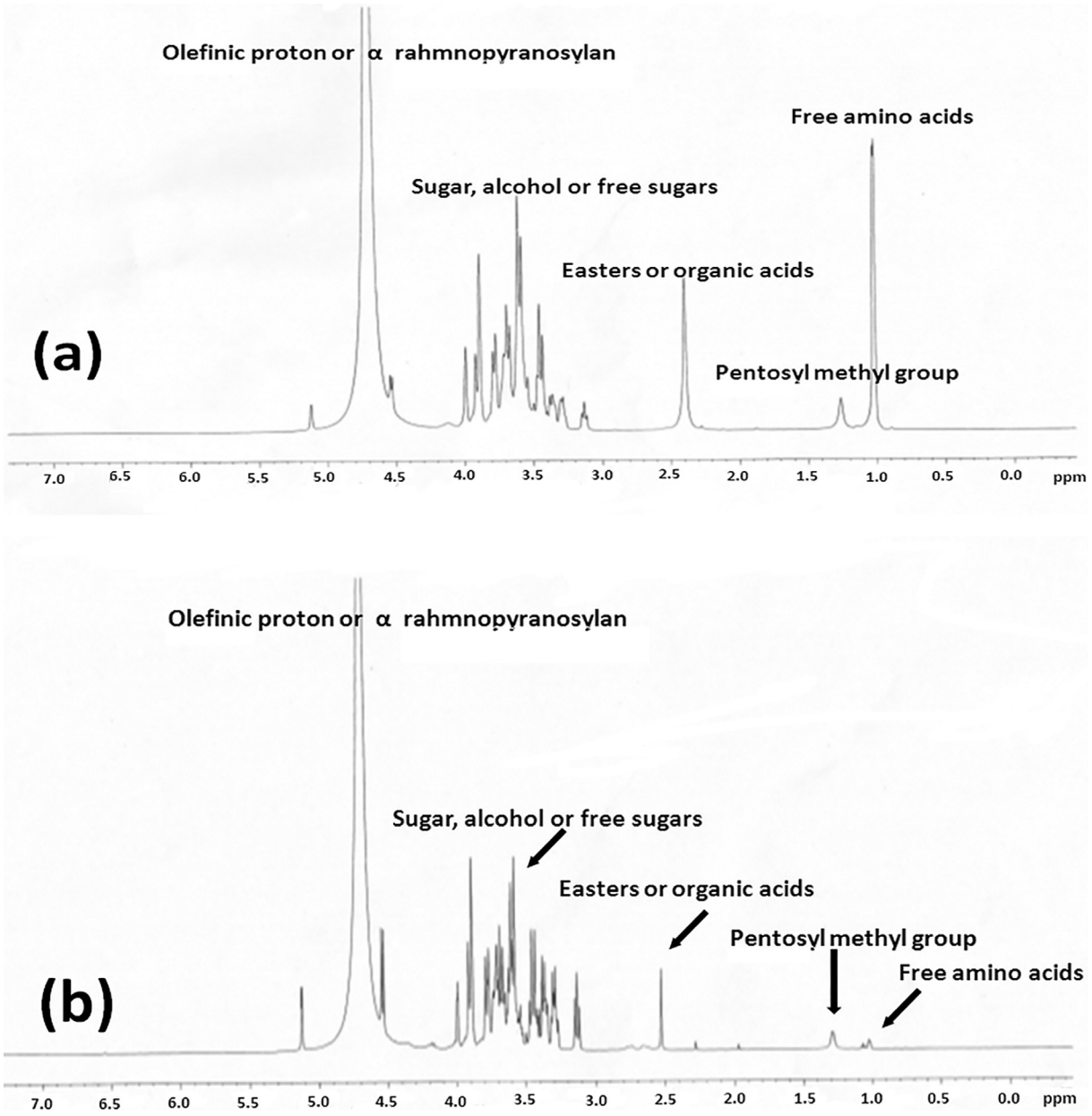

Fig. 3. Proton NMR of coconut water (a) *S1*, and (b) *S2*. Note the peak corresponding to sugar, alcohol or free sugars.

