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MURRAYA KOENIGII LEAVES EXTRACT FABRICATED NANOTEXTILE AS HOLY GRAIL FOR FEMALE HYGIENE

Biological Science

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ABSTRACT

Good feminine hygiene is important for preventing infections and other health issues. Medical literature has not given enough attention to intimate feminine hygiene, which makes education a priority. Inappropriate intimate hygiene can result in problems like urinary and reproductive tract infections. According to CDC these infections are treated with over the counter drugs, which lead to antimicrobial resistance. An incongruous level of antimicrobial-resistant microbes has been reached worldwide and is threatening global public health in the form of a silent pandemic. To deal with those microbes call for an advanced approach. Nanotechnology is widespread in the present era and the use of small particles known as nanoparticles is a part of nanomedicine, involving diagnosis and treatment of diseases. Antimicrobial nanotextiles are fabrics that have been treated to kill or inhibit the growth of microorganisms. Present study dealt with synthesis of silver nanoparticles (SNP) in an eco-friendly way by using leaves of Murraya koenigii. These nanoparticles were characterized and coated on selected fabric by the Pad-Dry-Cure method. The coated fabric appears darker in color, indicating the deposition of SNPs and coating on the fabric was further confirmed by Scanning Electron Microscopy (SEM). Various physical parameters of fabric were measured. The thickness of fabric is increased by 1.9%, air permeability and density also increases by 5.7% & 0.43% respectively, water absorption capacity decreases by 0.15% and there was no difference in Grams per Square Meter (GSM) of fabric, after coating with SNPs. The antimicrobial activity of the fabric was found against most prevalent urinary tract infection (UTI) causing bacteria and yeast. The results obtained by this eco- friendly bio-inspired method of SNPs synthesis and coating on fabric may contribute to advance the application of antimicrobial textile products especially for female hygiene.

KEYWORDS

female hygiene, Murraya koenigii, silver nanoparticle, nanotextile

INTRODUCTION

Despite the abundance of literature on the vaginal environment, there is little knowledge about the vulvar area and how personal hygiene practices can influence its biological and physiological stability. Poor feminine hygiene can lead to a number of health issues in women viz., infections of urinary tract and reproductive tract, which can cause infertility and childbirth complications, skin issues and life threatening diseases like pelvic inflammatory diseases, toxic shock syndrome etc.. Attention to feminine hygiene is highly sought after, which makes education and research a priority. (Chen *et al*., 2017)

Urinary tract infection (UTI) is a common health problem, particularly in women up to 30 times more often than men due to female anatomy. Nearly 50-60% of all women suffer from an episode of UTI at least once in their lifetime. The risk factors for UTI in women include sexual activity, birth control methods, menopause, pregnancy and various phases of age. The usual mechanism of infection is microbial colonization to the urethra or periurethral spaces and migration of infectious agents into the bladder. In women typically UTI causes by the gastrointestinal tract flora i.e., *Escherichia coli, Proteus* species, *Klebsiella* species and other flora i.e., *Staphylococcus* species, *Enterococcus* species together with opportunistic yeast *Candida* species. In reproductive age, 75% of women suffer from at least one episode of Vulvovaginal Candidiasis (VVC) a kind of infection caused mainly by *Candida albicans* and other *Candida spp*. (Mbakwem-Aniebo *et al*., 2020).

UTI and VVC affects women more often whose immunity is compromised (HIV infection), who have diabetes, immunodeficiency, or are on immunosuppressive therapy (corticosteroids). They are generally treated by broad spectrum antimicrobial drugs. These extraneous and imprudent usages of drugs are potential sources of Antimicrobial Resistance (AMR) (Puvača *et al*., 2022). Lack of clean water, sanitation, hygiene and inadequate infection prevention and control promotes the spread of AMR. UTI, VVC and AMR are major concerns that threaten the women's health.

Utilitarian fabrics have become important in the textile industry, especially the antimicrobial ones. Fabric generally provides good contact area and moisture absorption, both of which are required for microbial growth. The growth of microbes induces infection, allergy, malodor and fabric deterioration (Ammayappan and Moses; 2009). Hence, the embodiment of antimicrobial agents on textile products can overcome this issue, which is of sheer importance.

