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Original Research Article

Antimicrobial nanocomposite of Se, Ag, Cu nanoparticles for enhanced hygiene and comfort

Suneeta Panicker[1]*, **Saba Anjum**[1], **Sana Anjum**[1], **Abhishek Nair**[1]

[1]*Dr. D. Y. Patil Arts, Commerce and Science College, Pune, Maharashtra, India*

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ABSTRACT

Background: The prevalence of bacterial and fungal infections associated with sanitary napkins and diapers underscores the critical need for innovative antimicrobial solutions. Without antibacterial properties, bacteria can exacerbate existing skin conditions or cause new ones, such as diaper rash in infants or irritation around the genital area.

Objective: The aim of the study was to create a metal-based nanocomposite to enhance antimicrobial properties of sanitary napkins and diapers, thereby reducing the risk of microbial proliferation and associated infections.

Results: Here, we prepared nanocomposite (NC) comprising selenium, silver, and copper nanoparticles that were synthesized by utilizing *Cassia fistula* leaf extract, checked its antimicrobial properties and investigated its application onto a cellulose matrix commonly utilized in sanitary napkins and diapers. Antimicrobial assays of individual nanoparticles and their NC were conducted against *E. coli, Proteus, Staphylococcus, Bacillus, Candida,* and three strains of multidrug-resistant (MDR) bacteria using well diffusion assay. The NC was also compared with a commercial brand of sanitary napkin Sofy antibacterial (manufactured by Unicharm India Private Limited, Gururam, India). Selenium nanoparticles showed highest antimicrobial activity among the three. The NC incorporated cellulose matrix of sanitary napkins showed significant antimicrobial activity against all the test organisms compared to individual nanoparticles and even the known antibiotics like doxycycline and fluconazole, whilst the cellulose matrix of Sofy antibacterial napkin did not show any antimicrobial activity. Moreover these NCs were found to be effective against the three tested MDRs.

Conclusion: This research underscores the potential of nanotechnology in revolutionizing the design and functionality of everyday consumer products for addressing global health challenges. Further investigation of various other related medicinal properties of these NCs will allow their commercialisation in the sanitary napkins.

1. Introduction

The prolonged usage of sanitary pads and diapers, which come into direct contact with the genitalia, poses a significant health concern due to the conducive environment they create for microbial growth.[1] This environment, characterized by warmth and moisture combined with blood, can lead to the proliferation of harmful microbes. Conventional sanitary napkins and diapers lack antimicrobial properties, thereby increasing the risk of urinary tract infections, skin rashes, inflammation, toxic shock syndrome, and candidiasis.[2]

Currently, excessive antibiotic usage has resulted in the rise in antibiotic-resistant genes across different microbial strains. Nanoparticles (NPs), particularly metal NPs have gathered significant interest particularly for their ability

* *Corresponding author*.
E-mail address: suneetapanicker@gmail.com (S. Panicker).

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to interact effectively with microbial membranes. The conventional methods that are in practice for synthesizing NPs involve toxic chemicals. In contrast, green synthesis of metallic NPs involves biocompatible ingredients, the bioactive molecules found in the green route act as functionalizing ligands, thereby making the NPs more biocompatible.[3,4]

Recently, the Nanocomposite (NC) has been found to show enhanced antimicrobial activity compared to the individual nanoparticles, because the characters of hybrid NPs go beyond simply combining the benefits of each nanoparticle individually. Instead, they emerge from the synergistic interaction, forming a novel class of hybrid-NPs. Particularly, metal nanoparticles (MNPs) and metal NCs (MNCs) have shown significant antimicrobial properties against drug-resistant bacteria.[5]

Cassia fistula (Golden shower tree) is widely used worldwide to treat a wide range of diseases. Being a valuable medicinal plant, its therapeutic applications and phytochemical studies are worthy of attention. In Indian literature, this plant is recognized for its proven effectiveness in treating skin diseases, wounds, insect bites, liver issues, tuberculous glands, as well as conditions like haematemesis, pruritus, leucoderma, and diabetes. The leaf extract of *Cassia fistula* is also known for its antitussive and wound healing properties. The plant is utilized for various ethnomedicinal purposes by the tribe of the Similipal Biosphere Reserve (SBR).[6]

