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Research Article

Biological activity of peptides isolated from feather keratin waste through microbial and enzymatic hydrolysis

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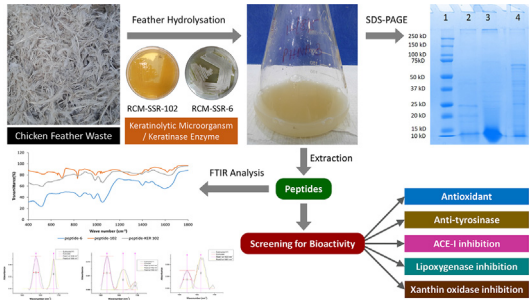
GRAPHICAL ABSTRACT



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ABSTRACT

Background: A major portion of poultry feather waste is constituted by keratin, which is recalcitrant to degradation by common proteases. Feather waste contributes to a significant volume of biowaste load to the environment. Valorization of these wastes into various products has been attempted by many researchers. The present study aimed to produce peptides (molecular weight < 10 kDa) from feather waste by the action of keratinolytic bacteria or keratinase enzyme and to screen the peptides for pharmaceutical and therapeutic properties. The feathers were subjected to hydrolysis by using locally isolated keratinolytic microorganisms, namely *Streptomyces tanashiensis*-RCM-SSR-6, *Bacillus* sp. RCM-SSR-102, and purified keratinase enzyme KER-102.

Results: The feather keratin hydrolysate obtained by hydrolysis with different bacterial species/enzymes showed different protein profiles in SDS-PAGE. As indicated by Fourier Transform Infrared Spectroscopy (FTIR) analysis, a difference was observed in the composition of α-helix and β-sheet in the peptides produced by different microbial/enzymatic methods. The peptides were screened for antioxidant potential,

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Keratinolytic bacteria
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antityrosinase property, and inhibitory activity against angiotensin-converting enzyme (ACE), lipoxygenase, and xanthine oxidase. The peptides showed promising results in all the assays, except peptide-102 that did not show ACE inhibitory activity. Interestingly, the crude peptide-6 (4.06 μg/mL) and peptide-102 (10.21 μg/mL) showed a lower EC_{50} value than the standard Kojic acid (27.04 μg/mL) in antityrosinase assay.

Conclusions: Degradation of chicken feather waste with microbial or enzymatic method is an eco-friendly approach to yield diverse bioactive peptides. Hence, the present study established that feather keratin could be a potential source of many health-beneficial peptides.

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1. Introduction

Chicken feather waste is generated in huge amounts as a byproduct of poultry processing plants [1]. They are very difficult to degrade and contribute to a significant volume to solid waste management. A feather is made up of a pure protein known as keratin, and almost 90% of feather weight is constituted by keratin alone [2]. Hence, improper management of feather waste not only causes environmental pollution but also wastage of protein-rich byproducts. Converting feather waste into valuable products has gained the attention of many researchers [3]. Recently, feather hydrolysate has been reported to show bioactivities such as angiotensin-converting enzyme (ACE) inhibitory, antioxidant, antidiabetic, and antimicrobial activities [4,5,6]. Hence, it could serve as the potential source of bioactive peptides (BPs). BPs are specific protein fragments that impart significant health benefits. They may be free or encrypted within the diverse protein matrices. However, most of the bioactive peptides are derived from expensive protein sources, thus making their use expensive. Therefore, researchers are looking for cheap and easily available protein sources. In this context, being rich in protein, poultry feathers could be a good source of BPs. Many BPs have been reported to show various bioactivities such as ACE inhibitory, antimicrobial, antioxidant, antiprotozoal, antithrombotic, antiviral, antityrosinase, cholesterol-lowering effects, and immunomodulatory activities [7,8]. Because of multiple health-beneficial properties, BPs are considered an indispensable component in functional foods and pharmaceuticals. BPs have been also considered the new generation of biologically active regulators that can prevent oxidation and microbial degradation in foods [9]. Production of BPs has been reported from agro-industrial and marine processing wastes [10,11]. In this context, poultry feather waste, an important protein-rich waste, could be a good substrate for the production of BPs. However, research on BP production from feathers has been sporadically undertaken, and only a few studies have been reported in the literature. The present study aimed to hydrolyze feather waste into feather hydrolysate to produce peptides by the action of keratinolytic bacteria and keratinase enzyme and to screen the peptides for therapeutic and pharmaceutical activities.

