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Research Article

BIOLOGICAL SYNTHESIS OF KERATIN NANOPARTICLES FROM DOVE FEATHER (*COLUMBA LIVIA*) AND ITS APPLICATIONS

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ABSTRACT

Objectives: The aim of the present study was to synthesize keratin nanoparticles from dove feathers.

Methods: Crude keratin was extracted by chemical method. The protein content was estimated by Lowry's method and it was found to be 0.18 mg/ml. The keratin nanoparticles were obtained using glutaraldehyde as cross-linking agent.

Results: A single peak maximum at 270 nm corresponds to the surface plasmon resonance of keratin nanoparticles was observed in the ultraviolet-visible spectrum. The size of keratin nanoparticles was 78 nm. The crystalline size of keratin nanoparticles was 79.6 nm and it was obtained by X-ray diffraction. The antibacterial activity of crude keratin and keratin nanoparticles was determined which revealed that keratin nanoparticles showed higher zone of inhibition than crude keratin protein against *Staphylococcus aureus* and *Salmonella typhi.* Keratin nanoparticles showed higher antioxidant activity than crude keratin.

Conclusion: Biological synthesis of nanoparticles has many advantages such as ecofriendly and low cost and can be synthesized in large scale. The keratin nanoparticles can be applied in wound dressing, biosorbent, and cosmetics.

Keywords: Dove feathers, Keratin nanoparticles, X-ray diffraction, Scanning electron microscopy, Antibacterial activity, Antioxidant activity.

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INTRODUCTION

Keratin is a fibrous structural protein. It is the key structural material making up feathers, hair, horns, claws, hooves, and the outer layer of human skin [1]. The earliest documented use of keratins for medicinal applications comes from a Chinese herbalist named Zhen in the 16th century [2]. Keratin proteins have several functions such as cytoarchitecture, proliferation and growth, apoptosis, and organelle transport [3,4]. It also protects epithelial cells from damage of stress [5].

Bird's feather contains 90% keratin protein among their total mass. The worldwide annual feather offal amounts to about 8×10^5 tonnes [6]. India has a large livestock population, in which the annual production of goatskin is 82%, sheepskin is 30%, cattle hides are 23%, and buffalo hides are 28 million among which the world production of bovine and ovine skins was 1192 million pieces during 2000. It is revealed that the quantity of keratins in the form of hairs, feathers, horns, and hoofs is wasted every year [7]. Keratin fibers extracted from chicken feathers are ecofriendly, non-abrasive, biodegradable, insoluble in organic solvents and having good mechanical properties, hydrophobic behavior, low density, and finally cheap [8].

Recently, nanoparticles have attracted the interest of many researchers due to their high surface area, low diffusion resistance, and more active sites [9]. Considering the advantages of nanoparticles and keratin biosorbents, keratin nanoparticles were synthesized in this study.

METHODS

Sample collection

The feather samples of dove (*Columba livia*) were collected from pet shops of Kilakarai locality, Ramanathapuram district, Tamil Nadu. Healthy feathers which appeared in the colors of white, half white, brown, and black were used for the experiment. Collected feathers were brought to the laboratory and washed with detergent and then they were chopped into small pieces and rinsed with distilled water followed by 70% ethanol and airdried.

Extraction of crude keratin protein

Dried feathers were dissolved in 5% of sodium hydroxide solution kept in shaker (Remi) at 70 rpm for 4 h of incubation. This mixture was then filtered using Whatman No. 1 filter paper. This crude extract was deep-frozen and lyophilized in Lyophilizer (Remi) at −40°C, from which the powder form of crude keratin protein was obtained and stored at 4°C.

Estimation of protein

Protein was estimated by the method of Lowry's *et al.* [10].

Synthesis of keratin nanoparticles

Crude keratin powder 100 mg was diluted by 2 ml of deionized water and was then mixed with 8 ml of absolute alcohol. To this, 1 µl of 8% glutaraldehyde was added. This mixture was stirred using magnetic stirrer at 40 rpm for 24 h. After that, stirred content was centrifuged by cooling centrifuge (Remi) at 10,000 rpm for 20 min. The supernatant was discarded and the pellet was collected and lyophilization process was performed to obtain keratin nanoparticles [11].

