Journal of Animal & Plant Sciences, 35(1): 2025, Page: 49-57
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2025.1.0003

COMPARATIVE ANALYSIS OF *Adansonia digitata* NANOPARTICLE AND ENCAPSULATION: SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL, AND ANTICANCER ASSESSMENT

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ABSTRACT

This study aims to further explore the synthesis, characterization, encapsulation, and biomedical applications of *Adansonia digitata* Baobab nanoparticles. Using a nano-precipitation technique, Gum Arabic and Polyvinyl alcohol were added to the nanoparticles that had been synthesized using the sonochemical process. Transmission electron microscopy was used to determine the physico-chemical properties of the synthesized and encapsulated nanoparticles, providing information about their morphology. Fourier Transform Infrared (FTIR) spectroscopy was employed to examine the chemical functional groups present in the samples. The particle sizes of ADNPs and Cap-ADNPs were verified by dynamic light scattering (DLS) analysis. While encapsulated Cap-ADNPs had a greater average size of around 230 nm with a PDI of 0.311, the average particle size for ADNPs was approximately 94 nm with a PDI of 0.208. Tests were conducted on the antibacterial activity of ADNPs and Cap-ADNPs against a range of specific Gram-positive and Gram-negative bacteria as well as certain fungi. Additionally, the nanoparticles' cytotoxicity toward human colon cancer cells (HCT-116) and human breast cancer cells (MCF-7) was assessed. With an IC50 of 73.6 mg/ml, ADNPs showed modest inhibitory action against HCT-116 cells; in contrast, Cap-ADNPs had a significantly greater impact, with an IC50 of 34.1 mg/ml. With an IC50 of 18.3 mg/ml, Cap-ADNPs have shown exceptional potency against MCF7 cells, whereas ADNPs had moderate inhibitory effects, with an IC50 of 64.7 mg/ml. According to preliminary findings, ADNPs and Cap-ADNPs have a great deal of promise to be effective therapeutic options in upgraded forms for use in bio-nanomedicine.

Keywords: *Adansonia digitata* nanoparticles, nano-encapsulation, antimicrobial activity, cytotoxicity

Published first online December 20, 2024 Published final February 18, 2025

INTRODUCTION

In the past ten years, nanotechnology has advanced significantly in the synthesis of several nanomaterials for biological purposes. The characteristics of nanomaterials drew much attention in the related prospective uses in tissue engineering, medication delivery, diagnostics, and medical imaging (Özeşer *et al*., 2024; Zheng *et al*., 2021; Mazayen *et al*., 2022). The current work describes the synthesis and characterization of *Adansonia digitata*, or baobab, nanoparticles from a biological viewpoint taking into account their nano-encapsulation potential.

One of the most important and related plants that is indigenous to the African continent is *Adansonia digitata*, also known as the baobab tree (Ibraheem *et al*., 2021). This plant has a wide variety of phytochemical components, from which polyphenols, flavonoids, and anthraquinones have been extracted and shown to exhibit a range of biological characteristics (Bentrad *et al*., 2022). To maximize therapeutic efficacy and enhance bioavailability, the nano-scaled carrier complex is easy to utilize with a variety of bioactive compounds that have the potential to be used in novel nanomaterials for a variety of purposes.

This innovative study highlighted a planned possibility for plant-based nanoparticle synthesis called "Green Synthesis," which is a low-scale, cost-effective, and ecologically friendly process. Consequently, green *Adansonia digitata* extracts used to make nanoparticles

may be less toxic and more biocompatible, making them a promising target for medication administration both in vitro and in vivo (Salah *et al*., 2020; Osman *et al*., 2024). According to reports, nanoencapsulation provides several benefits, such as increased stability for the bioactive substances, and envelopes them in nanoscale carrier systems (Alkholief *et al*., 2022; Saravana *et al*., 2024).

