Antimicrobial coating of fabric by biosynthesized silver nanoparticles from Panchakavya

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Abstract
Silver nanoparticles can be synthesized biologically by means of microbes and plants since they offer eco-friendly, non-toxic and uniform nanoparticles, though many researchers have done major work on biosynthesis there is very less research carried out on synthesis of nanoparticles by Panchakavya. Panchakavya is an Indian eco friendly fertilizer. Silver nitrate is treated with panchakavya to synthesize silver nanoparticles where in panchakavya acts as a reducing agent. The synthesized silver nanoparticles are monitored by UV-spectrophotometer then subjected to structural and morphological studies by XRD, SEM and AFM and silver nanoparticles of size 20–35 nm were obtained. The silver nanoparticles are then used against napkins containing urine sample for antimicrobial studies. Then the napkins are examined for the growth of colonies by carrying out bacterial culture method. The comparison study was done between the napkin without silver nanoparticles and with silver nanoparticles by using colony counting process. The antibacterial activity of silver nanoparticles were traced for two weeks and found same activity which was shown initially. Hence antimicrobial activity of AgNPs lasted even after weeks in napkin used.

1. Introduction
Nanotechnology is making revolution in each and every field since past three decades; the manipulation at atomic to molecular scale is truly amazing and can bring novel properties which were not existed before which can creates novel materials with better and excellent characteristics [1–4].

Silver nanoparticles (AgNPs) have got excellent antimicrobial property its one of the most widely studied nanostructure for antimicrobial activity so far [3–5].

Their use not restricted to only biomedical field but also they have been used to purify microbial contaminated water. Microbes can be utilized to synthesize AgNPs [4–6]. The AgNPs are widely used in several industries for their excellent antimicrobial property including textile industry [5, 6]. Some of the studies also demonstrated that AgNPs can be more effective antimicrobial agents than other metal nanoparticles including gold nanoparticles [7, 8].

Bacteria are single cellular microorganisms. Bacteria possess remarkable ability to reduce heavy metal ions. Bacterial species have the property of defense mechanism to reduce stresses like toxic effects of metals. At high metal ion concentration they have the ability to survive and grow. Silver nitrate is reduced by bacterial method to silver ions. Since bacteria have negative charge and silver nitrate have positive charge, electrostatic interaction takes place. The enzyme present inside the bacteria is called as nitrate reductase enzyme and they helps in reduction of nitrate. Thus silver nitrate is reduced to silver ions and hence silver nanoparticles are produced [8, 9].
Silver nanoparticles have wound bio burden reduction and anti-inflammatory properties, as silver they have positive charged ions by releasing at maximum rate when compared with bulk silver, because at nanoscale silver will have more surface to volume ratio which means surface is exposed more compare to bulk silver hence antibacterial, antifungal and antiviral activity will be more [9–18]. Though AgNPs have been used widely there are very less efforts for their usage in fabrics as an antimicrobial agent [9].

Silver nanoparticles have optical, conductive, and antibacterial properties that are utilized in various fields of research and since the technology is improving silver nanoparticles have wide range of applications and some of the main ones are listed as follows. Biosensors and numerous assays utilize the silver nanoparticles materials in fields of biological tags for the purpose of quantitative detection. They are used as antibacterial agents in shoes, paintings, wound dressing, cosmetic products, and polymers. Conductive inks make use of silver nanoparticles which are used in to composites to determine excellent optical, thermal and electrical conductivity [10–23].

Panchakavya can be used for many health benefits since hundreds of years in India which can be used to treat diseases HIV patients for gaining the weight, psoriasis and other skin disorders, neurological disorders, diabetes mellitus (reduces blood sugar), tuberculosis (act as anti-TB agent), arthritis and so on. It can be also used as a fertilizer to improve the immunity and growth of the plants. Panchakavya was used for on mice to study about diet and immune stimulation, both aspects important for combating diseases during re-convalescence, thus validating the traditional use of Panchakavya in treatment of hepatic diseases [11].

Cow products (cow-pathy) based treatment used in Ayurvedic medicine and of religious importance for Hindus. It is believed that this ‘Cow-pathy’ treatment (consumption of Panchakavya) is highly effective and it have no side effects, results in removal of physical as well as mental disorders and also enhances life force energy, physical strength & life span of an individual. Panchakavya can be used as natural fertilizer and essential for plant growth. Studies relating to ingesting individual components of Panchakavya and its properties are tested [12].