Characterization of keratin nanoparticles

The ultraviolet (UV)–visible absorption spectra of the keratin nanoparticles were recorded using UV–visible spectrophotometer (Cary 8454, Agilent, Singapore) operating in the UV to near-infrared (IR) (200–800 nm) spectral region. The surface properties and purity of keratin nanoparticles were examined by scanning electron microscopy (SEM)-energy-dispersive X-ray (EDX) (JSM 6360 JEOL, Japan) analysis. The particle size distribution was determined by dynamic light scattering (DLS) technique using a sub-micrometer particle size analyzer (Nanophox; Sympatec, Germany) with 10 mW intensity of He–Ne laser as a light source at a wavelength of 632 nm. The SEM was connected to EDX analyzer and the synthesized keratin nanoparticles were monitored for identifying their elemental composition. The X-ray

diffraction (XRD) technique was used to determine the structural nature and phase of nanoparticles. Fourier transmission IR (FTIR) spectroscopy involves absorption of electromagnetic radiation in the IR region of the spectra, which resulted in changes in the vibrational energy of molecule.

Antibacterial activity of crude keratin protein and keratin nanoparticles

Antibacterial activity was tested by agar well diffusion method using Mueller-Hinton agar. Test bacterial strains used in this study were *Salmonella typhi* and *Staphylococcus aureus*. Overnight culture of *S. typhi* and *S. aureus* was inoculated into Mueller-Hinton agar using sterile cotton swab. About 5 mm well was made using sterile cork borer. Crude keratin protein and keratin nanoparticles of various concentrations such as 20, 40, and 100 (µg/ml) were added into the wells using micropipette; each well was loaded with 40 µl of sample. It was incubated for 24 h at 37°C and the zone of inhibitions was observed.

Antioxidant assay of crude keratin protein and keratin nanoparticles

In this assay, diphenylpicrylhydrazyl (DPPH) was used as the standard. Working standard of keratin protein and keratin nanoparticles was 0.1 g/ml. From this series of 0.1, 0.2, 0.3, 0.4, and 0.5 ml was added in test tubes and 3 ml of DPPH taken were added to all test tubes including the blank, 3 ml of ascorbic acid was added and all the tubes were incubated in room temperature for 30 min. Optical density (OD) reading was observed in UV–visible spectrophotometer at 570 nm. Percentage of free radical property of nanoparticles was measured using the following formula:

$$\%\text{RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, RSA is the radical scavenging activity, Abs control is the absorbance of DPPH radical + ascorbic acids, and Abs sample is the absorbance of DPPH radical + keratin nanoparticles.

RESULTS AND DISCUSSION

Extraction of crude keratin

Dried feathers were soaked in 5% of sodium hydroxide solution and kept in shaker (Remi) at 70 rpm for 4 h. This mixture was then filtered using Whatman No. 1 filter paper. This crude extract was deep-frozen and lyophilized in Lyophilizer (Remi) at −40°C. 420 mg of crude keratin powder was obtained and stored at 4°C (Fig. 1).

Estimation of protein by Lowry's method

By plotting standard graph, the concentration of test keratin was found to be 0.18 mg/ml (Table 1 and Fig. 2). Similar results were reported by Deivasigamani and Alagappan [11] on keratinase production from soluble feather keratin. The amount of 1.44 mg/ml protein was obtained using the same method. Earlier research by:

Synthesis of keratin nanoparticles

Crude keratin powder 100 mg was diluted by 2 ml of deionized water and then mixed with 8 ml of absolute alcohol, to this 1 µl of 8% glutaraldehyde was added. This mixture was stirred by magnetic stirrer at 40 rpm for 24 h. After that, stirred content was centrifuged by cooling centrifuge (Remi) at 10,000 rpm for 20 min. Supernatant was discarded and pellet was lyophilized. 50 mg of keratin nanoparticles were obtained (Fig. 3).

Characterization of keratin nanoparticles

UV–visible spectrophotometer

The reduction of protein ions formation was confirmed by UV–visible spectrophotometric analysis. The UV–visible spectrum for keratin nanoparticles was obtained by exposing the sample to the UV light from a light source.

A single peak maximum at 270 nm corresponds to the surface Plasmon resonance of keratin nanoparticles was observed in the UV–visible spectrum. The gradual increase in OD value of reaction mixture indicates the aggregation of keratin nanoparticles in the solution (Fig. 4).

