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# GREEN SYNTHESIS OF SILVER NANOPARTICLES USING FRUIT EXTRACT OF PRUNUS DOMESTICA

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**Abstract:** A simple, economical and green method for the efficient synthesis of silver nanoparticles (AgNPs) using aqueous fruit extract of *Prunus domestica* (PD) has been described in the present study. UV-Visible spectroscopy was used to track the growth and stability of the produced silver nanoparticles (PD-AgNPs). Transmission electron microscopy, Dynamic light scattering, and Fourier transform infrared spectroscopy were used to characterize the nanoparticles further. The Surface Plasmon Resonance (SPR) band of the AgNPs was visible and centered at about 480 nm. The nanoparticles were spherical and between 20 and 30 nm in size, according to the TEM results. FT-IR spectral measurements showed that biomolecules such as phenols, amino acids, aldehydes, thiols, carboxylic acid, and others found in the fruit extract may have had a role in the reduction and capping of the formed nanoparticles. According to the DLS experiments, the particles had an average diameter of 198 nm, a zeta potential of -10.5 mV, and a polydispersity index (PDI) of 0.243. Additionally, the synthesized PD-AgNPs showed good antibacterial efficacy against both gram-positive and gram-negative bacteria. Hence, the synthesized PD-AgNPs could serve as an effective alternative antimicrobial for the treatment of pathogenic diseases.

**Keywords:** *Prunus domestica*; Green synthesis; Silver nanoparticles; Antimicrobial.

## I. INTRODUCTION

The production of nanoparticles is rising globally, making nanotechnology the most prominent area of study in material science. Due to specific characteristics, such as shape, structure, and size (1-100 nm), nanoparticles exhibit entirely new or better features.[1] AgNPs have become very popular because of their versatility in various applications like construction materials, water filtration systems, medical devices, and antibacterial and personal care products.[2] The three basic nanoparticle synthesis methods are chemical, physical, and biological. The physical synthesis of nanoparticles will be carried out under constant pressure, energy, and temperature. Chemical techniques for creating nanoparticles include laser pyrolysis, molecular condensation, chemical etching, spray pyrolysis, sputtering and sol-gel processes.[3] The conventional techniques for making nanoparticles are expensive,

toxic, and unfavourable to the environment. In order to solve these issues, scientists have pinpointed the specific green pathways or naturally occurring sources and their components that can be used to create nanoparticles. Three sources of green synthesis can be distinguished: (a) using membranes, virus DNA, and diatoms; (b) using plants and their extracts; and (c) using microorganisms like fungus, bacteria, actinomycetes, and yeasts.

Compared to physical and chemical processes, which consume more chemicals and energy and produce hazardous by-products, green synthesis offers the advantages of being affordable, environmentally friendly, and using biocompatible agents to create silver nanoparticles. Plants and their extracts are helpful in the synthesis of metal nanoparticles because they are readily available, can be handled safely, and have a variety of metabolites that may aid in the reduction process.[4] Plant extracts can act as both reducing and stabilizing agents for metal nanoparticles. Silver salt is reduced, and the resulting silver nanoparticles are stabilized by biomolecules like amino acids, proteins, enzymes, alkaloids, polysaccharides, phenols, tannins, terpenoids, vitamins, and saponins. The size, shape, and antibacterial capabilities of plant-produced nanoparticles are all influenced by the kind and concentration of phytoconstituents in the plant and reaction time and synthesis temperature.[5]

The following considerations are crucial for plant extract-mediated green synthesis: (i) the phytochemical makeup of plant extracts; (ii) their accessibility; (iii) the amount of time needed for synthesis; (iv) the effect of seasonal variation; and (v) the stability of the produced AgNPs. [6] During the reaction, it is essential to consider the pH of the reaction medium, light, time, temperature, and the ratio of plant extract to silver salt. During synthesis, the primary and secondary metabolites of the extracts are primarily involved in redox processes.[7]