AMR is the main problem in medicine worldwide. Advancement in nanotechnology gives a strong base to use nanoparticles (NPs) in the fight against pathogens (Sharma *et al*., 2022). Nanoparticles are exceedingly small structures and properties demonstrated by nanoscale structures are extraordinary in terms of their application. Nanotechnology research mainly nanopartical synthesis approaches gained notable propulsion as it is complicated to synthesize especially for biomedical applications. Silver nanoparticles (SNPs) have been the prime focus as it has an anti-bacterial property and extremely used in the health industry (Jain and Pradeep, 2005), food storage (Costa *et al*., 2011), textile coatings (Perelshtein *et al*., 2012) and also for a number of environmental applications (Li *et al*., 2008). Among novel alternatives for controlling this infection, the use of metallic nonmaterial such as silver nanoparticles is one most promising technology (Higa *et al*., 2013).

Nowadays various methods are available to synthesize SNPs, but the "**Bio-inspired**" methods are best as they are eco-friendly and less toxic (Rodrigues *et al*., 2013). SNPs can be synthesized at low concentration of plant extract without using any harmful chemical or physical methods. The approach taken in this study is green synthesis of SNPs, which is simple, cost-effective, less cumbersome, and ultimately sustainable.

MATERIALS AND METHODS

Extract preparation from the *Murraya koenigii* leaves (*M.koenigii*) (Qais *et al*., 2019) Firstly the leaves were collected, washed thoroughly then dried in as shade and grind to make powder. 5 g of powder was added in 100 mL deionized water and boiled for an hour in a water bath. Centrifugation followed by filtration was carried out to collect extract. The extract was stored at 4℃ for further use.

Green synthesis of SNPs were achieved by mixing 10 mL of 1mmol/L $AgNO_3$ solution with 0.125 mL of *M.koenigii* extract. This setup was incubated in a dark chamber to minimize photo-activation of silver nitrate at room temperature. Reduction of Ag^+ to Ag^0 was confirmed by the color change of solution from colorless or yellow to reddish brown. For optimum SNPs synthesis various parameters were studied for the reaction viz. pH at 5, 7, 9; temperature at 25℃, 50℃, 70℃; and time duration for 1h, 2 h and 24 h.

Synthesized SNPs were characterized by visual observation and UV–Vis spectrophotometry. Nanoparticle size was confirmed by Scanning Electron Microscopy (SEM).

Antimicrobial Sensitivity Test (AST) assays were determined. For antimicrobial activity we have selected common UTI causing pathogens- *Escherichia coli* (ATCC 25922), & *Escherichia coli* (ESBL- Extended Spectrum Beta-Lactamase producing strain) (ATCC 35218), *Klebsiella pneumoniae* (ATCC 700603), *Staphylococcus aureus* (ATCC 25923), *Proteus mirabilis* (ATCC 12453), *Pseudomonas aeruginosa* (ATCC 27853) and VVC causing *Candida albicans* (MCC 1151), *Candida tropicalis* (MCC 1163) *and Candida glbrata* (MCC 1554). The sensitivity of the microbes was evaluated by using standard Kirby-Bauer disc diffusion method following CLSI guidelines (CLSI M07-A11 2018, M100-32nd edition 2022 & M44A). Antibacterial sensitivity was done by using Muller Hinton Agar plate (MHA) and anticandidal sensitivity was done using MHA supplemented with 2% dextrose (MHA-D). Antibiotic disc used in this study were as shown in figure 3 and procured from HiMedia Laboratories PVT. Ltd., India. Microorganisms inoculated plate containing distilled water control disc, standard antibiotic disc for bacteria/yeast species and biosynthesized SNPs containing disc were incubated at 25-30°C for 24-48 hours. After incubation plates were observed for the zone of inhibition and the zone size was measured in mm.