Adansonia digitata has the potential to be a contemporary, sustainable functional food with therapeutic effects due to its total nutritional content. There are several uses for nutraceuticals and functional foods to enhance human health due to the quickly developing area of nanoscience nowadays. The current study was conducted to investigate *Adansonia digitata* nano and nanocapsulated formulations for therapeutic potential with improved bioavailability and efficacy employing a natural polymer as an encapsulating medium (Manal *et al*., 2017).

MATERIALS AND METHODS

Synthesis of *Adansonia digitata* Nanoparticles in Methanol: 30 milliliters of methanol were used to dissolve 200 milligrams of Adansonia digitata fruit powder. 100 mL of boiling water was sprayed with 3 mL of this solution dropwise for 5 minutes at a flow rate of 0.2 mL/min using ultrasonic technology (power: 100 W, frequency: 30 kHz). After 30 minutes of sonication, the solution was stirred for 10 minutes at ambient temperature at 200–800 rpm. *Adansonia digitata* nanoparticles were obtained as a beige powder by drying the fluid (Manal *et al*., 2017).

Synthesis of Encapsulated *Adansonia digitata* Nanoparticles: The nanoprecipitation (Wu *et al*., 2008; Bilati *et al*., 2005) technique was used with a weight ratio of *Adansonia digitata*: Gum Arabic: PVA (1:5:3; w/w/w). 100 mg of *Adansonia digitata* and an appropriate amount of Gum Arabic were dissolved in 50 mL of ethanol to form an internal organic phase solution. The internal organic phase solution was rapidly injected into 130 mL of an external aqueous solution containing the appropriate amount of PVA. The solution was homogenized at 22,000 rpm for 25 minutes for encapsulated *Adansonia digitata* nanoparticle formation. The ethanol was removed by evaporation, followed by drying to obtain the nanoparticle powder.

Using transmission electron microscopy (TEM), the nanoparticles' morphology was examined. Using the dynamic light scattering (DLS) approach, the average particle size of the produced *Adansonia digitata* nanoparticles (ADNPs) and encapsulated ADNPs (Cap-ADNPs) was measured using a zeta sizer. Fourier Transform Infrared (FTIR) spectroscopy was used to identify the functional groups that were present in the samples (Perkin-Elmer FTIR Spectrum BX, Waltham, MA, USA).

Antimicrobial Activity Evaluation: Both ADNPs and Cap-ADNPs' antibacterial activities were evaluated against a range of gram-positive and gram-negative bacteria and fungi. The examination was conducted using the diffusion agar well plate technique. To ascertain the antibacterial action, the zone of inhibition was measured in millimeters (López-Malo *et al*., 2020).

Cytotoxicity Evaluation: *Adansonia digitata* extract, ADNPs, and Cap-ADNPs were tested for their possible cytotoxic effects on the human breast cancer cell line (MCF7) and the human colon carcinoma cell line (HCT-116). Standard growth conditions for the HCT-116 and MCF-7 cell lines were 37 °C and 5% CO2. Cell viability was assessed using the 0.4% trypan blue exclusion test. The proportion of viable cells in comparison to the control group will be used to express the results. (ZiaSarabi *et al*., 2018).

Statistical Analysis: Data were analyzed statistically using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test with Origin 2019b software. Results are presented as the mean ± standard deviation (SD) from three independent experiments.

RESULTS AND DISCUSSION

Characterization of Nanoparticles: The shape of the encapsulated nanoparticles may be better understood from the pictures in Figure 1 (A and B). Compared to the unencapsulated nanoparticles, these images enable us to determine if the encapsulation procedure resulted in any notable morphological changes. Both *Adansonia digitata* nanoparticles (ADNPs) and their encapsulated counterparts (Cap-ADNPs) have a distinct spherical shape, according to Transmission Electron Microscopy (TEM) research. The TEM pictures demonstrate that the encapsulated Cap-ADNPs (Figure 1B) and the produced ADNPs (Figure 1A) have consistent particle sizes, signifying effective synthesis and encapsulation procedures. The polymer covering of the Cap-ADNPs sets them apart from the unencapsulated ADNPs and explains their bigger size. Similar findings on the size and shape of nanoparticles before and during encapsulation have been reported in other studies. For example, when curcumin nanoparticles were coated with a polymer, Ha *et al*. observed a steady rise in particle size, but the spherical shape stayed the same (Ha *et al*., 2012). Likewise, Vijayakurup and colleagues found that encapsulating silver nanoparticles in a chitosan matrix led to a noticeable increase in size without altering their spherical shape (Vijayakurup *et al*., 2019). These results imply that the synthesis and encapsulation procedures employed for ADNPs and Cap-ADNPs in this