*P. domestica* is a small shrubby tree grown at high altitudes and belongs to the Rosaceae family. Leukorrhea, irregular periods, and debility following miscarriage can all be treated medicinally with the fruits of *P. domestica*. It has been demonstrated that the fruit lowers low-density lipoprotein (LDL) cholesterol in human plasma as well as plasma and liver lipids in rats[8], prevents and ameliorates ovariectomy-induced hypercholesterolemia in rats[9], and ameliorates loss of bone mineral density in postmenopausal women.[10] It also has antiemetic action.[11] The dried fruit of *P. domestica* contains significant amounts of antioxidant compounds, including chlorogenic acid, cryptochlorogenic acid, neochlorogenic acid, (+)-abscisic acid, (+)-D-glucopyranosyl abscisate.[12] The fruit also contains carbohydrates (sucrose, fructose, sorbitol, glucose), vitamins (tocopherol, carotene), organic acids (citric acid, malic acid), flavonols (kaempferol, myricetin, and quercetin), as well as minerals (potassium, sodium, calcium, magnesium, zinc, iron).[13] Chlorogenic acid and neochlorogenic acid are the two antioxidant compounds found in high concentrations in prunes.[14] The involvement of polyphenols in the production of silver nanoparticles has been described in several papers.[15] *P. domestica* was selected due to its phytochemical profile and documented antimicrobial activity.[16] The present work used *Prunus domestica* fruit extract to produce AgNPs and examined the antibacterial activity of the synthesized AgNPs against gram-positive and gram-negative bacteria

## II. MATERIALS AND METHODS

### 2.1 Materials and chemicals:

*P. domestica* fruits were collected from Rohtak, Haryana (India). The chemicals were procured from Loba Chemie, CDH, Rankem, and HiMedia, India.

### 2.2 Extract preparation

*P. domestica* fruits were washed properly first with running tap water and afterwards with Milli-Q water. The washed fruits were sliced into small pieces and crushed. About 25 g of the fruit paste was added to 100 ml Milli-Q water. The resulting mixture was stirred on a magnetic stirrer at 50˚ C for 10-15 minutes and filtered through Whatman filter paper; the resulting filtrate was used afresh for the synthesis of AgNPs.[17]

### 2.3 Green synthesis of AgNPs:

A series of preliminary experiments were performed to select the optimum conditions for *Prunus domestica* mediated AgNP (PD-AgNP) synthesis. The optimum conditions were found to be 25 % w/V fruit extract, 1 mM silver nitrate solution, incubation temperature of 60º C and incubation time of 15 hours, and extract to $AgNO_3$ ratio of 1:2. After selecting the optimum condition, 10 ml of the prepared extract was slowly added to 20 ml of 1mM aqueous $AgNO_3$ solution. The mixture was stirred on a magnetic stirrer for 10 minutes. After, sometime the color of the reaction mixture started changing its color to reddish brown with further deepened to brownish as time passed. The resulting AgNPs were centrifuged at 13000 rpm for 15 minutes, and the pellet thus obtained was given several washings with Milli-Q water. The purified nanoparticles were dried using a lyophillizer and characterized further using different analytical techniques.[17]

### 2.4 Characterization of the synthesized PD-AgNPs:

Using quartz cuvettes with a 1 cm path length and a UV-Vis Spectrophotometer (UV-1800 Schimadzu, Japan), the PD-AgNPs were characterized in the wavelength range of 200-800 nm. The morphological features (shape and size) of the synthesized AgNPs were investigated by TEM (Talos F200X TEM for Materials Science) using carbon coated copper grids. FT-IR spectroscopic analysis was done to find the possible functional groups involved in the reduction and capping of AgNPs using KBr pellets using FT-IR (Bruker Alpha II) spectrophotometer. Size and stability of the particles was evaluated using zeta sizer instrument (Malvern Zeta sizer, Nano ZS90).[17]

### 2.5 Antimicrobial activity of the synthesized PD-AgNPs

Two gram positive bacteria i.e. *Bacillus subtilis* (ATCC 1272), and *Staphylococcus aureus* (ATCC 6538), and two gram negative bacteria i.e. *Escherichia coli* (ATCC 8739), and *Klebsiella pneumonia* (ATCC 13882), were used to test the antimicrobial activity of the synthesized PD-AgNPs and *P. domestica* fruit extract by agar disc diffusion method. The chosen bacteria were cultured in tryptone broth and kept at 37˚ C for 24 hours. The bacterial strains

were inoculated on Soya Bean Casein Digested Agar plates for zone of inhibition investigation. Paper discs containing 5 µl of the samples (synthesized PD-AgNPs & PD fruit extract)/standard were added to the agar plates. Ciprofloxacin was taken as the standard antibacterial drug. The treated plates were incubated for 24 h at 37˚ C. The plates were examined for inhibitory zones around the wells following the incubation time.[18]