SEM

SEM was used to record the photomicrograph images of synthesized keratin nanoparticles. A small volume of keratin nanoparticles suspension was taken for SEM analysis on electron microscope stub.

The stub placed briefly in a drier and then coated with titanium in an ion sputter. The pictures were taken by random scanning of the stub. The morphology of keratin nanoparticle was crystalline shape and aggregated into the larger structure. The SEM micrograph showed the presence of keratin nanoparticles and its polydispersity. The crystalline size of keratin nanoparticles was 79.6 nm (Fig. 5). Similarly, Meignanalakshmi *et al*. [12] reported that the size of keratin nanoparticles was 78 nm. Keratin nanoparticles complexes are spherical in nature, particle size was found to be 100–160 nm [13].

Table 1: Estimation of protein by Lowry's method

S. No.	Sample name	Volume of sample (ml)	Optical density at 660 nm
1.	Blank	-	0.00
2.	S1	0.2	0.42
3.	S2	0.4	0.63
4.	S3	0.6	0.81
5.	S4	0.8	0.92
6.	S5	1.0	1.13
7.	T1	0.2	0.23
8.	T2	0.4	0.40

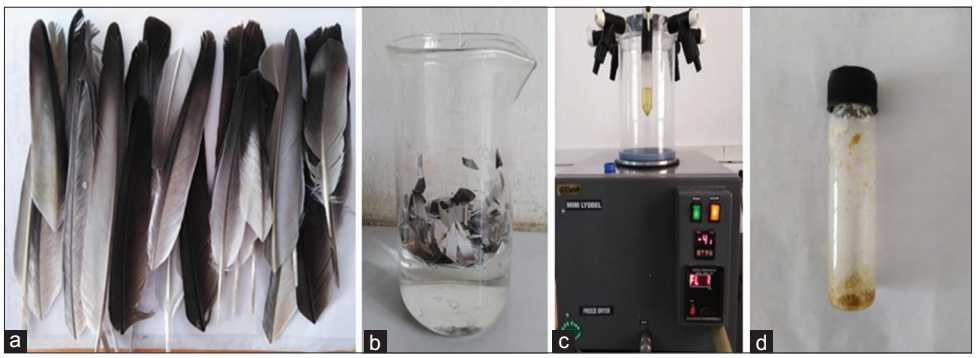

Fig. 1: Extraction of crude keratin (a) Collection of dove feathers. (b) Dove feathers dissolved in sodium hydroxide solution. (c) Obtained extract was lyophilized. (d) Crude keratin powder was collected

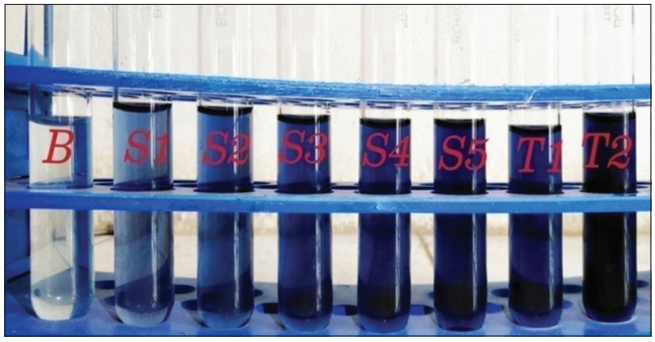

Fig. 2: Protein estimation of Lowry's method

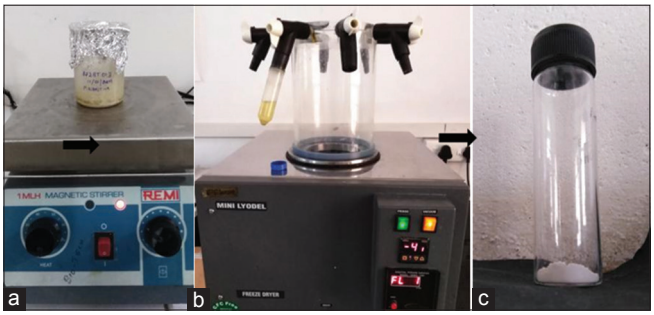

Fig. 3: Synthesis of keratin nanoparticles (a) Crude keratin with glutaraldehyde was kept in magnetic stirrer. (b) Obtained extract was lyophilized. (c) Keratin nanoparticles were obtained