investigation are consistent with accepted nanoparticle encapsulation techniques, guaranteeing the uniformity and structural integrity of the nanoparticles. The Shape Filter plugin in ImageJ was used to determine the average particle diameter from the TEM images, and the result was 9.247 nm.

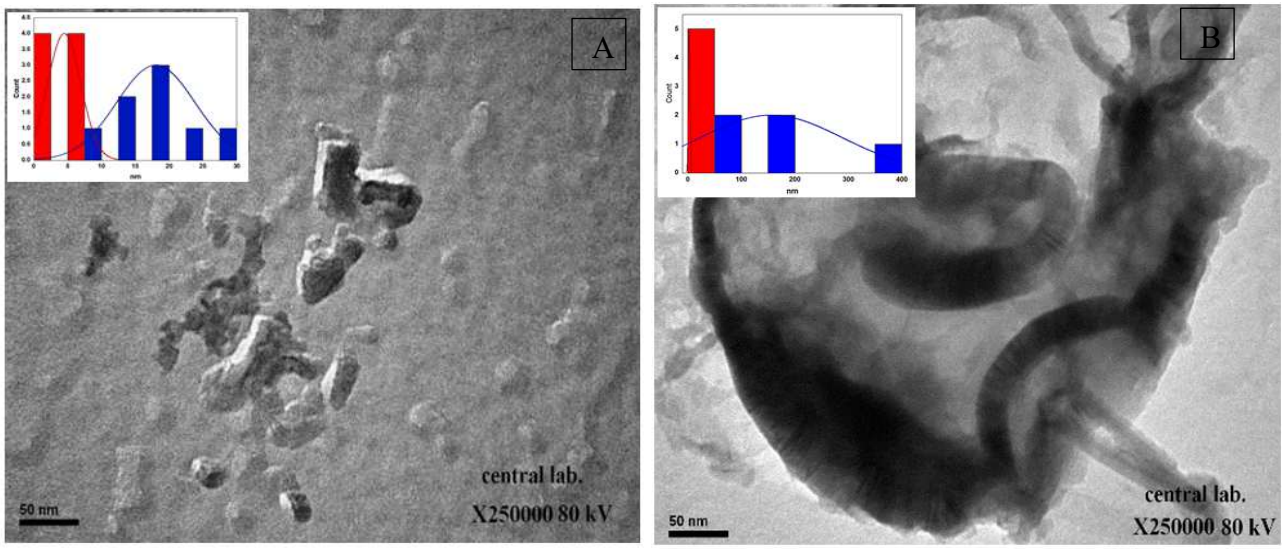

Figure 1: shows TEM photographs and particle size histogram of (A) ADNPs, and (B) Cap-ADNPs.

The hydrodynamic diameter of the nanoparticles is determined using the Dynamic Light Scattering (DLS) data, which provides information on their size in suspension. The polydispersity index (PDI) and average particle size may be calculated from this data. The average particle size of the produced nanoparticles is confirmed by DLS analysis of both ADNPs and Cap-ADNPs. The average particle size for ADNPs was found to be around 94 nm, with a PDI of 0.208 (Figure 2A). The encased Cap-ADNPs, on the other hand, showed a somewhat higher average size of around 230 nm (and PDI of 0.311) (Figure 2B). The encapsulation procedure, which surrounds the core nanoparticles with a coating of gum arabic and PVA, is responsible for this size growth.