## III. RESULTS AND DISCUSSIONS

### 3.1 UV-Vis spectral analysis:

The emergence of a brownish tint in the reaction mixture was indicative of the synthesis of AgNPs. AgNPs are well known to have a characteristic surface plasmon oscillation that gives them a yellowish brown colour in aqueous solution.[19] The phytochemicals present in the PD extract might have carried out the reduction of silver ions. *P. domestica* fruit is rich in polyphenols such as neochlorogenic acid and chlorogenic acid[14] and flavonols (myricetin, quercetin, and kaempferol).[13] A distinguishing characteristic of silver nanoparticles is a surface plasmon resonance band in the 400–500 nm range. A clear SPR band centered at around 480 nm can be clearly seen in Fig. 1, which is assumed to be an indication of silver nanoparticles.

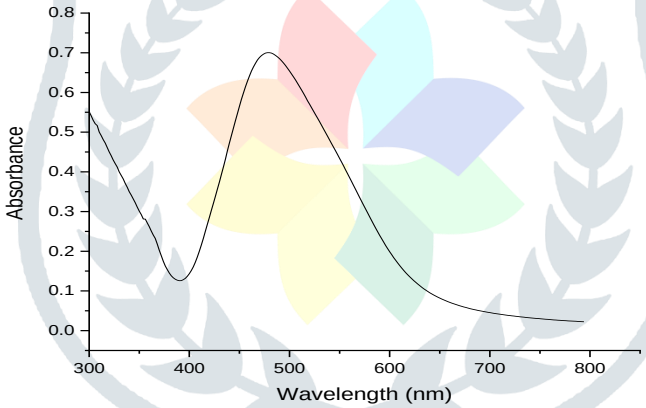



**Fig. 1:** UV-Vis spectra showing the formation of PD-AgNPs

### 3.2 Transmission Electron Microscopy (TEM) analysis:

To assess the size and shape of the nanoparticles, TEM examination was performed. The results of TEM (Fig. 2) showed that the PD-AgNPs were monodispersed and almost spherical in shape. The nanoparticles were found to be between 20 and 30 nm in size.

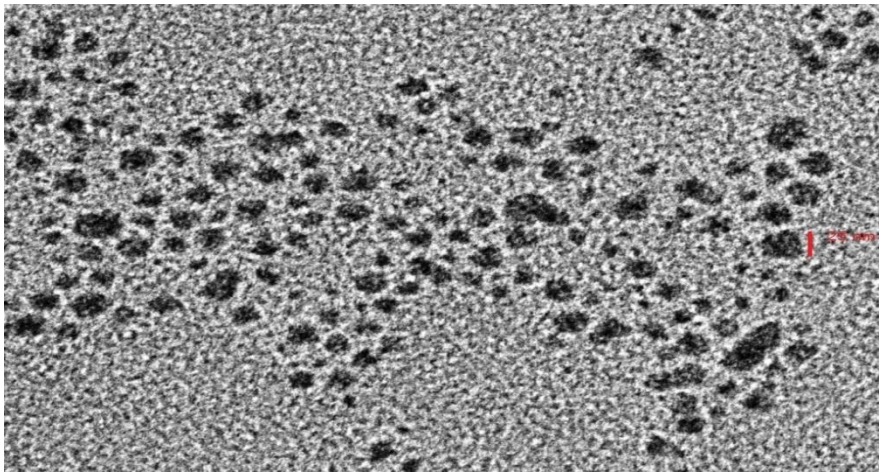
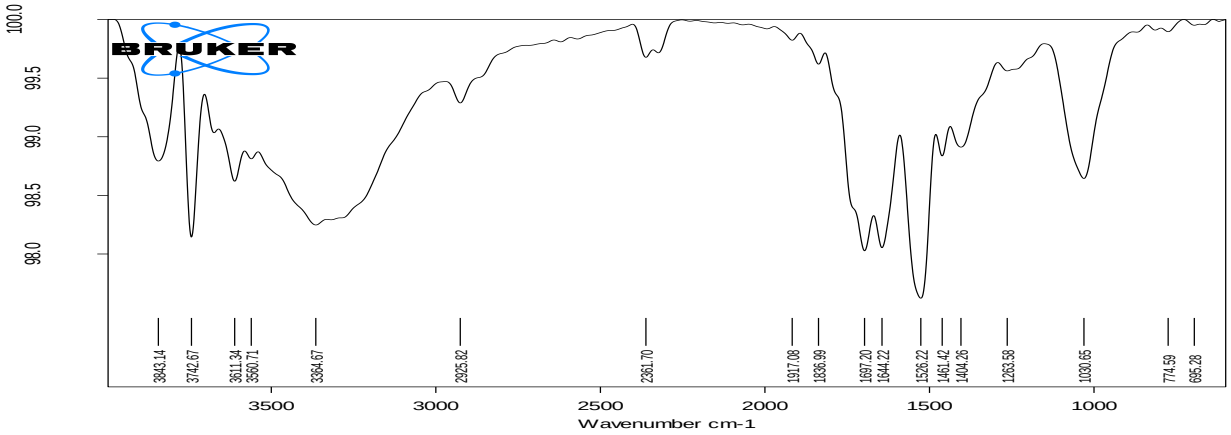