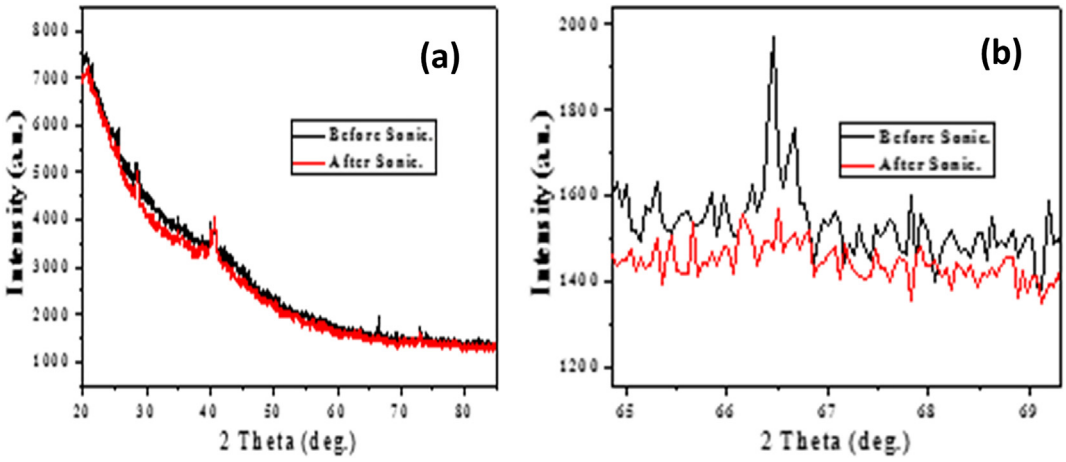

Fig. 4. (a) XRD pattern of precipitate of coconut water and (b) magnified view (a) in specific range of diffraction angles.

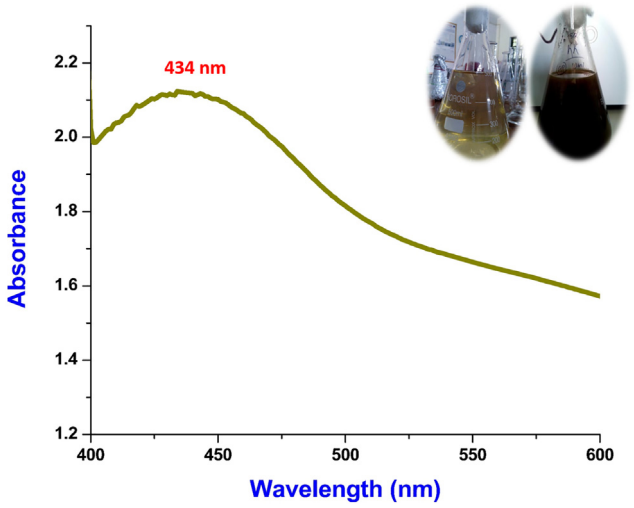

Fig. 5. UV–vis spectrophotometry analysis of AgNPs from *G. pinnata* leaf extract.

mediated synthesis of AgNPs, that plays a crucial role in determining the overall structural characteristic of the as-prepared nanoparticles (Khan et al., 2011). Such irregularity in the basic structure of the AgNPs resulted into the differential charge potentials distributed in the overall structure of the prepared material (Kent and Vikesland, 2012) as evident from the color bar index of the electrostatic potential. Due to the differential electrostatic potential (−2.2 V to +1.1 V) of the material, the nature of interactions with the biological entity like coconut DNA becomes a significant interplay for the overall charge transfer mechanism (Li et al., 2014) among the AgNP-DNA composite, resulting into an avenue in the food preservative applications.