2. Materials and methods

2.1. Feather source

The white broiler feathers were supplied by Avian House, Manipur, India. Feather preparation was performed according to Kshetri et al. [12]. Feathers of 45-day-old broiler chickens were collected, washed thoroughly with tap water followed by distilled water, and dried in an oven. The dried feathers were cut into pieces (2–3 cm) and used for hydrolysis.

2.2. Preparation of feather peptides

The feathers were subjected to hydrolysis by using previously reported keratinolytic microorganisms *Streptomyces tanashiensis*-RCM-SSR-6 [13], *Bacillus* sp. RCM-SSR-102, and purified KER-102 keratinase enzyme from *Bacillus* sp. RCM-SSR-102 [14]. Feather degradation was performed in a basal medium containing feather 10.0 g/L; K_2HPO_4 3.0 g/L, and KH_2PO_4 1.0 g/L. The degradation process was allowed for 72 h at 30°C and 120 rpm. For enzymatic hydrolysis, 1.0 g feather was dissolved in 50 ml glycine-NaOH buffer (50 mM, pH 10), and 5000 U or (100 U/mL) of keratinase enzyme was added. One unit of keratinase is defined as the amount of enzyme that could increase 0.01 absorbance at 595 nm when keratin azure is used as the substrate. The reaction mixture was then incubated at 45°C for 24 h in a shaker (120 rpm). To determine feather weight loss, the whole hydrolysate was filtered through a sieve (approximately 1 mm) and washed twice with distilled water. The undigested feather was dried in an oven (60°C), and the final weight was then measured [12]. To prepare crude peptides, the feather hydrolysate was centrifuged at 10000 rpm for 30 min. The supernatant was filtered through a 0.2 μM syringe filter. The cell-free filtrate was again filtered using Amicon Ultra 10 kDa (Merck, USA). The collected permeate filtrate was lyophilized and used for bioactivity assays. The absorbance of the peptide (<10 kDa) was measured at 205 nm, and the concentration was calculated using the equation developed by Anthis and Clore [15]. (**Equation 1**).

$$\textbf{peptide concentration}\ (\textbf{mg/ml}) = \textbf{absorbance at}\ 205\ \textbf{nm}/31 \qquad \text{Equation1}$$

The crude peptides hydrolysed by RCM-SSR-6 and RCM-SSR-102 were named peptide-6 and peptide-102, and the peptide produced by KER-102 was named peptide-KER102.

2.3. SDS-PAGE

Laemmli SDS-PAGE of the feather hydrolysate was performed using 12% (w/v) acrylamide gel. The samples (20 μg) were run in a Bio-Rad protein gel electrophoresis system at a constant voltage of 80 V for stacking gel and 100 V for resolving gel. The crude peptide was analysed in Tricine-SDS-PAGE using a 4% stacking gel, 10%, spacer gel, and 16.5% resolving gel [16]. The samples (15–20 μg) were run at 30 V in a stacking gel, at 80 V in a spacer gel, and at 140 V in a resolving gel. A standard molecular weight protein marker consisting of proteins between 10–250 kDa (Bio-Rad, USA) was included.

2.4. Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectra of the crude feather peptide were recorded in a Fourier Transform Infrared Spectrometer (FTIR) (Perkin Elmer, Spectrum Two, USA). The infrared spectra were measured in the wavenumber range from 400 to 1800 cm^{-1} in the transmittance mode at room temperature. To determine the secondary structure of the peptide, the deconvoluted graph of the amide I region (1600–1700 cm^{-1}) was plotted using Origin Lab 8.0 software.

2.5. Bioactivity assays

The bioactivity assays included assays for antioxidant, ACE inhibitory, antityrosinase, xanthine oxidase (XO) inhibitory, and lipoxygenase (LOX) inhibitory activities.