Selenium NPs (SeNPs) show numerous advantages including low toxicity, high degradability, excellent anticancer, antimicrobial and antiviral activities. The strong ability of SeNPs to inhibit bacterial growth also provides new strategies against drug-resistant bacteria infections.[7] Silver NPs (AgNPs) show low toxicity to human cells, have effective broad-spectrum activity against bacteria and a far lesser probability to cause microorganism resistance than conventional antibiotics.[8] Antimicrobial activity of Copper NPs (CuNPs) has been well known since ancient times. CuNPs have demonstrated antimicrobial activity against a wide range of microorganisms.[9]

In current study, green synthesis of SeNPs, AgNPs and CuNPs using *Cassia fistula* leaf extract as a reducing agent and capping agent was performed. The aim was to create a metal-based Se/Ag/Cu nanocomposite to enhance their antimicrobial properties synergistically compared to individual nanoparticles. By incorporating this NC into sanitary napkins and diapers, we aimed to introduce antimicrobial features to these personal hygiene products, thereby reducing the risk of microbial proliferation and associated infections such as UTIs, skin infections, and yeast infections ensuring enhanced hygiene and comfort along with environment sustainability by opting green synthesis method.

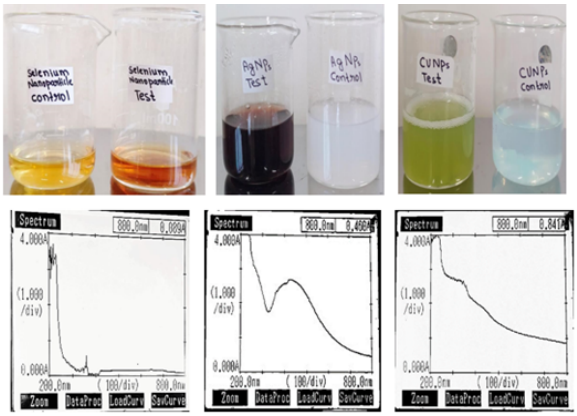

Figure 1: Confirmation and characterization of Se/ Ag/ Cu nanoparticles

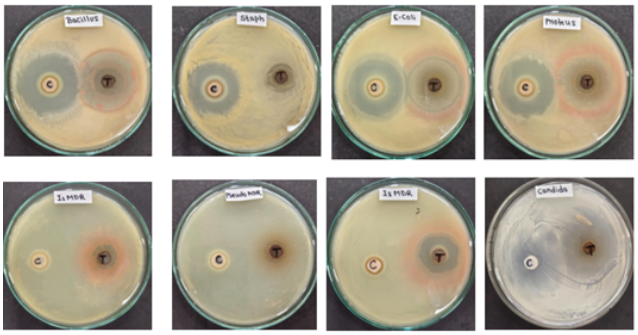

Figure 2: Antimicrobialactivity of nanocomposite against test organisms. C=control; T=test

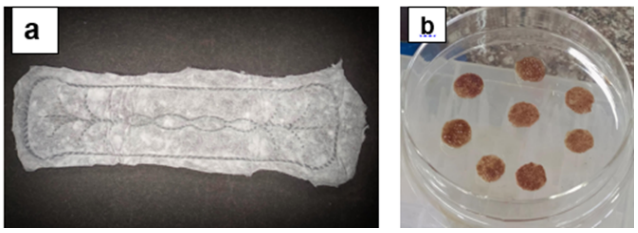

Figure 3: a:) Cellulose matrix of sanitary napkin (Stayfree), **b:)** discs of cellulose matrix incorporatedwith nanocomposite

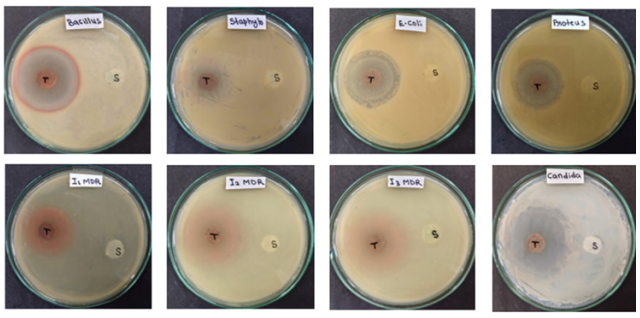

Figure 4: Antimicrobialactivity of NC incorporated cellulose matrix and Sofy antibacterial sanitarynapkins cellulose matrix. T= test; S=sofy antibacterial napkin cellulose

Table 1: Antimicrobial activity of crude form nanoparticles against test organism