3.4. Interaction of silver nanoparticles with the tender coconut extracted DNA

The implication of AgNPs over the isolated DNA extracted from tender coconut is analyzed by Gel electrophoresis (Bio Rad, USA). The DNA base-induced differential AgNPs aggregation has been quantified from surface plasmon resonance spectroscopy (SPRS)

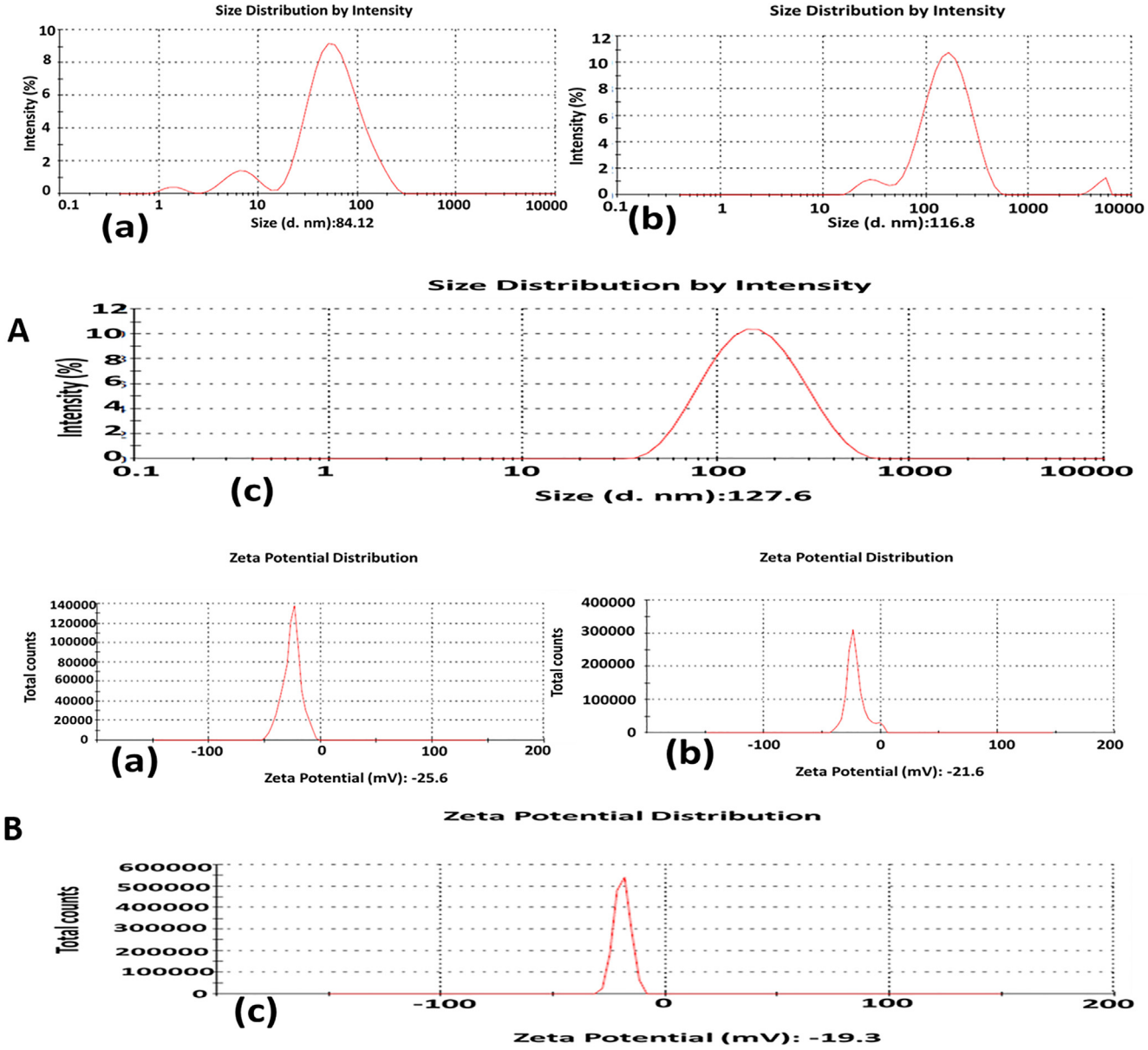

Fig. 6. (A) Size distribution and (B) Zeta potential of different sized AgNPs employed for interaction with DNA.