2.5.1. Antioxidant assays

Antioxidant activity of the samples was determined by two different methods, namely 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay and ferric ion reducing power (FRAP) assay. ABTS assay was performed according to Arnao et al. [17] with some modifications. In this method, a stock solution of ABTS was prepared by mixing an equal volume of 7.4 mM ABTS in ethanol and 2.6 mM potassium persulphate solution for 12 h at room temperature (RT) in dark. The working solution of ABTS was freshly prepared by dilution with ethanol (1:20, v/v). Next, 100 µl of the sample (different concentrations) was reacted with 1.9 mL of ABTS solution. The tubes were incubated for 1 h at RT in dark, and the reduction of stable ABTS free radical (decolourization of green color) was read at 734 nm against ethanol as blank by using a UV–visible spectrophotometer (Merck, USA). Ascorbic acid was used as the positive control. The percentage of radical scavenging activity was calculated using **Equation 2**:

$$\textbf{Percent Inhibition}(\%) = \frac{\textbf{Ac} - \textbf{As}}{\textbf{Ac}} \times 100 \qquad \text{Equation2}$$

where Ac is the absorbance of control and As is the absorbance of the sample. The concentration of the sample required for scavenging 50% ABTS radicals (EC_{50}) was also determined.

FRAP assay was performed according to the method of Benzie and Strain [18] with some modifications. The FRAP reagent was prepared using 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tris-(2-pyridyl)-5-triazine in 40 mM HCl), and 20 mM $FeCl_3$ in the proportion of 10:1:1. Before use, the FRAP reagent was incubated at 37°C for 30 min. The reaction mixture contained 950 µl of FRAP reagent with 50 µl of the sample (different concentrations). The calibration curve of $FeSO_4$ containing 5–100 nMoles was also prepared to calculate the FRAP value. The intense blue colour thus developed was then analyzed after 5 min using a UV–Visible spectrophotometer (Merck, Germany) at 593 nm (**Equation 3**).

$$\textbf{FRAP value or mM Ferrous Equivalents} = \frac{\textbf{D}}{\textbf{V}} \times \text{F} \qquad \text{Equation3}$$

where D = Sample dilution factor of the sample to fit within the standard curve range; V = Sample volume in µL; F = Ferrous amount from the standard curve (nmol).

2.5.2. Antityrosinase assay

The antityrosinase activity was evaluated using mushroom tyrosinase [19] and kojic acid as a positive control. Tyrosinase enzyme was dissolved in pH 6.5 phosphate buffer (50 mM). Next, 20 µl enzyme was mixed with 100 µl of peptide sample dissolved in assay buffer (100 mM phosphate buffer pH 6.8). The volume of the reaction mixture was made up to 360 µl with assay buffer. Next, 360 µl dihydroxyphenylalanine (16 mM in assay buffer) was added and incubated for 5 min at room temperature. The amount of dopachrome produced was measured using a spectrophotometer at 480 nm. The absorbance of the reaction mixture without the sample was taken as the control. Percent enzyme inhibition was calculated using **Equation 4**.

$$\text{Percent Inhibition}(\%) = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100 \qquad \text{Equation4}$$

where Ac = Absorbance of the control at 480 nm, As = Absorbance of the sample at 480 nm. The concentration of the sample required for 50% inhibition of the enzyme (EC_{50}) was also determined.

2.5.3. ACE inhibitory assay

ACE inhibitory activity was performed according to Hayakari et al. [20]. Briefly, the 0.5 ml reaction mixture contained 40 µmol potassium phosphate buffer (pH 8.3), 300 µmol NaCl, 1.5 µmol hippuryl-L-histidyl-L-leucine (HHL), different concentrations of the peptide, and the enzyme. The reaction mixture was incubated at 37°C for 30 min, and the reaction was terminated by keeping the reaction tubes in a boiling water bath for 10 min. Next, 3.5 ml of 0.2 M phosphate buffer (pH 8.3) and 1.5 ml of triazine solution (3% in dioxane) were added to the reaction mixture. It was vigorously stirred until the turbid solution became transparent and tubes were centrifuged at 10000 rpm for 10 min. The absorbance of the supernatant was recorded at 382 nm. Control samples (without inhibitor) and standard samples (inhibitor captopril) were also run. Percent enzyme inhibition was calculated using the following formula (**Equation 5**).

$$\text{Percent inhibition}(\%) = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100 \qquad \text{Equation5}$$

where Ac = Absorbance of the control at 382 nm, As = Absorbance of the sample at 382 nm. The concentration of the sample required for 50% inhibition of the enzyme (EC_{50}) was also determined.