Silver nanoparticles can be synthesized using Panchakavya which is the combination of nine as ingredients including five cow products; dung, urine, milk, ghee, curd, and other derivatives jiggery, banana, tender coconut and water. The silver nitrate can be reduced to silver ions then followed by the silver nanoparticles by proteins involved in the Panchakavya. The produced silver nanoparticles were used for antibacterial studies against the Aeromonas sps. and Citrobacter sps., resulted excellent reduction in the growth rate of these two bacteria’s [12]. Silver nanoparticles synthesized by cow milk are tested for their antibacterial activity herbal treated cotton fabric [13].

2. Materials and methods

2.1. Preparing Panchakavya

Panchakavya was prepared by using 2500 g cow dung mixed with 250 g of ghee in a plastic container and stirred it twice a day for three days. On 4th day 1 litre of milk was added, 1.5 litre cow urine and 1 litre curd and it is mixed twice a day for 15 days. Also 6 ripened banana was added to avoid foul smell.

2.2. Synthesizing silver nanoparticles from Panchakavya

The 10 ml of Panchakavya was mixed with 40 ml of 1 mM AgNO₃. The yellowish brown mixture was formed and it is kept for 2 h in a magnetic stirrer (600 rpm at 30 °C). The colour change from yellow to dark brown (as seen in Figure 1) indicated the presence of AgNPs which is primarily confirmed by the UV–vis spectrophotometer. The solution was then centrifuged for 1 h and 30 min at 2800 rpm to get to discard supernatant. The contents was filtered and kept for drying in hot plate for 10 min and powdered sample was

![Figure 1. Silver solution.](image-url)
Figure 2. Napkin A dipped in 5 ml AgNPs solution.

Figure 3. Napkin B dipped in 10 ml AgNPs solution.

Figure 4. Napkin C without AgNPs solution.
obtained. The synthesized Ag nanoparticles were then subjected to morphological studies by XRD, SEM and AFM.

2.3. Producing silver nanoparticles fabricated infant napkins
Three napkins were taken which were of dimension 8 × 6.5 cm, which is named as napkin A (figure 2), B (figure 3), and C (figure 4), respectively. Napkin A was dipped in 5 ml nano silver solution and the napkin B was dipped in 10 ml of nano silver solution, were napkin C is kept as it is without coating AgNPs. The napkins were dried and kept for 1 day at room temperature. The next day, urine sample was collected and 15 drops were added in sample A, B, and C respectively. The napkins were again kept for 1 day. Later, the cotton was taken and was dipped in three different water containers separately and serial dilution is carried out in all the three samples.

2.4. Media preparation
Media was prepared by taking 5.6 g of nutrient agar and added in 200 ml of water. The nutrient agar and glass wares were kept in autoclave for sterilization. Nutrient agar medium was added to three petri plates and kept in laminar air flow in order to avoid contamination. The final solution of the three samples after serial dilution was taken and added to the nutrient agar medium. Sample is spread using L shaped spreader. Silver nanoparticles of 5 ml with urine was added to petri plate A, 10 ml nano silver solution with urine was added to petri plate B, and petri plate C with only urine. Then it is kept for incubation at 36.5 °C for 24 h.

3. Results and discussion
After silver nanoparticles are synthesized, for preliminary conformation, the obtained silver nanoparticles are characterized in UV–vis spectrophotometer [8, 15, 18]. Then the solution of AgNPs is centrifuged to get pellet for characterization [8, 14].

3.1. UV–vis spectrophotometer
The colour change observed from yellow to dark brown indicates presence of AgNPs (figure 5). The colour change is because of the reduction of AgNO₃ to silver ions followed by silver nanoparticles from panchakavya which acts as a reducing agent.

The peak is obtained at 417 nm which is in the range for the light absorption maximum by silver nanoparticles (400–530 nm). The nanoparticles formation can be monitored by UV–vis spectrophotometer and the known range for silver nanoparticles is compared where in the peak ranges from 400–530 nm according to their size, the larger the wavelength (peak) more is the size. The result shows the maximum absorbance at 417 nm, which indicates that synthesized silver nanoparticles are smaller in nanometre range (figure 6).

3.2. XRD
The obtained XRD peaks matches JCPDS card number 04-0783, by Braggs law the crystalline size obtained is 17.47 nm. The intensities of XRD were recorded from 300 to 800 at 2 theta angles. The peaks in XRD pattern show pure silver nanoparticles (figure 7). The peaks also show the structure of silver nanoparticles is FCC as
demonstrated by [15]. Similar peaks were observed in the studies which confirm the presence of Nanoscale structures [21, 23]. However, according to the work of Jansen et al that compares the particle size estimation by XRD and microscopy techniques, the former technique usually requires a confirmatory approach as the x-ray Diffraction Analysis is well equipped for crystalline particles. At times, the smaller amorphous particles co-exist along with the crystalline nanoparticles. Hence, this study complements the results with other microscopy techniques such as SEM and AFM [24].