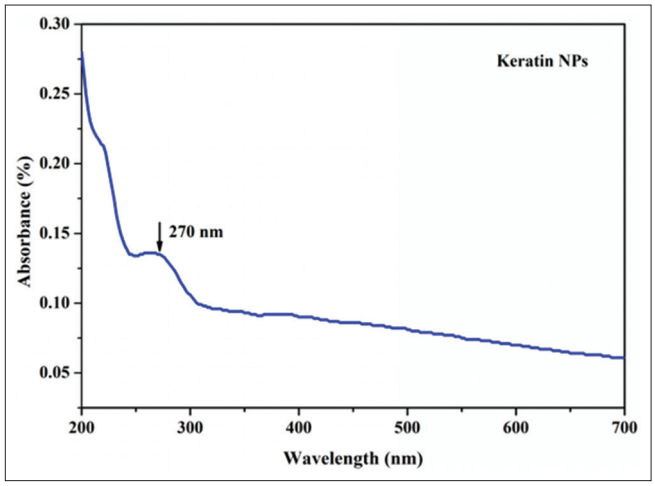

Fig. 4: Absorbance of keratin nanoparticles

DLS

The prostate-specific antigen results showed that the synthesized keratin nanoparticles possessed an average size of about 11.26 nm (Fig. 6). Likewise, keratin nanoparticles from controlled gastric mucoadhesion with an average size ranged from 200 to 400 nm, which was consistent with the result of particle size detection [15]. Keratin silver nanoparticles are in the range of 50–100 nm with an average size of 71.8 nm and keratin gold nanoparticles are in the range of 8–30 nm with mean size of 14.0 nm [13].

EDX

Analysis through EDX spectrometer confirmed the presence of elements of keratin nanoparticles. The vertical axis displays the number of X-ray counts with the horizontal axis displayed energy in keV. Identification lines for the major emission energies for keratin were displayed and corresponded with peaks in the spectrum, thus giving confidence that keratin has been correctly identified.