2.5.4. Lipoxygenase (LOX) inhibitory assay

LOX inhibitory activity of the peptides was performed using LOX Inhibitor Screening Assay Kit (Cayman, USA) in accordance with the manufacturer's instructions. Nordihydroguaiaretic acid was used as a positive control. The concentration of the sample required for 50% inhibition of the enzyme (EC_{50}) was determined.

2.5.5. Xanthine oxidase (XO) inhibitory assay

Xanthine oxidase inhibitory activity was determined according to Azmi et al. [21] with some modifications. The enzyme was dissolved in 0.1 M phosphate buffer (pH 7.5) to achieve the concentration of 0.2 U/mL. The reaction mixture containing 50 µL enzyme, 200 µL phosphate buffer (50 mM, pH 7.5), 50 µL distilled water, and 50 µL peptide sample was pre-incubated at 37°C for 15 min. Then 100 µL substrate (0.15 mM xanthine) was added to the reaction mixture and incubated at 37°C for 30 min. The reaction was terminated by adding 100 µL HCl (0.5 M), and absorbance was measured at 293 nm against a blank. Allopurinol, a well-known xanthine oxidase inhibitor was used as a positive control. A control reaction (Ac) without the peptide sample was also included. Percent enzyme inhibition was calculated using the following formula (**Equation 6**).

$$\text{Percent Inhibition}(\%) = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100 \qquad \text{Equation6}$$

where Ac = Absorbance of the control at 293 nm, As = Absorbance of the sample at 293 nm. The concentration of the sample required for 50% inhibition of the enzyme (EC_{50}) was also determined.

2.6. Statistical analysis

All the assays were performed three times in triplicate. and the data were presented as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's HSD test was performed to analyze the significant variation (p-value $\leq$ 0.05) between the data using SPSS 16.0 software. EC_{50} values of the crude peptides were determined using the Origin 8.0 software (OriginLab Corporation, USA).

3. Results and Discussion

For the past three decades, researchers are vigorously working on the valorization of feather keratin using keratinolytic microorganisms and their enzymes. In the early 1990s –2010, the research was mainly oriented to the production of feather meal for use as an animal feed supplement or a nitrogen fertilizer. Recently, the orientation of the research has been shifted toward the exploration of other medicinally important compounds/peptides extracted from feather hydrolysate. The present study focused on the bioprospecting of peptides prepared from feather keratin by the action of keratinolytic bacteria and the keratinase enzyme.

3.1. Peptide preparation and characterization

The bacterial strains, namely *Bacillus* sp. RCM-SSR-102 and *Streptomyces tanashiensis* RCM-SSR-6, could degrade feathers completely (100% weight loss), whereas KER-102 keratinase could achieve 85% feather weight loss. Here, 100% weight loss implies that all the feather barbules and rachis are degraded into 1 mm or less. The pattern of soluble protein/peptides present in feather hydrolysate (without passing through the 10 kDa filter) and crude peptides (filtered through the 10 kDa filter) prepared with different microbial and enzymatic methods was analyzed by SDS-PAGE. The crude peptide samples showed peptide bands below the 10 kDa standard protein marker. This indicates that the molecular masses of the petides were less than 10 kDa (Fig. 1). For the feather hydrolysate, protein bands with MW > 10 kDa were observed (Fig. 2). A