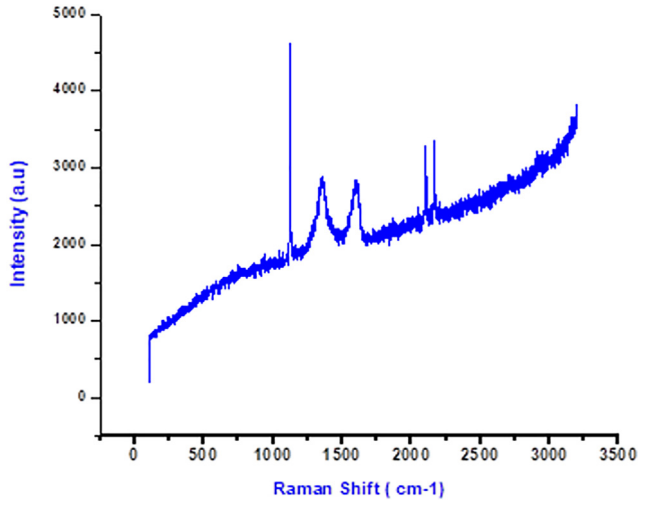

Fig. 7. Raman Spectroscopy study of *G. pinnata* mediated silver nanoparticles.

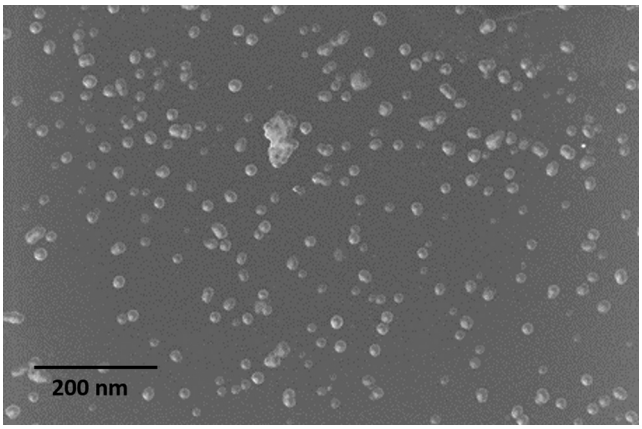

Fig. 8. Surface morphology of *G. pinnata* synthesized silver nanoparticle by scanning electron microscopy.

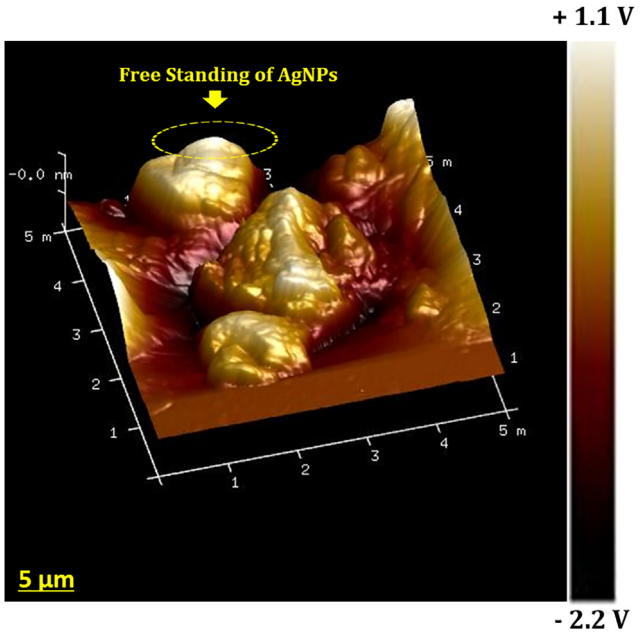

Fig. 9. Atomic Force Microscopy study of the synthesized silver nanoparticles from the leaf extract of *G. pinnata*.