Fabrication of textile with synthesized SNPs was done by immersing the fabric in the SNPs solution for up to 24 h for deposition of SNPs on fabric followed by the Pad-Dry-Cure method. The SNPs deposited fabric was first padded with SNPs preparation with 80% wet pickup and then dried in a thermal oven at 90℃ for 5 minutes and cured at 130℃ for 45 seconds. The fabricated nanotextile was stored under standard conditions at 25± 2℃ for 24 h before further testing. (Hasan *et al*., 2020)

Physical properties of SNPs coated fabric were accessed. Coated and uncoated fabrics properties i.e., weight grams per square meter (GSM), thickness, density, water absorption, air permeability were evaluated by following methods of Textile study center the online library for textile engineers using given formula **(**https://textilestudycenter.com/colio/fabric-testing/**).**

$$GSM = \frac{\text{weight of the sample in gram X 1000}}{\text{Area of sample in cm2}}$$

$$\text{water absorption\%} = \frac{W2 - W1}{W1} x100\%$$

(W1= Weight before immersion, W2= Weight after immersion)

$$D = \frac{m}{A}$$

(D=density of fabric, m=mass per unit area of fabric, A=measure thickness of fabric)

Characterization of SNPs coated textile was carried out. SNPs fabricated textile material was subjected for SEM using Zeiss SUPRA 35VP model, which was operated at 5 kV (Zhang *et al*., 2009). The morphology and distribution of SNPs on textile fabric was examined.

Antimicrobial activity of SNPs coated fabric was evaluated by following ISO 20645:2004 protocol using coated fabric pieces.

Synthesized SNPs' cytotoxicity study was carried out. The SNPs' vaginal irritation potential was screened using vaginal-ectocervical (VEC) tissues (Ayehunie *et al*., 2011). The in vitro cytotoxicity of the SNPs were screened against the HeLa cell line by a dose-dependent method of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays (Mosmann,1983 & Sukirtha, *et al*., 2012).

RESULT AND DISCUSSION

Green synthesis and characterization of SNPs. In our study we approached green synthesis of silver nanoparticles (SNPs) using aqueous extracts of plant *M.koenigii* leaves. *M.koenigii* is a well-known medicinal plant and flavonoids, phenolic acids and tannins are the main types of phenolic compounds present. According to Kumar *et al*., 1999, the plant contains a variety of essential oils, including-pinene (39.93%), sabinene (13.3%), and trans caryophyllene (9.02%) which has been shown to have antibacterial activity against *Bacillus subtilis, Proteus vulgaris*, and *Corynebacterium pyogenes*. The plant's leaves have many pharmacological applications (Kumar *et al.,* 2024) and explicitly contain carbazole alkaloid, which have antibacterial, antifungal and antioxidant activities (Balakrishnan *et al*., 2020). The interest in green synthetic methods and SNPs has a number of reasons. They are simple, inexpensive compared to its effectiveness; provide large quantities, harmless and environment friendly.

The *M. koenigii* leaves extract was prepared by non-chemical simple boiling method in order to green synthesized SNPs and treated with aqueous $AgNO_3$ solution (1 mM). For optimum SNPs synthesis various parameters were considered and the optimized reaction pH was 9; incubation temperature was 25℃; and time duration of reaction was 2 Hrs. The visual color change observed and absorption spectra were recorded with Systronics ER 2203 double beam spectrophotometer using 1 cm matched quartz cells. It is an efficient and rapid method, which showed similarity with the results obtained by other researchers who worked with different plant systems (Chandran *et al*., 2006). Change in color was due to the excitation of Surface Plasmon Resonance (SPR) in the metal nanoparticles (Jalal *et al*., 2022).

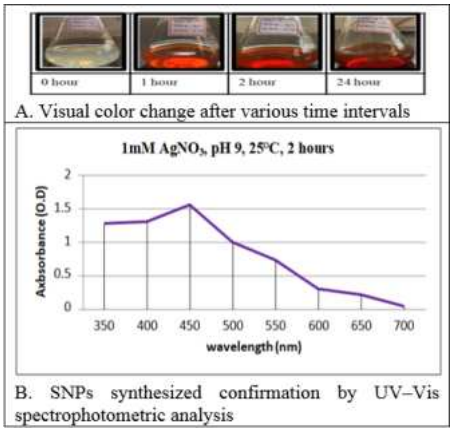

Figure 1. Results of SNPs synthesis confirmation A. Visual method and B**.** UV–Vis spectrophotometric analysis.