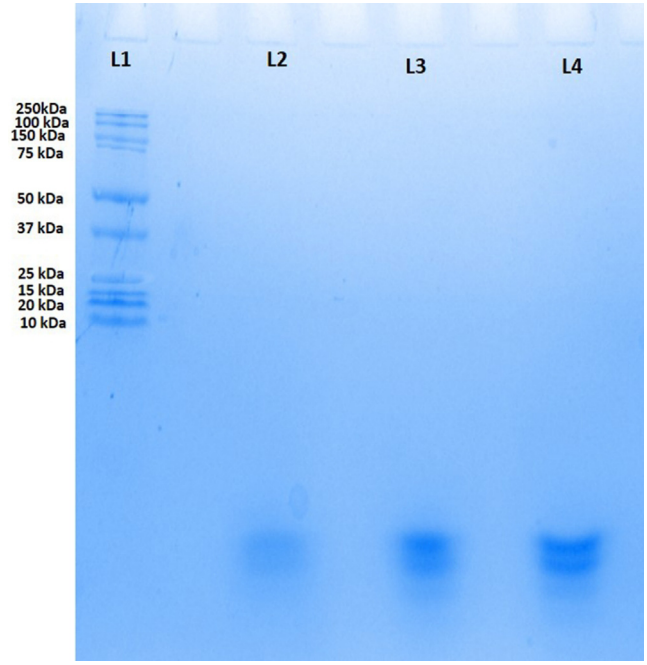


Fig. 1. Tricin SDS-PAGE of crude peptides, lane (1) Standard protein marker, (2) peptide-6 (3) peptide-102, and (4) peptide-KER102.

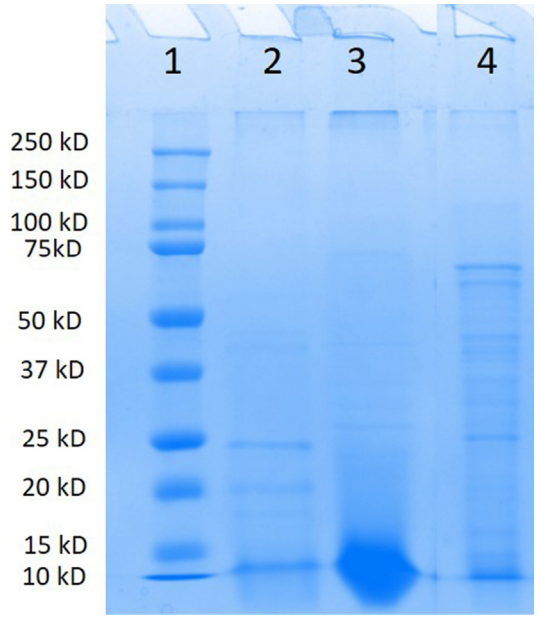


Fig. 2. SDS-PAGE of feather hydrolysates, lane (1) Standard protein marker, (2) RCM-SSR-6 feather hydrolysate, (3) RCM-SSR-102 feather hydrolysate, and (4) KER-102 feather hydrolysate.

difference in the protein/peptide bands was observed in both crude peptide and feather hydrolysate samples (Fig. 1 and Fig. 2). This indicates that a plethora of BPs can be generated from keratin using different microorganisms or keratinase enzymes. Similarly, hydrolysis of milk protein by different strains of *Lactobacillus* spp. or proteolytic enzymes could generate various peptides of different lengths and amino acid compositions with various bioactivities [22]. Low-molecular-weight bioactive peptides have many advantages over macromolecular peptides; hence, they are preferable for therapeutic use. For instance, small peptides from soybean hydrolysate have a higher rate of absorption than large peptides in the rat intestinal perfusion model [23]. Moreover, keratin hydrolysates have gained popularity in cosmetic industries, and low-molecular-weight keratin peptides can easily penetrate into the skin and protect skin by providing nutrition and moisturization [24].