It is commonly known that the extra coating materials used in nanoparticle encapsulation usually lead to an increase in hydrodynamic diameter. In line with the results of this investigation, Nguyen *et al*.'s work on the encapsulation of nanoparticles with polyvinyl alcohol (PVA) revealed a comparable rise in particle size (Nguyen *et al*., 2021). Additionally, their DLS study showed that the hydrodynamic diameter of encapsulated nanoparticles was greater than that of their unencapsulated counterparts. Additionally, one important metric that shows how uniform the size distribution of the nanoparticles is is the polydispersity index (PDI). In general, a more uniform size distribution is indicated by a low PDI score. Likewise, a study by Danaei showed that encapsulation using biopolymers, such as Gum Arabic, may retain a low PDI and uniform size distribution (Danaei *et al*., 2018). To identify any size changes brought on by the encapsulation process, the DLS data not only offers information on the hydrodynamic diameter of the nanoparticles but also enables a comparison of the size distribution between the unencapsulated ADNPs and the encapsulated Cap-ADNPs. According to several studies, DLS provides larger particle size measurements than TEM images (Danaei *et al*., 2018; Filippov *et al*., 2023).

FTIR analysis was conducted to investigate the chemical constituents of the PVA-gum Arabic composite, ADNPs, and encapsulated Cap-ADNPs (Figure 3). In the ADNPs spectrum (Figure 3(blue line)), carboxylic acid (O-H) stretching appears at ~2931 and 3003 cm^{-1}, corresponding to organic acids like citric and ascorbic acid, which are abundant in baobab fruit. A carbonyl (C=O)-stretch at ~1612 cm^{-1} is associated with aldehydes, ketones, organic acids, sugars, and possibly esters. Ether (C-O) stretching vibrations at ~1427 cm^{-1} are linked to glycosidic bonds in carbohydrates such as pectin, while polysaccharide (C-OH)-bending at ~1056 cm^{-1} is associated with pectin and cellulose (Tadda *et al*., 2021). In Figure 3(red and black lines) PVA, rich in hydroxyl groups due to its polyvinyl alcohol structure, shows strong hydrogen bonding, while Gum Arabic contains numerous hydroxyl groups in its sugar units, with a characteristic peak at ~3471 cm^{-1}. Gum Arabic's ether (C-O) stretching appears at ~1072 cm^{-1}, and both PVA and gum Arabic (Figure 3(black line)) show C-H stretching at ~2931 cm^{-1} and alcohol (O-H) bending vibrations at ~1612 cm^{-1} (Kuo *et al*., 2017).