in a previous work (Basu et al., 2008). There is rarely any report that discusses *in vitro* AgNPs-DNA interaction with DNA being derived from the tender product. Moreover, no study is available regarding effect on band intensity due to the AgNPs-DNA interaction with respect to concentration variation, size and surface charge of the nanoparticles. Herein, three different AgNPs of average size, ~84 nm, 116 nm and 127 nm (Fig. 6A, B) have been assessed after DNA conjugation. Due to the similarity in the molecular size of the coconut DNA, because of the variation in the age, geographical region etc. the size of the DNA doesn't gets altered and remains conserved in its attributes. Due to that reason, S2 (tender coconut) was chosen for the extraction of DNA. It is found out that the size of the double stranded DNA of the coconut is ~10 kbp (kilo base pair).

AgNPs of the range 200–1200 ng were co-incubated with high purity DNA (conc. 600/120 ng) of OD_{260}/OD_{280} ~ 1.8 for 30 min at the room temperature. The next step follows an electrophoretic analysis as shown in Fig. 6. It could be seen clearly from the figure of Gel electrophoresis, that adequate migration of DNA bearing the band size approximation ~1.8 kbp in the well, whereas upon treatment with the AgNPs the decrease in the band intensity at 18 kbp which is indicated by the decrease in the fluorescence intensity could be easily seen (lane 4–6). Owing to the constant amount of DNA concentration in all the wells in the well range from 4 to 6, the increase in the AgNPs creates a competition for the available DNA to be conjugated to the available AgNPs. Such underlying reason is the crucial factor for the decreasing in the fluorescence intensity while having a paradigm shift from DNA to AgNPs concentration mark. Also, a peculiar agglomeration in the migration of DNA into wells is noticed. The reason behind such observation lies in the fact that due to the binding of the negatively charged DNA with the negatively charged AgNPs, the movement of the composites gets effected as compared to the normal DNA migration. The adsorption of DNA and silver nanoparticles is more of the surface adsorption form. The fragmentation in the lanes of the electrophoresis is not been observed under the study. The intensity of the control is similar to that of the increased DNA and AgNP content bearing ratio 2: 1 and 1: 1 ratio, exhibiting similar position in the band. The trend of the decreasing in the fluorescence intensity of the NPs-DNA with the increase in the nanoparticular concentration has been previously shown by Kumar et al. (2011). The underlying cause of the conjugation was the overloading of DNA molecules by the nanoparticles. It could be clearly seen (Fig. 10a-c) from Lane 4 for each case, band intensity seems to be increased with the increase in the particle size of AgNPs. The decrease in the DNA band intensity could also be attributed to the fact that smaller particles bears higher zeta potentials of ~25.6 mV, due to which the rate of DNA expellation is higher in magnitude. On the other hand, because of the lower zeta value of ~19.3 mV, could experience a more binding sites for DNA binding indicating a brighter band under fluorescence light.

Since AgNPs are known for strong antimicrobial activity against major pathogenic microbes and the activity refers to the high level of toxicity of AgNPs to the microbial DNA altering the DNA regulation (McShan et al., 2014). It is understood that the main role of the vita biomacromolecules in the living entity is mainly due to the generation of Reactive Oxygen Species (ROS) molecules. Such peroxide radicals are the main player for the protein damage, denaturation of DNA etc. in the living system (Reidy et al., 2013). Although numerous studies have been addressed on nano-toxicity, the understanding aspect is still open to formulation of samples, size distribution, surface charge, concentration and several other factors. Considering all these major parameters, investigating the interaction of AgNPs with select molecules and definite cellular organelles or and stimulating signaling pathways along with obtaining secondary responses in different cells is quite cumber-