The visual color changes from colorless light yellow to reddish brown and UV-Vis spectrum indicating the formation of SNPs (Figure 1 A & B). This analysis showed the sharp absorbance at around 450 nm (Figure 1 B), which was specific for SNPs (Chandran *et al*., 2006). Huang *et al*., 2007 reported similar results, in which they observed that when sun-dried leaf extracts of *Cinnamomum camphora* and *Capsicum annuum* were challenged with aqueous silver ions, the reaction mixture containing SNPs showed the absorption peak at about 420 nm due to the excitation of plasmon resonance vibration.

SEM analysis of reaction mixture demonstrates size, shape and dispersion of synthesized SNPs. A particle size distribution histogram determined from the SEM images showed the large variation in the particle size. The particles are in the range of 50–90 nm with average diameter size of 58.80 nm; spherical in shape and polydisperse as shown in figure 2.

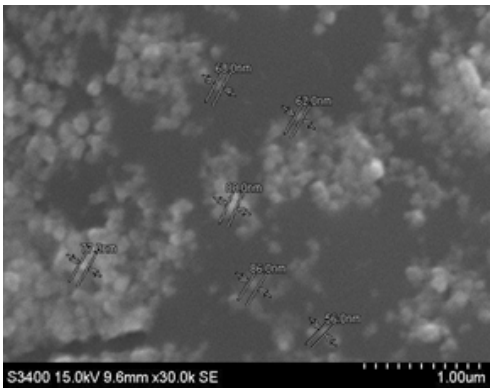

Figure 2. Photograph showing SEM analysis of *M .koenigii* mediated green synthesized SNPs

Antimicrobial activity of *M. koenigii* mediated green synthesized SNPs was tested with potential UTI (Table 1) and VVC (Table 2) causing organisms against standard antibiotics as positive control (PC 1&2) and distilled water used to prepare the solutions of SNPs was employed as negative control (NC). As expected, the negative control showed no inhibition zone against all the organisms tested. The inhibition zones of SNPs ranged from 11 mm to 18 mm. Zone of inhibition for positive control and SNPs for each tested organism is given in table 1 and 2. Gram negative bacilli remained the predominant causative agents of UTIs in previous studies (Silago *et al.*, 2022; Chaula *et al.*, 2017; Kaduma *et al.,* 2019) and somewhere else (Mlugu *et al.,* 2023), *E. coli* remained the most prevalent agent of UTIs, highlighting its persistence as a major pathogen, followed by *K. pneumoniae* (Silago *et al.,* 2022; Chaula *et al.,* 2017). The predominance of these two pathogens is unsurprising as these are gut microbiota which can easily contaminate perineum and genital regions, resulting into mounting UTI mostly among women.

The synthesized SNPs have potent antibacterial activities against *S. aureus, E. coli, Klebsiella pneumonie, Proteus mirabilis, Pseudomonas aeruginosa* and strains of the *candida spp*. which. The findings of (Hwan *et al*., 2010) complementing with our results. These findings substantiate with the findings reported by Shahverdi and his coworkers in 2007, who found that *E. coli* showed the maximum sensitivity to SNPs. In study of Kidwai *et al*.,2017 they showed that ciprofloxacin and ofloxacin had only 40-50% sensitivity to the commonly prevalent pathogens, similarly co- trimoxazole which was the most commonly prescribed and recommended antibiotic in the international guidelines was only sensitive to 16% of the total *E. coli*, 35% of *S. aureus* and 26% of *Klebsiella* strain.

The strongest risk factor for treatment in patient with UTI were previous use of antibiotic, especially β-lactams, Fluoroquinolones - ciprofloxacin. The most common UTI causing organism in adult is *E. coli,* which accounts 75-90% of bacterial isolates (Hoban *et al*., 2011). According to World Health Organization (WHO) report published in 2021 ESBL producing *Enterobacteriaceae* i.e., *E. coli* are part of the group causing the highest risk to public health (WHO, 2021). In our study we have tested *E. coli* (ATCC 35218) a β-lactamase producing strain which also showing zone of inhibition with the synthesized SNPs.

The *C. albicans* in our study showed sensitivity to fluconazole, similar to the findings of Kumar and Pandya, 2023; who stated that all the *Candida* isolates were tested for Azole group and all *C. albicans* were sensitive to fluconazole. The susceptibility profile of *Candida* isolates showed 89.3% sensitivity to fluconazole (Kumar *et al*., 2016). Sonu Panwar *et al*., 2016 reported the susceptibility profile of *Candida* isolates as 58.3% sensitive to Fluconazole.