The crude peptides were characterized by FTIR analysis (Fig. 3a). The FTIR spectrum is one of the widely used techniques to study the secondary structure of a protein. The secondary structure of protein/peptides consists of α-helix, β-sheet, β-turn, and random coil. The feather peptides showed a typical amide I and II peak at 1700–1600 cm^{-1} and 1600–1500 cm^{-1} respectively. The secondary structures are mainly present in the amide I of protein. In amide I region, the featured peak of α-helix is at 1646 to 1664 cm^{-1}, and for the β-sheet, the featured peaks are at 1610–1640 cm^{-1} and 1682–1700 cm^{-1}. The β-turn typical peak is reported to be absorbed at 1664–1681 cm^{-1}, and the random coil configuration peak is found at 1637–1645 cm^{-1} [25,26]. The deconvoluted graph of the amide I region is presented in Figs. 3c-d. The crude peptides exhibited different combinations of secondary structures. For instance, peptide KER-6 contained 100% β-structure, KER-102 contained 43.60% α-structure and 56.40% β-structure, and peptide-KER102 contained 68.38% α-structure and 31.62% β-structure (Table 1). Feather keratin contains both α-helix and β-sheets secondary structure; however, the proportion of β-sheets is higher than that of α-helix [27,28]. Peptide-102 contained higher β-sheets than α-helix, while in peptide-KER102, the secondary α-helix structure was slightly higher than β-sheet. This observation further established that hydrolysis of keratin by whole-cell bacteria and cell-free keratinase enzyme yields peptides