Test organism	Zone of Inhibition (in mm)			
	Positive control (doxycycline and fluconazole)	Selenium Nanoparticle (crude)	Silver Nanoparticle (crude)	Copper Nanoparticle (crude
Proteus	36+0.21	25+0.42	-	14+0.36
Staphylococcus	30+0.33	30+0.35	10+0.51	20+0.41
Bacillus	33+0.25	35+0.43	-	20+0.47
E. coli	33+0.37	30+0.48	10+0.38	20+0.32
Candida	18+0.23	36+0.43	-	-

Table 2: Antimicrobial activity of nanoparticles dispersed in DW

Test organism	Zone of Inhibition (in mm)			
	Positive control (doxycycline and fluconazole)	Selenium NPs	Silver NPs	Copper NPs
E. coli	34+0.22	9+0.25	-	20+0.42
Bacillus	34+0.32	-	-	14+0.27
Staphylococcus	33+0.36	-	-	-
Proteus	38+0.28	-	-	20+0.34
Candida	20+0.22	-	-	-

Table 3: Antimicrobial activity of nanoparticles dispersed in DMSO

Test organism	Zone of inhibition (mm)			
	Positive control doxycycline	Selenium NPs	Silver	Copper
Staphylococcus	32+0.16	10+0.34	15+0.31	15+0.26
Bacillus	34+0.23	-	30+0.29	9+0.25
E. coli	34+0.28	10+0.28	29+0.26	-
Proteus	37+0.13	15+0.31	31+0.33	31+0.37
Candida	19+0.25	-	17+0.13	18+0.42

Table 4: Antimicrobial activity of Se/Ag/Cu nanocomposite

Test organism	Zone of inhibition (mm)	
	Positive control (doxycycline and fluconazole)	NC
Bacillus	35+0.25	36+0.42
Staphylococcus	33+0.22	24+0.33
E. coli	36+0.32	34+0.37
Proteus	36+0.37	36+0.38
MDR *Enterobacter*	-	25+0.23
MDR *Pseudomonas*	-	30+0.28
MDR *Klebsiella*	-	19+0.34
Candida	20+0.23	36+0.35

Table 5: Antimicrobial activity of individual nanoparticles and Se/Ag/Cu nanocomposite incorporated cellulose matrix

Test organism	Zone of inhibition (mm)			
	SeNPs disc	AgNPs disc	CuNPs disc	Nanocomposite disc
Bacillus	30+0.35	27+0.21	20+0.27	38+0.32
Staphylococcus	27+0.42	14+0.11	19+0.44	28+0.43
E. coli	29+0.48	26+0.48	16+0.42	37+0.31
Proteus	24+0.12	27+0.45	22+0.14	37+0.18
MDR *Enterobacter*	11+0.34	13+0.51	14+0.33	23+0.13
MDR *Pseudomonas*	20+0.26	14+0.34	18+0.31	22+0.24
MDR *Klebsiella*	38+0.23	-	-	40+0.35
Candida	30+0.35	20+0.19	-	38+0.32

2. Materials and Methods

2.1. Sample collection

The *Cassia fistula* leaves were collected from MIDC Bhosari, Pune- Maharashtra 411026. The plant was identified as *Cassia fistula* by the experts from the Department of Botany, Dr. D.Y. Patil Arts, Commerce and Science College, Pimpri Chinchwad, Pune-Maharashtra 411018, India.

2.2. Cassia fistula leaf extract preparation

Fresh leaves of *Cassia fistula* were taken and cleaned thrice using distilled water (DW); the excess water was removed by blotting paper. Leaves were crushed using motor and pastel. 60 gm of crushed leaves were added to 600 ml of DW and boiled until it became 200 ml. The solution was filtered using Whattman filter paper and then the obtained filtrate was preserved at 4ºC for further experiment.[10]

2.3. Phytochemical analysis of leaf extract of Cassia fistula

Qualitative phytochemical analyses for the extracts were performed according to a previous study.[11]

2.4. Green synthesis of selenium nanoparticles using leaf extract of Cassia fistula

2 ml of leaf extract was added into 10 ml of 10 mM sodium selenite solution using a burette on a magnetic stirrer. The mixture was incubated for 24 h at room temperature on a rotatory shaker. The color change was observed by comparing it with the control, followed by UV-Vis spectrophotometry analysis. The crude solution was centrifuged at 10,000 rpm for 30 min. The obtained NP pellet was washed twice with DW and was resuspended in DW & or DMSO (dimethyl sulfoxide).[12]