**Fig. 2:** TEM image of the synthesized PD-AgNPs

### 3.3 FT-IR spectroscopy analysis:

Understanding the potential role of biomolecules found in PD fruit extract in the synthesis of AgNPs requires an understanding of FT-IR spectral research. The principal peaks of the FT-IR spectra of the PD extract and the PD-AgNPs are depicted in Fig. 3 and their transmittance values with the matching functional groups are presented in Table 1, respectively. A broad peak at 3364 cm-1 was visible in the FT-IR spectrum of the PD extract, which may have been caused by intermolecular H-bonded -OH groups of phenolic compounds or -NH groups of amino acids. A distorted peak at 3365 cm-1 was visible in the FT-IR spectra of PD-AgNPs, which may have been caused by novel interactions between the extract's biomolecules and the silver metal. Alkane stretching frequency was ascribed to the peaks at 2925 (in extract) and 2926 (in PD-AgNPs). The -N=C=S, -N=C=O, or -SH group is represented by the peaks at 2361 (in extract) and 2363 (in PD-AgNPs). The presence of carbonyl, carboxyllate, and aromatic C=C groups could be seen in the extract and extract-capped nanoparticles by looking for peaks around 1644–1681 in both samples. Peaks at 1526 in the extract and 1533 in the PD-AgNPs may be the result of C=C aromatic ring stretch. These peaks demonstrated that the biomolecules present in the PD fruit extract, including phenols, amino acids, aldehydes, thiols, carboxylic acid, and others, were used to cap the nanoparticles. By inhibiting their aggregation, these functional groups' presence on the surface of the produced PD-AgNPs gives them stability. Previous studies that showed the role of flavonoids, phenolics, amino acids, aldehydes, and carboxylic acid in the formation of AgNPs provide strong support for our findings.[20]