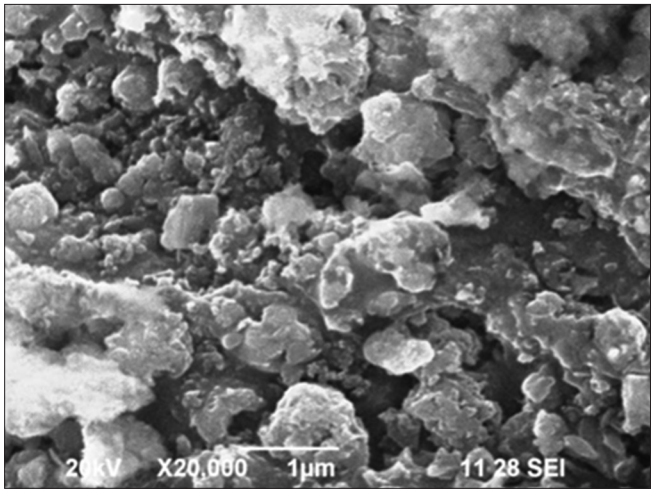

Fig. 5: Scanning electron microscopy image of keratin nanoparticle

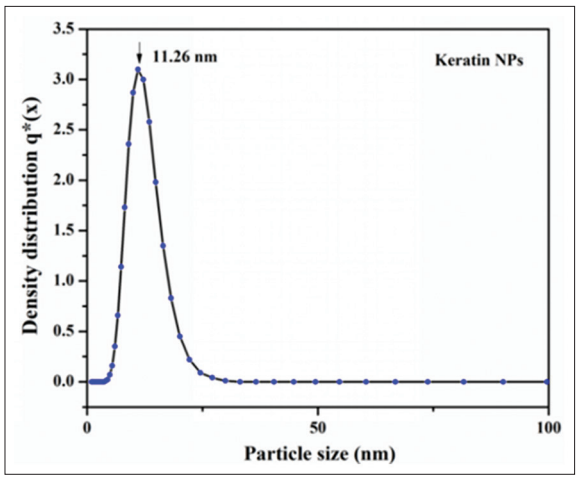

Fig. 6: Dynamic light scattering of keratin nanoparticles

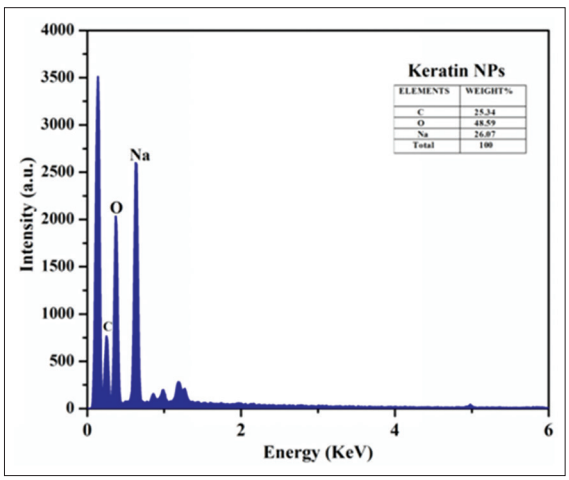

Fig. 7: Energy dispersive X-Ray analysis results of keratin nanoparticles

The peaks around 0.3 keV, 0.4 keV, and 0.7 keV correspond to the binding energy of C, O, and Na, respectively (Fig. 7). The peaks around 0.5 keV, 1.5 keV, 2 keV, and 5 keV for the samples of keratin nanoparticles correspond to the binding energy of C, O, Na, and Ag, respectively [13].

XRD

The XRD diffractogram obtained to assess the physical characterization of keratin nanoparticles in shown in Fig. 8; different peaks at θ = 16, 31, 37, and 45 indicated that the nanoparticles had a mixed amorphous crystalline structure. Selvaraj *et al* [14]. (2018) described that the nanoparticles had a mixed amorphous-crystalline structure on diffraction peaks at 2θ=5, 22, and 43 (Fig. 8). Diffractogram of CEL+KER+Ag+NPs composite exhibits three major peaks at 2θ=27.94, 32.35, and 46.37 which are characteristics of the peaks of keratin-derived silver nanoparticles [16].

FTIR

For the extracted keratin nanoparticles, band at 1653 cm^{-1} is assigned to the amide I of protein, respectively, and the band observed at 1119 cm^{-1} can be assigned to the C–N stretching vibrations of the amines (Fig. 9). Earlier research showed that the FTIR spectra of the extracted keratin showed 400–4000 cm^{-1} [16]. It demonstrated the stretching of ether group on 1035 cm^{-1}. The vibration band on 866 cm^{-1} ascribed to alcohol (Fig. 10). The characteristic frequency of hydrogen-bonded N-H has been observed at 3142 cm^{-1}. The Ag microparticle showed peak on 2467 cm^{-1}. The extracted keratin forms two bands 1653 cm^{-1} and 1583 cm^{-1} were assigned to the amide I and II bands of protein [17].

Antibacterial activity of crude keratin protein and keratin nanoparticles

Antibacterial activity of keratin nanoparticles and crude keratin was tested against *S. aureus* and *S. typhi.* The diameter of the zone of inhibition for keratin nanoparticles against *S. aureus* and *S. typhi* was 24 mm and 28 mm, respectively. The diameter of the zone of inhibition for crude keratin against *S. aureus* and *S. typhi* was 23 mm and 27 mm, respectively (Table 2).

The zone of inhibition of keratin was in the diameter of 9.5 mm against *S. aureus* and 11 mm against *Escherichia coli* 9. Human hair keratin exhibited the antibacterial activity against *E. coli* (3.2 cm in diameter) and *Klebsiella pneumonia* (4.4 cm in diameter) [13].

Antioxidant activity of crude keratin protein and keratin nanoparticles

DPPH is a stable free radical that showed maximum absorbance at 517 nm. The DPPH free radical scavenging activity was investigated at various concentrations such as 0.1, 0.2, 0.3, 0.4, and 0.5 µg/ml. The maximum