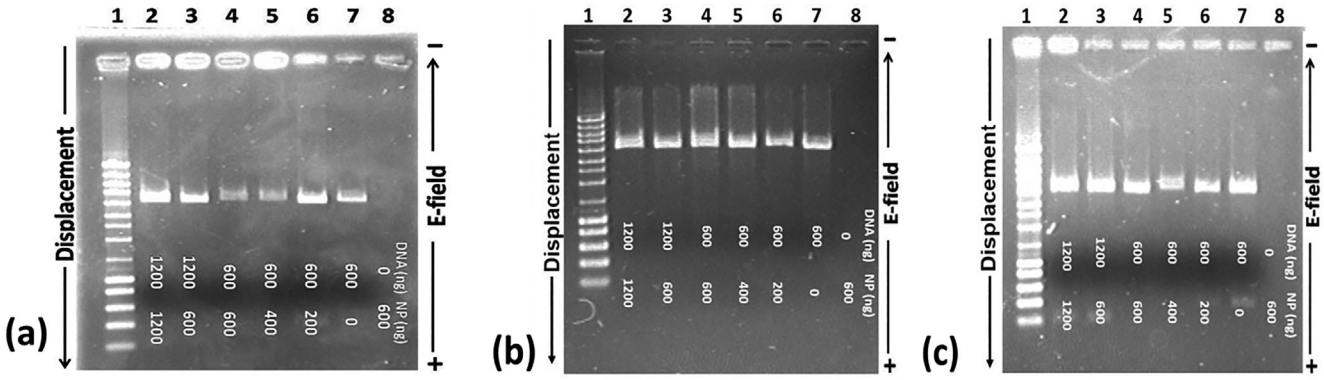

Fig. 10. Electrophoresis study of DNA and AgNPs-DNA conjugates. Lane 1: molecular weight marker; Lane 2–6: DNA-AgNPs conjugates (amount mentioned in the figure); Lane 7: only DNA (control); lane 8: only nanoparticles. Nanoparticles of size and Zeta potential used (a) 84.12 nm; −25.6 mV, (b) 116.8 nm; −21.6 mV, and (c) 127.6 nm; −19.3 mV.

some. But it is crucial to secure nanomaterials as a means of consumer products without any adverse side effects.

4. Conclusion

Coconut water is one of the universally appealing drinks for proper health and metabolism owing to the presence of plentiful naturally occurring bioactive enzymes, such as, simple sugars, amino acids, electrolytes, vitamins and others. But the major drawback of this drink is with regard to its shelf-life for commercialization as functional food item in packets and bottle. It has the chances of microbial contamination, as can be seen via observation of low pH value with elapsed time. Moreover, manifested optical absorption spectra can be revealed with aging. The NMR study of tender coconut water characterized the presence of pentosyl methyl group and frees amino acids apart from esters and phenols. Considering 1:1 concentration, the DNA interaction with AgNPs gave intense band for larger particles. Since DNA and AgNPs are negatively charged, their interaction may not necessarily be electrostatic but surface adsorption via chemical bonding. A reduced zeta potential of large sized AgNPs keep DNA on hold, resulting in bright band. Since AgNPs are known to be good anti-infectant, can be considered in beverages and packaging of coconut drinks. Further investigation, with more focused goal is needed to assess the exact AgNP-DNA interaction both in wet lab as well as *in silico* study. Hence AgNPs which are also earlier known to be good anti-infectant can be smoothly used in packaging of coconut drinks. Moreover, the future research will be carried out on the impact of implementation of the metal nanoparticles on the nutraceutical value of the tender coconut water is a major interest during development of coconut based functional foods. Here green nanotechnology will be more useful in the food industry.

Declaration of Competing Interest

Authors declares no any conflicts of interest related to the publication of the manuscript.

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