2.5. Green synthesis of silver nanoparticles using leaf extract of Cassia fistula

Using a burette, 2 ml of leaf extract was added to 36 ml of 1 mM silver nitrate solution on hot plate magnetic stirrer at 65ºC and maintained at that temperature for another 30 min with constant stirring at 600 rpm, the color change from light yellow to dark reddish brown was observed. This was followed by UV-Vis spectrophotometry analysis. The crude solution was centrifuged at 10,000 rpm for 30 min. Obtained NP pellet was washed twice with DW and the pellet was resuspended in DW & or DMSO.[13]

2.6. Green synthesis of copper nanoparticles using leaf extract of Cassia fistula

Using a burette, 2 ml of leaf extract was added to 20 ml of 1 mM of copper sulfate solution on a hot plate magnetic stirrer at 60ºC and stirred until the color changed to pale yellow. The mixture was covered with aluminium foil and incubated for 24 h at room temperature on a rotatory shaker. The color change from pale yellow to green was observed by comparing it with the control, followed by UV-Vis spectrophotometry analysis. The crude solution was centrifuged at 10,000 rpm for 30 min. Obtained NP pellet was washed twice with D.W and the pellet was resuspended in DW & or DMSO.[14]

2.7. Preparation of selenium-silver-copper nanocomposite

SeNPs, AgNPs, and CuNPs were mixed in equal proportions and incubated at 37°C for 3 h to make the selenium-silver-copper nanocomposite.[15] CuNPs in DW, AgNPs in DMSO and SeNPs in crude form were used to synthesize the NC. This composition was decided based on their individual antimicrobial activity in different solvents.

2.8. Antimicrobial assay of NPs and NC against Gram positive bacteria, Gram negative bacteria and Candida

Well diffusion assay was performed for determination of antimicrobial activity of all the synthesized nanoparticles. Gram negative - *E. coli* and *Proteus*; Gram positive - *Staphylococcus* and *Bacillus;* yeast - *Candida* and multi drug resistant (MDR) *Enterobacter*, *Klebsiella* and *Pseudomonas* were procured from the Department of Microbiology, Dr. D.Y. Patil Arts Commerce and Science College, Pimpri, Pune, Maharashtra-411018. The bacterial cultures were inoculated in nutrient broth and *Candida* in potato dextrose broth respectively and incubated at 37°C for 24 h to bring them in a logarithmic phase. As a positive control, doxycycline to a final concentration of 100 μg was used for bacteria, and fluconazole of 100 μg was used for *Candida* in every assay. Water / DMSO were used as negative control.

For antimicrobial assay of nanoparticles, 100 μL of log phase bacterial culture was spread on Mueller Hinton Agar (MHA) plate and 100 μL of *Candida* was spread on Potato Dextrose Agar (PDA) plate. Wells were dug in the plate using 8 mm sterile cork borer. 100 μL of respective NPs, positive control and negative control were added to respectively labelled wells, followed by pre-diffusion for 15 min at 4°C. The plates were incubated at 37°C for 24 h and results were observed.[16]

For NC testing, on each spread plate, NC incorporated cellulose matrix disc was placed along with the 'Sofy antibacterial' sanitary napkin's 12 mm cellulose matrix disc, using sterile forceps and kept for pre-diffusion at 4°C for

15 min. Sofy antibacterial, *Unicharm India Private Limited, Gururam, India,* is a commercially available antibacterial sanitary napkin and thus was used as positive control. The plates were incubated at 37°C for 24 h and results were observed.[17] Every assay was performed in triplicates and the standard deviation was calculated to represent the data.

2.9. Incorporation of nanocomposite into cellulose matrix used in sanitary napkins and diapers

Sanitary napkins (Brand-Stayfree, JNTL Consumer Health (India) Private Limited, Mumbai, India) were taken and its first cellulose layer was separated in aseptic conditions. The separated cellulose layer was cut into 12 mm diameter discs and 100 μL of synthesized NC was impregnated on each disc, the discs were kept at room temperature to dry under aseptic conditions. Similarly 100 μL of SeNPs in crude form, AgNPs dispersed in DMSO and CuNPs dispersed in DW were also impregnated separately on 12 mm disc of cellulose matrix for further assays.[18] Every assay was performed in triplicates and then the standard deviation was calculated.