Table 1. Results of AST for potential UTI causing organisms

Name of organism	Image	NC (Distilled water)	PC1	PC2	SNPs
		Name of antibiotics - zone of inhibition in mm			
Escherichia coli (ATCC 35218)		No zone	Co-trimoxazole -	Imipenem-27	17
Escherichia coli (ATCC 25922)		No zone	Ciprofloxacin – 29	Ceftriaxone – 29	12
Klebsiella pneumoniae (ATCC 700603)		No zone	Ciprofloxacin – 27	Ceftriaxone – 24	11
Staphylococcus aureus (ATCC 25923)		No zone	Ciprofloxacin – 21	Ceftriaxone – 22	13
Proteus mirabilis (ATCC 12453)		No zone	Ciprofloxacin – 27	Ceftriaxone – 27	12
Pseudomonas aeruginosa (ATCC 27853)		No zone	Ciprofloxacin – 27	Ceftriaxone – 23	12

Table 2. Results of AST for potential VVC causing organisms

Name of organism	Image	NC (Distilled water)	PC1	PC2	SNPs
		Name of antibiotics - zone of inhibition in mm			
Candida albicans (MCC 1151)		No zone	Fluconazole – 22	Itraconazole – 22	18
Candida glabrata (MCC 1554)		No zone	Fluconazole – 25	Itraconazole – 18	13
Candida tropicalis (MCC 1165)		No zone	Fluconazole – 25	Itraconazole – 13	13

* Ciprofloxacin CIP (SD060A) 1 mcg , Ceftriaxone CTR (SD065)30 mcg, Imipenem IPM (SD073) 10 mcg, Co-Trimoxazole COT (SD010) 25 mcg, Clotrimazole CC (SD115) 10 mcg, Fluconazole FLC (SD232) 25 mcg

Figure 3.Antibiotic Susceptibility Test results

The fabric chosen was knitted cotton purchased from Ginza Industries Ltd, Udhana, Surat as best suited fabric for women intimate apparel. The fabric was coated with SNPs by Pad-dry-Cure method with speed of 3 meter/minute and 3 pound pressure with 80% wet pick up on laboratory padding mingle machine at Man-made Textiles Research Institute (MANTRA) Surat. Color transformation of the textile fabrics from white to light brown as shown in Figure 4 indicates the adsorption of SNPs on the surface of textile fabric (Ballottin *et al*., 2017).

Figure 4. Photograph showing Padding mingle machine & Deposition of SNPs on fabric

After color transformation, the surface morphology of the SNP-coated fabric was investigated under SEM. Figure 5 shows the SEM micrograph images of the uncoated and SNP-coated fabric at lower and higher magnification. The uncoated fabric with smooth surface and without any contaminating particles on their surfaces (fig 5 A-1, B-1) and the coated fabric with rough surface with a hierarchical structure confirmed the deposition of SNPs on fabric (fig 5 A-2, B-2).

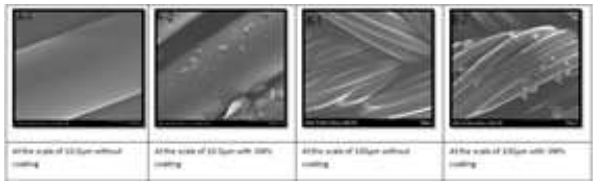

Figure 5.SEM micrograph of coated and uncoated textile fabric Surface

After color transformation, the surface morphology of the SNP-coated fabric was investigated under SEM. Figure 5 shows the SEM micrograph images of the uncoated and SNP-coated fabric at lower and higher magnification, the uncoated fabric with smooth surface and without any contaminating particles on their surfaces (fig 5 A-1, B-1), the coated fabric with rough surface with a hierarchical structure confirmed the deposition of SNPs on fabric.

Various physical parameters of fabric were also inspected. The thickness of fabric was increased by 1.9%, air permeability and density also increased by 5.7% & 0.43% respectively, water absorption capacity decreased by 0.15% and there was no difference in Grams per Square Meter (GSM) of fabric, after coating with SNPs. Syafiuddin et al., 2020 also showed that the presence of SNPs deposition on the surface of textile fabric can decrease the capacity of water absorption and density of textile fabric increases with SNPs deposited on its surface.