3.3. Morphological studies with SEM & AFM
The synthesized silver nanoparticles are then subjected to morphological analysis by SEM. The SEM result (figure 8) shows that the average particle sizes of prepared nanoparticles are 25–35 nm. Where in the Shareef et al [15], Ukkund et al [20, 21], Dhabalia et al [22] had got the particle size of 75 nm, 30–45 nm, 55–65 and 30–70 nm respectively, with the similar method by fungi where in by using Panchakavya smaller silver nanoparticles were obtained, thus Panchakavya acted as more efficient reducing agent with compare to previous studies [15, 18, 20–22]. The AFM results also confirm the particle size of synthesized AgNPs as 25–35 nm (figure 9).

3.4. Microbial growth studies
By carrying out the characterization technique, the presence of AgNPs is confirmed and then these silver nanoparticles were poured to plates (A and B) containing culture media and kept for incubation for 24 h, it was noted that petri plate A (5 ml AgNPs) and petri plate B (10 ml AgNPs) had less colonies compared to petri plate C (without AgNPs). It was also observed that petri plate B (10 ml AgNPs) had less colonies compared to petri plate A (5 ml AgNPs). In order to count the bacterial colonies we followed, petri plate A is divided into 4 parts, out of which the first part had 44 colonies, part 2 had 19 colonies, part 3 had 5 colonies, and part 4 had 6 colonies. Therefore the total colony in petri plate A is 74 colonies with one huge colony (figure 10(A)). When petri plate B was divided into 4 parts, part 1 had 2 colonies, part 2 had 3 colonies, part 3 had 2 colonies, and part 4 had 4 colonies. Therefore the total number of colonies in petri plate B is 11 colonies (figure 10(B)). In petri plate C is
divided into 4 parts, out of which first part had 58 colonies, part 2 had 44 colonies, part 3 had 32 colonies, and part 4 had 50 colonies. The total count of the colonies was 184 colonies (figure 10(B)), thus plate B with 10 ml of silver nanoparticles had lesser microbial colonies (table 1) which indicates that silver nanoparticles effective against killing microbes. This antimicrobial study was comparable with previous studies reported by Wikkinson et al[9] where most of the studies reported antimicrobial activity of silver nanoparticles of size ranging from
Table 1. Effect of silver nanoparticles on microbes.

| Sl No. | Sample name | Quantity of AgNPs (ml) | Colonies formed | % of reduction of colonies (C) |
|--------|-------------|------------------------|-----------------|-------------------------------|
| 1.     | Sample A    | 5                      | 74              | 248                           |
| 2.     | Sample B    | 10                     | 11              | 1677                          |
| 3.     | Sample C    | Nil                     | 184             | 1                             |

20–90 nm and also the research done by Shareef et al [8] has showed excellent antibacterial property of silver nanoparticles of size 50–75 nm and triangle shaped. Hence can be used for coating in clothes [9], in this way AgNPs will find a great importance in textile engineering to avoid bacterial, viral and other microbial infections. Speaking of pandemic AgNPs can also be utilized in the masks and other clothes which are proven anti-viral activity too. Thus AgNPs has great potential role in textile engineering field in future.

4. Conclusions

The pure form of Panchakavya is synthesized by using cow dung, cow urine, cow ghee, milk, curd and banana. Silver nitrate solution was reduced to silver nanoparticles by Panchakavya. Panchakavya acted as a reducing agent. The presence of AgNPs was confirmed by UV–vis spectrophotometer and XRD patterns. In UV–vis spectrophotometer plot the peak obtained is at 417 nm which is in the range of absorption wavelength silver nanoparticles (400–530 nm). In case of XRD characterization the XRD peaks matches JCPDS card number 04-0783, by Braggs law the crystalline size obtained is 17.47 nm. The SEM and AFM result reveals presence of silver nanoparticles with size range 20–35 nm. Among three napkins the one which is coated with 10 ml of silver nanoparticles with urine had less bacterial colonies compared to the napkin which is coated with 5 ml of silver nanoparticles. The napkin that was not coated with silver nanoparticles had more bacterial colonies compared to the two other napkins that were coated with silver nanoparticles. The outcome from the project was that the count of bacterial colonies in napkin A fabricated with AgNPs had 74 colonies which is more compared to count of bacterial colonies in the napkin B that is fabricated with AgNPs of 10 ml which had only 11 colonies. Napkin C which is not fabricated with AgNPs had 184 colonies, which is more compared to napkin A and B. Hence it was proved that silver nanoparticles exhibit excellent antimicrobial property since the growth of bacteria were less in the napkin fabricated with silver nanoparticles.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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