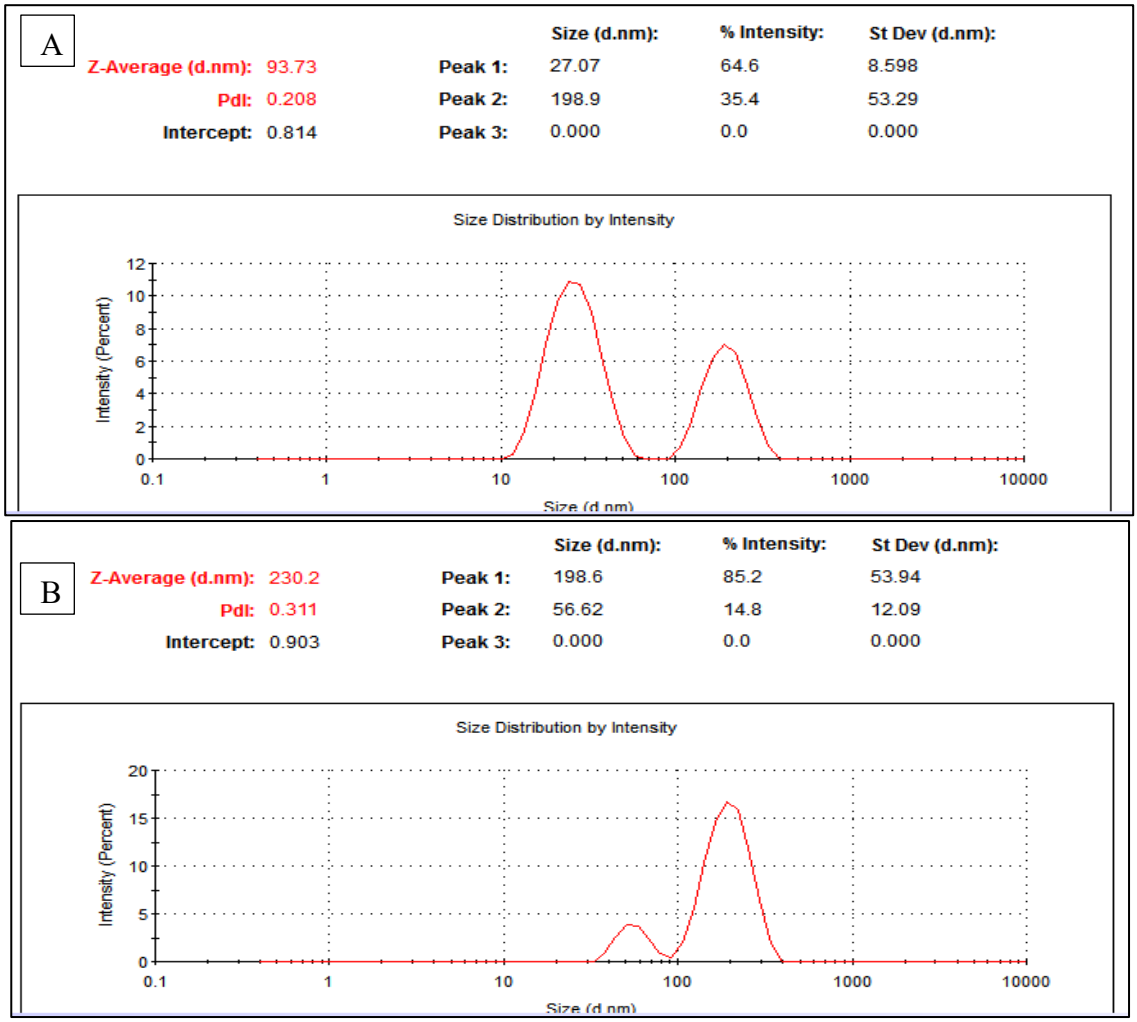

Figure 2: DLS analysis of (A) ADNPs, and (B) encapsulated ADNPs,

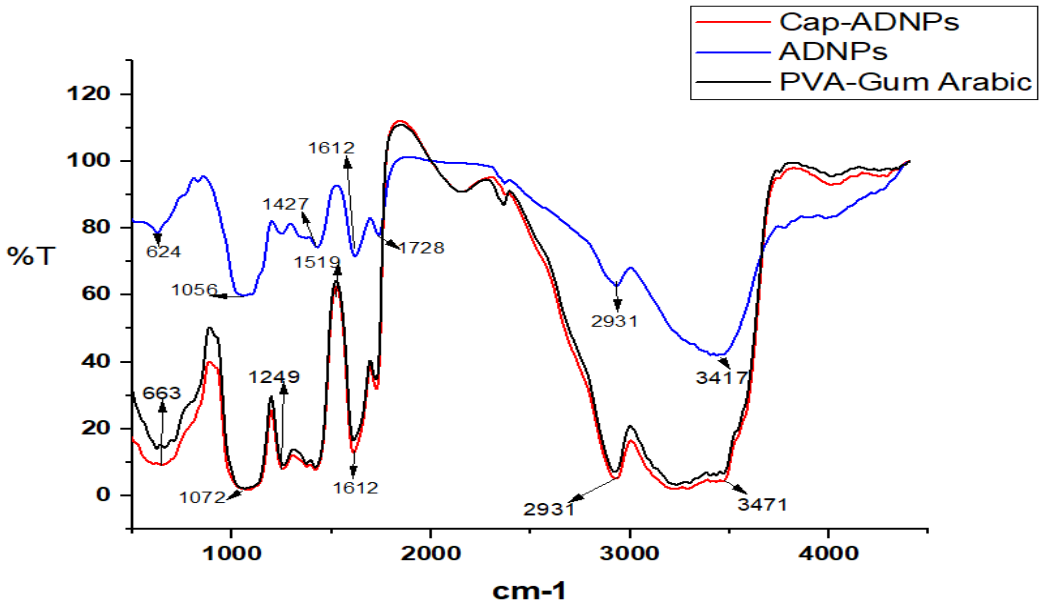

Figure 3: FTIR spectra of Cap-ADNPs, ADNPs, and PVA_Gum Arabic samples.

Antimicrobial Activity: The samples' antibacterial activity was evaluated against several microorganisms. Table 1 provides a summary of the findings.

Table 1: Antimicrobial Activity of Synthesized Samples (Zone of Inhibition in mm ± SD)

Tested Microorganisms	AD Extract	ADNPs	Cap-ADNPs	Standard (St.)
Fungi				Amphotericin B
Aspergillus fumigatus (RCMB 02567)	16.0±1.0	22.0±1.0	22.0±1.0	21.7±1.5
Gram-Positive Bacteria				Ampicillin
Streptococcus pneumoniae (RCMB 010011)	18.0±2.0	20.3±1.5	21.3±1.5	21.0±1.0
Bacillis subtilis (RCMB 010068)	20.3±1.2	24.0±1.0	25.3±1.2	31.3±1.5
Gram Negative Bacteria				Gentamicin
Escherichia coli (RCMB 010054)	17.7±1.5	24.0±2.0	24.7±0.58	20.3±0.58

According to the results of the antibacterial activity, both ADNPs and Cap-ADNPs have notable antimicrobial qualities; however, Cap-ADNPs have somewhat larger zones of inhibition than ADNPs. The enclosed nanoparticles showed improved efficacy, especially against Escherichia coli and Bacillus subtilis. ADNPs are nanoparticles without encapsulation, whereas Cap-ADNPs are nanoparticles encapsulated in polymers. Therefore, Cap-ADNPs are bigger than ADNPs in size. The stability and bioavailability of nanoparticles are often improved by encapsulating, as can be shown when comparing our findings with published research. The effectiveness of the active ingredients can be increased, for example, by using