Table 1. Fabric parameter before and after coating of SNPs.

Fabric parameters	Thickness	Air permeability	Density (g/m^2)	Water absorbtion (%)	GSM (Gm/m^2)
Uncoated	0.504	82.5	2.096	223.46	208.34
Coated	0.523	88.12	2.087	222.13	208.34

As presented in figure 6 the zone of inhibition surrounding the SNPs coated fabric against lawn growth of microbes; indicates the successful coating of fabric which leads to fabrication of nanotextile.

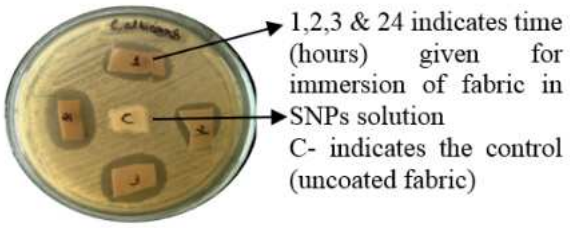

Figure 6.AST by using coated and uncoated fabric

The results for AST of SNPs coated fabrics of this study indicates that SNPs can be used as effective antibacterial and anticandidal materials against potent UTI and VVC causing microorganisms which can endanger human beings. The study results of Hiragond et al., 2020 on masks incorporated with SNPs and Valdes-Salas et al., 2021 on disinfectant with SNps shows the growth inhibition of E. coli, S. aureus and Klebsiella.

Vaginal irritation potential of synthesized SNPs was screened using vaginal-ectocervical (VEC) tissues. The morphology of SNPs treated cells were observed under microscope after an interval of 1 hour up to 24 and 48 hours. We have observed that there was no hazardous effect of SNPs on the cell membrane, nucleus and overall integrity of the cell after exposure of 1, 2, 3 & 24 hours but some distortment was seen after 48 hours as shown in figure 7. It proves that SNPs are safe to use if they are of intermediate size that is average 50-100 nm, <30 nm are toxic as per literature. SNPs showed little adverse effect after long exposure only.

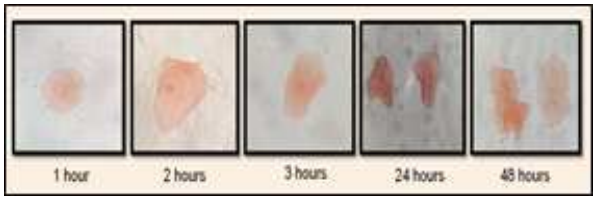

Figure 7. Effect of SNPs on VEC

Cytotoxicity of SNPs was carried out on HeLa cell line using MTT assay. Study indicated that the cell mortality or the % of cell growth inhibition of HeLa cell line was in dose dependent manner. LC50 value was estimated using y=0.753x + 6.301 and it tends to be 58.03 µg/mL. The highest inhibition was observed at the concentration of 100µg/mL which was about 76.32 %. On the other hand, the SNP solution showed variations in cell viability as a function of dose and time. Cell viability reached values below 70% only at SNP concentrations ≥ 29 µg/mL after 24 h of incubation, but was above 70% after 72 h of incubation, indicating the non-cytotoxicity of the synthesized SNPs at the tested concentrations.

CONCLUSION

Silver nanoparticles were successfully produced using M. koenigii leaves extract mediated green synthesis technique. The synthesized SNPs were coated on knitted cotton fabric so as to enhance the multifunctional properties of the fabric. The deposition of SNPs on fabric was accomplished through the pad-dry-cure method. Nanoparticles were firmly adsorbed on the surface of the fabric. They were stable and could not be washed away with water. Owing to physical and chemical interaction, the nanoparticles were bound uniformly on the fabric surface. The fabric coated with SNPs exhibited good antimicrobial and anticandidal activity. The advantages of this process are its ease to carry out and its efficiency. The substances involved in this process are nontoxic, low cost, and readily available. The fabrics thus coated with SNPs can be used in several applications especially as a nano textile for the production of women intimate products viz. sanitary pads, panty liners etc. to maintain feminine hygiene.

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