a




b

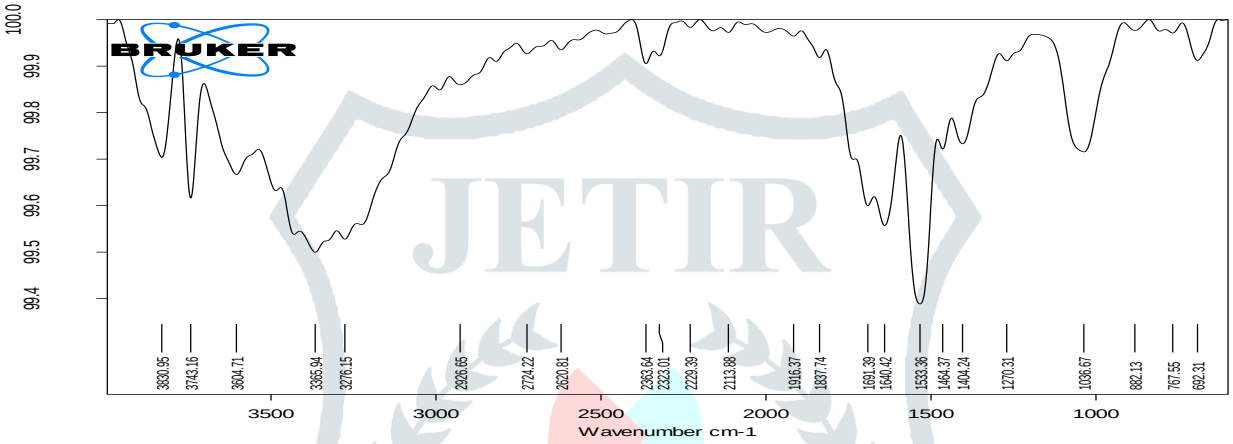



**Fig. 3:** FT-IR spectra of PD fruit extract (a) and PD-AgNPs (b).

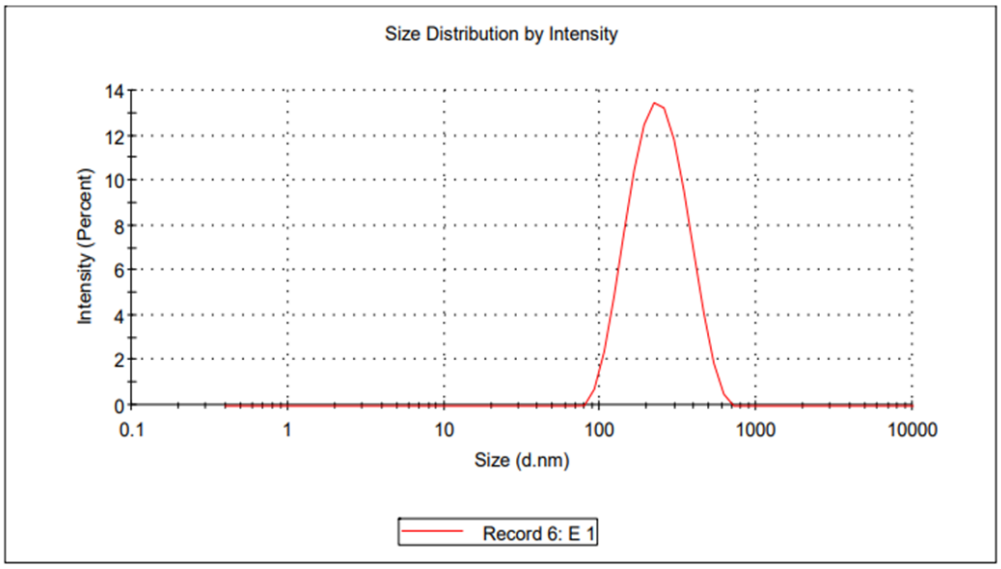
Table 1: Major functional groups in the FT-IR spectra of PD extract and PD-AgNPs with their transmittance values.

| S. No. | Functional groups | Transmittance ($cm^{-1}$) | |
|---|---|---|---|
| | | PD extract | PD-AgNPs |
| 1. | C=C (aromatic stretch) | 1526 | 1533 |
| 2. | -C=O, $COO^-$, C=C (aromatic) | 1644, 1687 | 1640, 1681 |
| 3. | -N=C=S, -N=C=O, S-H | 2361 | 2363 |
| 4. | -OH (H- bonded), -NH (amino acid, amine) | 3364 | 3276, 3365 |
| 5. | -CH (aliphatic) | 2925 | 2926 |

### 3.4 Dynamic light scattering (DLS) analysis of AgNPs:

It was discovered that the nanoparticles had an average size of 198 nm. According to the findings, the size of PD-AgNPs were found to be greater when measured using DLS than when measured using TEM. This distinction demonstrates that DLS assesses the particle diameter in addition to molecules or ions adhering to the surface of AgNPs, whereas TEM solely relies on a number base size distribution and is free of any capping agent.[21] An essential factor determining the stability of aqueous nano suspensions is zeta potential. The stability of the nanoparticles toward agglomeration increases with their zeta potential values, whether positive or negative. The nanoparticles' zeta potential value was discovered to be -10.5 mV, indicating that the particles have a fair amount of stability. The PD-AgNPs had PDI of 0.243 indicating that they were nearly monodisperse in nature. The results are presented in Fig. 4.