polymer-encapsulated nanoparticles, which provide targeted distribution and controlled release (Opoku-Damoah *et al*., 2022; Ahmed *et al*., 2022; Özçiçek *et al*., 2022; Villemin *et al*., 2019). Encapsulation also protects the nanoparticles from degradation, thereby extending their shelf life and functional activity (Salah *et al*., 2020). The following processes contribute to nanoparticles' antibacterial properties: the microbial cell membrane may become physically disrupted and more permeable because of the nanoparticles' attachment. This results in the leaking of cellular contents and ultimately the cell death (Wang *et al*., 2017). Reactive oxygen species (ROS), which cause oxidative stress and harm essential biological constituents including DNA, proteins, and lipids, can be produced by nanoparticles. One of the main ways that nanoparticles have an antibacterial impact is through the production of ROS (Hajipour *et al*., 2012). Furthermore, nanoparticles can disrupt vital enzymes and microbial metabolic pathways, endangering the bacteria's ability to survive. Cell development and replication may be inhibited as a result (Ahmad *et al*., 2024). Research has indicated that encapsulating nanoparticles greatly increases their antibacterial activity. For instance, the regulated release of silver ions and extended engagement with microbial cells in polymer-encapsulated silver nanoparticles have demonstrated enhanced antibacterial activity when compared to their non-encapsulated counterparts (Mohamed *et al*., 2024; ALRashdi *et al*., 2023; Rosli *et al*., 2021; Zhang *et al*., 2020).