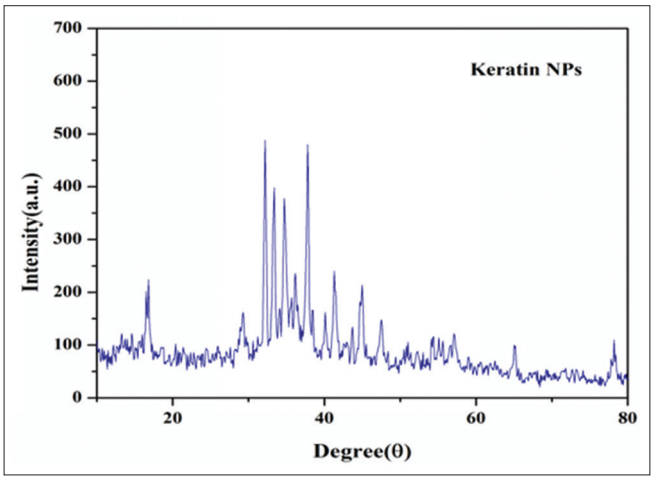

Fig. 8: X-ray diffraction patterns of keratin nanoparticles

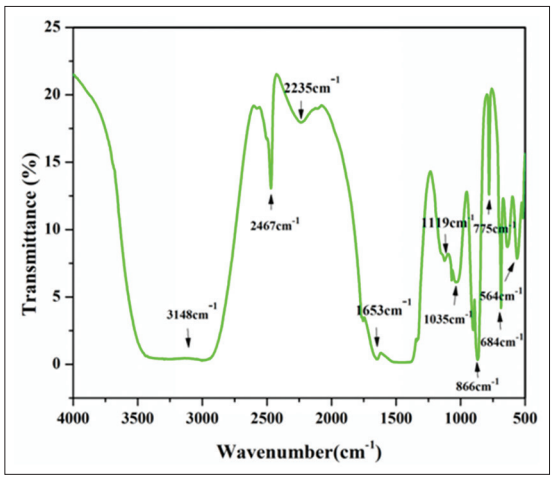

Fig. 9: Fourier transmission infrared result of keratin nanoparticle

Table 2: Antibacterial activity of crude keratin protein and keratin nanoparticles

Sample	Zone of inhibition	
	Staphylococcus aureus	*Salmonella typhi*
Keratin protein	23 mm	27 mm
Keratin nanoparticles	24 mm	28 mm
Positive control	25 mm	29 mm
Negative control	-	-

Table 3: Antioxidant activity of crude keratin protein and keratin nanoparticles

Sample	Concentration (µg/ml)	Free radical scavenging (%)
Standard (ascorbic acid)	0.1	81.23
Keratin protein	0.1	56.33
	0.2	61.11
	0.3	76.11
	0.4	81.66
	0.5	85.55
Keratin nanoparticles	0.1	72.22
	0.2	77.22
	0.3	81.66
	0.4	91.66
	0.5	94.44

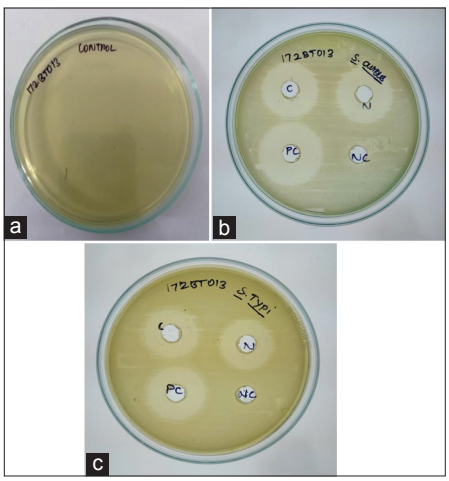

Fig. 10: Antibacterial activity of keratin protein and keratin nanoparticles (a) Control. (b) Keratin protein and keratin nanoparticles against *Staphylococcus aureus.* (c) Keratin protein and keratin nanoparticles against *Salmonella typhi*. C: Crude keratin, N: Keratin nanoparticles, PC: Positive control (streptomycin 0.1 g/ml), NC: Negative control (sterile distilled water)

percentage of radical scavenging activity of keratin protein by ascorbic acid was 85.55% and at 0.5 µg/ml (Table 3). The percentage of antioxidant activity increased with respect to the concentration of keratin protein and keratin nanoparticles. The percentage of free radical scavenging activity for keratin nanoparticles is 94.44% at 0.5 µg/ml which is higher than the antioxidant activity of keratin protein. Keratin nanoparticles obtained from chicken feather wastes showed 77.9% at 350 µg/ml [13].

CONCLUSION

Utilization of feathers may lead to reduction in waste management. The management of keratin-based waste biomass by reconversion into commercially used product will not solely save the ecosystem from great amount of sludge, however, will economically improve the industries. The synthesized keratin nanoparticles can be applied in wound dressing, biosorbent, and cosmetics.

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AUTHORS' CONTRIBUTIONS

All authors have equally contributed to the research study.

CONFLICTS OF INTEREST

None.

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