**a**




**b**

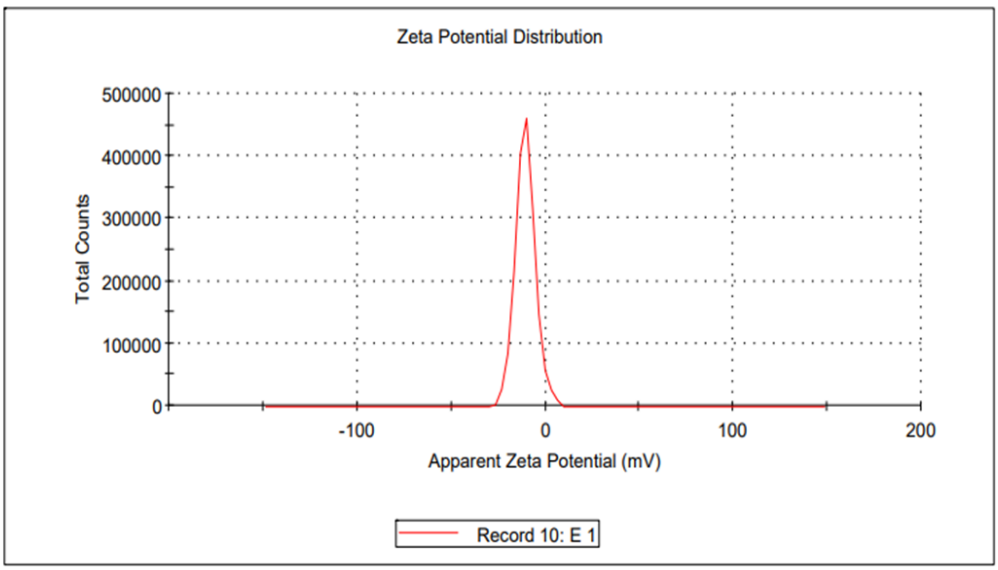



**Fig. 4:** a) Particle size and b) zeta potential of PD-AgNPs

### 3.5 Antimicrobial activity of the synthesized PD-AgNPs

Two gram positive bacteria i.e. *Bacillus subtilis* (ATCC 1272), and *Staphylococcus aureus* (ATCC 6538), and two gram negative bacteria i.e. *Escherichia coli* (ATCC 8739), and *Klebsiella pneumoniae* (ATCC 13882), were used to test the antimicrobial activity of the synthesized PD-AgNPs and PD fruit extract by agar disc diffusion method. From the results, it was observed that the zone of inhibition observed for PD-AgNPs was greater than PD extract. The zone of inhibition against *B. subtilis*, *E. coli*, *S. aureus*, and *K. pneumoniae* was found to be 10, 12, 10, 12 mm respectively for PD-AgNPs. While with PD extract, the inhibition zone against *E. coli*, *S. aureus*, and *K. pneumoniae* was found to be 10, 8 and 9 mm respectively. No zone was observed against *B. subtilis* with the PD extract. The standard drug showed zone of inhibition of 16 mm for all the bacterial strains. AgNPs' capacity to kill bacteria may be attributed to the production of positively charged $Ag^+$ ions, which interact with protein thiol groups to reduce membrane permeability and deactivate vital enzymes, leading to cell death. It was observed that the PD-AgNPs demonstrated higher activity against gram negative bacteria compared to gram positive bacteria which might be due to the difference in sensitivity and cell wall thickness between Gram negative and Gram positive bacteria.[22] Table 2 represents the results of antimicrobial activity.

**Table 2:** Zone of inhibition values of PD fruit extract and PD-AgNPs against the test organisms

| Test organism | Zone of inhibition (mm) | | |
|---|---|---|---|
| | PD fruit extract | PD-AgNPs | Standard |
| *B. subtilis* | - | 10 | 16 |
| *E. coli* | 10 | 12 | 16 |
| *S. aureus* | 8 | 10 | 16 |
| *K. pneumoniae* | 9 | 12 | 16 |

## IV. CONCLUSION

The study reported here successfully produced AgNPs adopting a green approach using *P. domestica* fruit extract. The technology outperformed the traditional physical and chemical approaches since it was simple, quick, affordable, and environmentally benign. Both gram-positive and gram-negative bacteria were effectively combated by the synthesized AgNPs' antibacterial properties. Further, the biosynthesized AgNPs can be used to treat pathogenic diseases.

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