Cytotoxicity Results: The cytotoxicity of the samples was tested using the colon cancer cell line HCT-116 and the breast cancer cell line MCF7; the results are displayed in Figures 3 and 4, respectively. Against HCT-116 cells, the AD extract showed only little inhibitory effect, with an IC50 value higher than 100 mg/ml. In contrast, ADNPs had an IC50 value of 73.6 mg/ml, indicating moderate inhibitory action. Interestingly, Cap-ADNPs had an IC50 value of 34.1 mg/ml and showed a strong inhibitory impact on HCT-116 cells. With an IC50 value of 18.3 mg/ml, the Cap-ADNPs also showed a significant inhibitory effect against MCF7 breast cancer cells. With an IC50 value of 64.7 mg/ml, ADNPs also demonstrated some inhibitory effect against these cells, albeit to a lower degree. Comparatively, the IC50 value of the AD extract was more than 100 mg/ml, indicating a modest inhibitory effect against the MCF7 cells once more. These cytotoxicity findings imply that the encapsulating procedure greatly increases the *Adansonia digitata* nanoparticles' inhibitory activity. The fact that Cap-ADNPs had lower IC50 values than ADNPs for both the HCT-116 and MCF7 cell lines suggests that encapsulation increases the nanoparticles' capacity for cytotoxicity. This result is in line with other research that shows encapsulation can increase the effectiveness of medication delivery systems based on nanoparticles. For example, Zhang *et al*.'s study showed that polymer-encapsulated nanoparticles had more cytotoxicity than non-encapsulated ones because of better cellular absorption and longer-lasting release of active ingredients (Zhang *et al*., 2020). Cap-ADNPs have encouraging cytotoxic behavior, indicating that they may have substantial therapeutic promise as alternative treatments.

Other studies (Zhang *et al*., 2020; Machtakova *et al*., 2022) have shown that plant-based nanoparticles may be useful anticancer medicines, providing a less hazardous substitute for traditional chemotherapy. The results of published research indicate that encapsulation enhances nanoparticle stability and bioavailability,

allowing for controlled release and targeted distribution and ultimately improving therapeutic efficacy. This is consistent with the improved cytotoxicity of Cap-ADNPs in comparison to ADNPs. Several hypothesized pathways might explain the anticancer actions of nanoparticles. Because of the leaking vasculature, nanoparticles tend to aggregate in tumor tissues, which enhances medication delivery (Mohammadzadeh *et al*., 2019), and can be made functional by adding ligands that bind to receptors that cancer cells overexpress, so guaranteeing targeted delivery and reducing side effects (Herdiana, *et al*., 2023). Additionally, NPs can cause oxidative stress in cancer cells, which can result in programmed cell death or apoptosis (Nakamura *et al*., 2016). They can also interfere with vital signaling pathways that are necessary for the survival and growth of cancer cells (Dutta *et al*., 2021). These processes highlight Cap-ADNPs' potential as strong antibacterial and anticancer drugs, with the encapsulation process being essential to boosting the drugs' stability, bioavailability, and therapeutic effectiveness (Dinçer *et al*., 2019).