2.10. Data analysis

Microsoft Excel® was used to calculate the standard deviation for the triplicate values of zone of inhibition.

3. Results

3.1. Phytochemical analysis of Cassia fistula leaf extract

In phytochemical test of *Cassia fistula* leaf extract showed the presence of flavonoid, tannin, protein, reducing sugar, steroid, anthocyanin, Emodol, saponin, alkaloid, coumarin, quinone and glycoside while starch was absent. The presence of these phytochemicals make *C. fistula* suitable for green synthesis of nanoparticles, as they can act as reducing agents in the process.

3.2. Confirmation and characterization of SeNPS, AgNPs and CuNPs

The change of color from yellow to orange indicated the formation of SeNPs. UV-Vis spectrophotometry analysis between wavelength ranges 200-800 nm showed λ_{max} at 232 nm confirming the formation of SeNPs.

The color change from colourless to light yellow followed by dark reddish brown indicated the formation of AgNPs. UV-Vis spectrophotometry analysis showed λ_{max} at 424 nm confirming the formation of AgNPs.

The color change from light blue to dark yellowish green indicated the formation of CuNPs. UV-Vis spectrophotometry analysis showed λ_{max} at 210 nm confirming the formation of CuNPs. These results are depicted in Figure 1.

3.3. Antimicrobial assay of SeNPs, AgNPs, CuNPs when dispersed in DW, DMSO and in crude form

The well diffusion assay performed for testing the antimicrobial activity of the synthesized NPs, SeNPs in crude form showed the highest zone of inhibition against all the test organisms as compared to when dispersed in DW and DMSO (Tables 1, 2 and 3). AgNPs showed the highest zone of inhibition against all the test organisms when dispersed in DMSO as compared to DW or in crude form. CuNPs showed the highest zone of inhibition when dispersed in DW as compared to DMSO or in crude form. Based on these results, the NPs that showed the highest zone of inhibition were chosen to prepare the nanocomposite.

3.4. Antimicrobial assay of Se/Ag/Cu nanocomposite

In one well 100 μL of positive control was filled, in another well 100 μL of NC was filled. All the test organisms were highly susceptible to the NC (Figure 1). *Proteus* and *Bacillus* were more susceptible to the NC than that of positive control; the zone of inhibition of NC was larger as compared to most of the individual NPs, indicating the synergistic activity of NPs in the NC (Tables 3 and 4). MDR strains of bacteria were also susceptible to the NC but were resistant to positive control (doxycycline) as shown in Figure 2 indicating the strong antimicrobial activity of NC.

3.5. Incorporation of nanocomposite into cellulose matrix used in sanitary napkins and diapers

The separated cellulose membrane from the sanitary napkin (Figure 3 a) was used to impregnate the NCs. SeNPs and AgNPs imparted their reddish color to the NCs and due that the cellulose when incorporated with these NCs appeared dark reddish in color as shown in Figure 3 b. This could be the limitation of the study and needs to work towards diminishing this color to maintain the aesthetic look of the sanitary napkin / the diapers during commercialization of such a product.

3.6. Antimicrobial assay of nanocomposite incorporated cellulose matrix

The NC incorporated cellulose matrix disc showed a large zone of inhibitions against all test organisms as compared to Sofy antibacterial sanitary napkin's cellulose matrix disc, which didn't show zone of inhibition on any test organism as shown in Figure 4. *Bacillus*, *E. coli*, *Proteus* and *Candida* showed most susceptibility to the NC incorporated cellulose matrix disc with an average zone of inhibition 43+0.35 mm, while *Staphylococcus* and MDR *Klebsiella* showed lesser susceptibility to NC with a zone of inhibition of 33+0.57 mm. This shows high antimicrobial activity of

the NC incorporated cellulose matrix, compared to the commercially available Sofy antibacterial sanitary napkin cellulose matrix.

3.7. Comparative antimicrobial assay of individual nanoparticles and nanocomposite incorporated cellulose matrix:

Cellulose matrix discs incorporated with SeNPs, AgNPs, CuNPs and NC respectively were used for antimicrobial assay by disc diffusion method and the results are shown in the Table 5. NC incorporated cellulose matrix discs showed the highest zone of inhibition against all the test organisms followed by SeNPs, indicating the synergistic antimicrobial activity of NPs together.