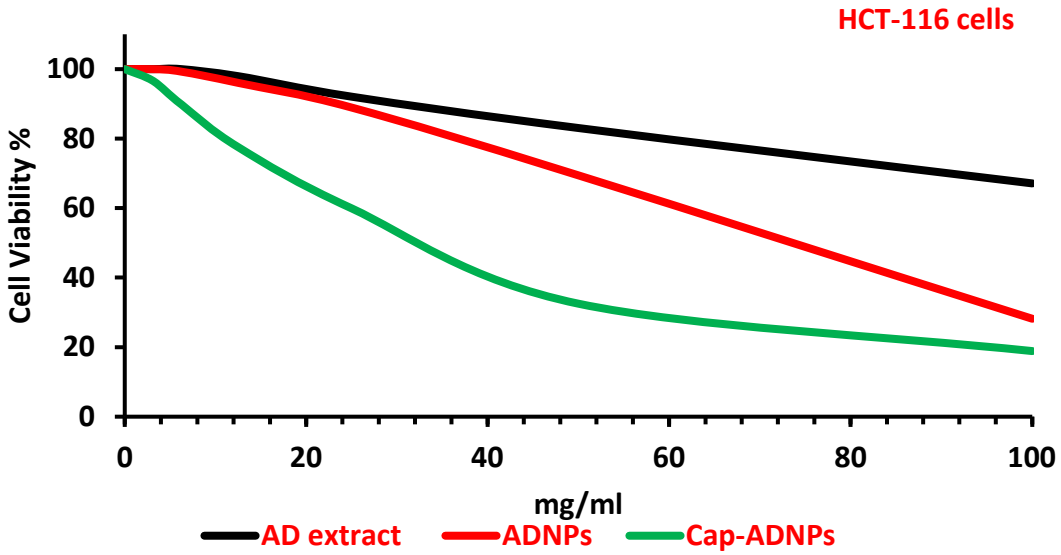

Figure 3: Cell Viability of HCT-116 cell line evaluated using MTT assay. HCT-116 cells were treated with AD extract, ADNPs, and Cap-ADNPs at various concentrations

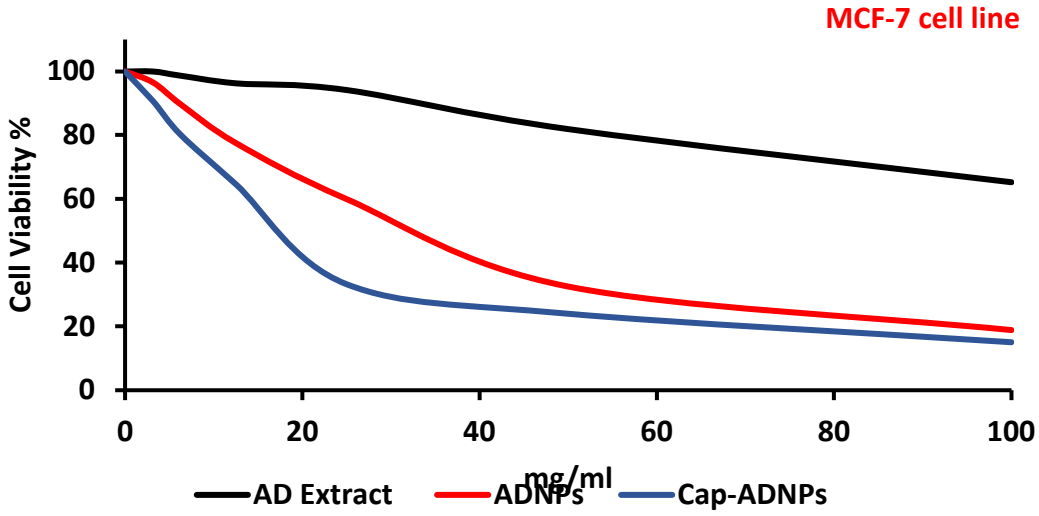

Figure 4: Cell Viability of MCF7 cell line evaluated using MTT assay. MCF7 cells were treated with AD extract, ADNPs, and Cap-ADNPs at various concentrations

Conclusion: In conclusion, *Adansonia digitata* nanoparticles (ADNPs) and the encapsulating nano-formulation (Cap-ADNPs) of these nanoparticles were effectively generated in this study for possible use in biomedicine. The stabilization, antibacterial activity, and anticancer properties of the nanoparticles were enhanced by their encapsulation in Gum Arabic and polyvinyl alcohol. When compared to non-encapsulated ADNPs, cap-ADNPs demonstrated increased cytotoxicity and improved antibacterial qualities against breast and colon cancer cells. These results demonstrate the potential of Cap-ADNPs in antimicrobial therapies and cancer therapy, indicating the need for more research to further investigate their therapeutic use.

Acknowledgements: Researchers Supporting Project number (RSPD2024R1065), King Saud University, Riyadh, Saudi Arabia

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