4. Discussion

Most women of reproductive age use sanitary pads during their menstrual periods for an average of 1800 days in their lifetime. Similarly, the diaper is a hygiene product that is in direct contact with the external genitalia of infants and toddlers for several months to years and even adult diapers. The warmth and moisture along with blood creates an excellent environment for the growth of microbes. The commercially available sanitary napkins and diapers lack antimicrobial properties leading to issues like urinary tract infections, rashes, inflammation, toxic shock syndrome and candidiasis.

The phytochemical analysis of aqueous leaf extract of *Cassia fistula* in the current study revealed the presence of flavonoids, Phenols, tannins, alkaloids, Anthocyanin, Emodol, saponin and carbohydrates. A different group[19] studied the phytochemical presence in Cassia fistula methanolic leaf extract and found only alkaloids, saponins, flavonoids, anthraquinone and phenolic compounds. The can be attributed to the different solvents used for extraction.

In present work, Cassia fistula leaf extract was utilized to synthesize Se, Ag, Cu NPs, similarly, another study[20] utilized Cassia fistula leaf for synthesis of SeNPs. The λ_{max} at 232 nm was determined for SeNPs in contrast their study obtained λ_{max} at 270 nm at the same concentration of 10 mM of sodium selenite. Their antibacterial results showed that the highest zone of inhibition for 10 mM SeNPs against *P. fluorescence* was 26.8 ± 0.07 mm, whereas the present study showed zone of inhibition for 10 mM SeNPs against the MDR *Pseudomonas* spp. was 31.2 ± 0.21 mm. Antibacterial activity of SeNPs against MDRs was shown similar to another study which used orange peep waste to synthesis SeNPs.[21]

In our work SeNPS in crude form showed highest zone of inhibition against *Bacillus*, *Proteus*, *E. coli, Candida*, MDR; *Enterobacter*, *Pseudomonas* and *Klebsiella*, similar results.[22] showed that biogenic selenium nanoparticles were highly active against clinical isolates of *P. aeruginosa* but had lower efficacy toward *C. albicans*. A group[23] studied the antibacterial effect of SeNPS against *Staphylococcus* aureus and the bacterial growth was inhibited approximately 20 times that of control used as similar to the current study where the SeNPS showed a inhibition zone of 27+0.3 mm against *Staphylococcus* aureus.

Silver nanoparticles dispersed in DMSO showed the highest zone of inhibition against Gram negative bacteria *E. coli*, *Proteus* and multi drug resistant *Pseudomonas* and *Klebsiella*, similarly a different study[24] reported that AgNPs show greater antibacterial activity against *E. coli* and S. aureus.

Various studies[25,26] demonstrated high antibacterial activity of CuNPs against Gram negative bacteria as were reported in our findings.

A group of researchers[17] worked on the synthesis of zinc nanocomposite incorporated textile using Crescoating technology in which the NCs are synthesized on the textile itself, in contrast we first prepared the NCs using green synthesized NPs and then impregnated them on the cellulose matrix of the sanitary napkin. Functionalized textiles were subjected to antibacterial and antifungal testing using AATCC Test Method 100–2004 in their study while we used disc diffusion method. The results of both studies aimed to utilize NCs for antimicrobial activities in fabrics but the methods differ. Thus the results cannot be compared though the organisms used in both studies were the same. A study demonstrated similar antibacterial activity of Graphene oxide GO-Ag nanocomposite against Gram- negative *E. coli* as in present work.[27]

5. Conclusion

The purpose of this study was to mitigate the risk of urinary tract infections, skin irritations, inflammation, toxic shock syndrome, and candidiasis associated with prolonged use of sanitary pads and diapers. By enhancing the antimicrobial properties of these essential hygiene products, we sought to improve the overall health and well-being of users. In conclusion, the date presented in this study can be included in the use of biodegradable materials in the nanocomposite based sanitary napkins and diapers to focus more on environmental sustainability and comfort of the user. Further biocompatibility assays of the prepared nanocomposite needs to be done to ensure user safety and concerns.

6. Source of Funding

None.

7. Conflicts of interest

No conflicts of interest, financial, or otherwise.

8. Acknowledgment

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Author biography

Suneeta Panicker, Assistant Professor https://orcid.org/0000-0002-3228-7718

Saba Anjum, Student

Sana Anjum, Student https://orcid.org/0000-0002-3228-7718

Abhishek Nair, Student https://orcid.org/0000-0002-3228-7718

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