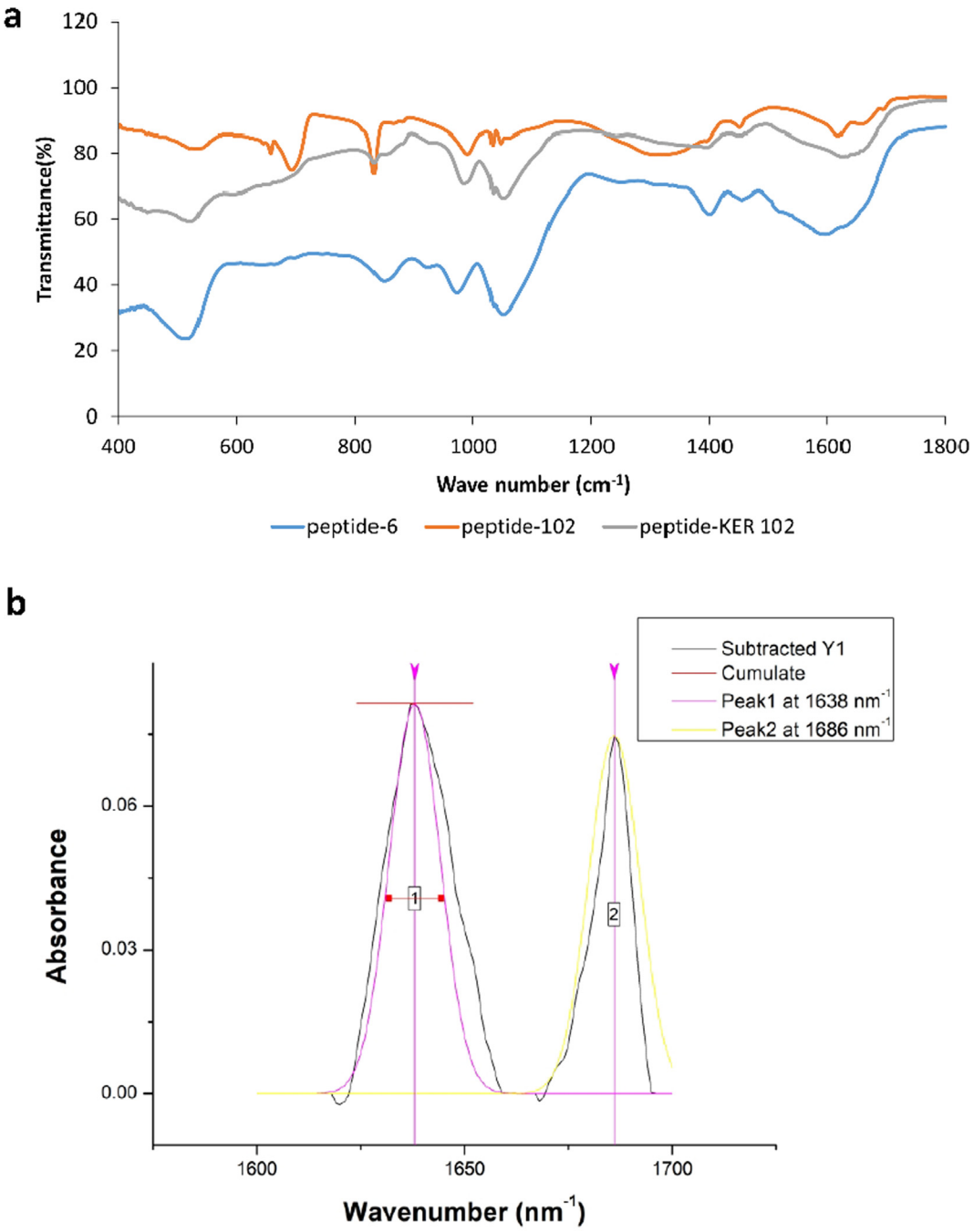


Fig. 3. FTIR spectra of peptides (a) Deconvoluted graph of peptide-6 (b) peptide-102 (c) and (d) peptide-KER102.

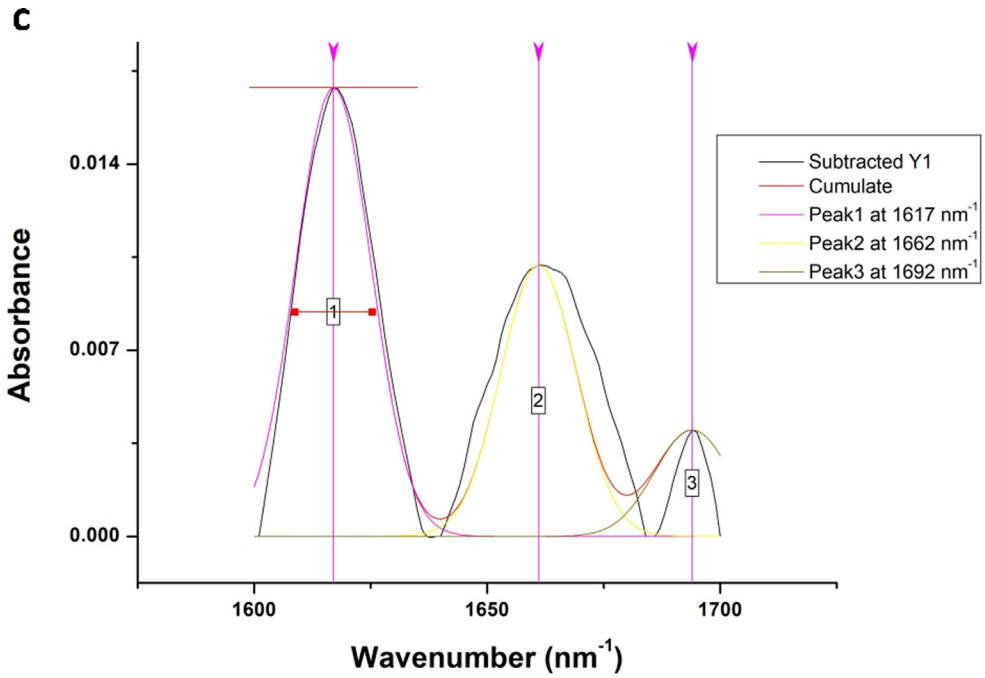
of different secondary structures. Characterization of peptides in terms of their secondary structure is very important as there is a significant correlation between the peptide's secondary structure and bioactivity. For instance, Yuan et al. [26] demonstrated that the conversion from α-helix to β-sheet of milk protein peptide enhances the antioxidant activity of the peptides.

3.2. Bioactivity of peptides

To explore the therapeutic and pharmaceutical potential of feather peptides, the three crude feather peptides were screened for five bioactivities, namely antioxidant, antityrosinase, ACE inhibitory, lipoxygenase inhibitory, and xanthine oxidase inhibitory activity.

3.2.1. Antioxidant activity

The antioxidant activity of the feather peptide samples is presented in Table 2. The peptides sample showed good antioxidant activities as revealed by their EC_{50}. The highest antioxidant activity was exhibited by peptide-KER102 followed by peptide-6 and peptide-102. Several studies have reported the antioxidant activity of feather peptides or hydrolysates [2,4,14]. Purification and identification of the amino acid sequence of antioxidant peptides (Asn-Leu-Cys-Arg-Pro-Cys-Gly) from feathers have been reported in the literature [6].



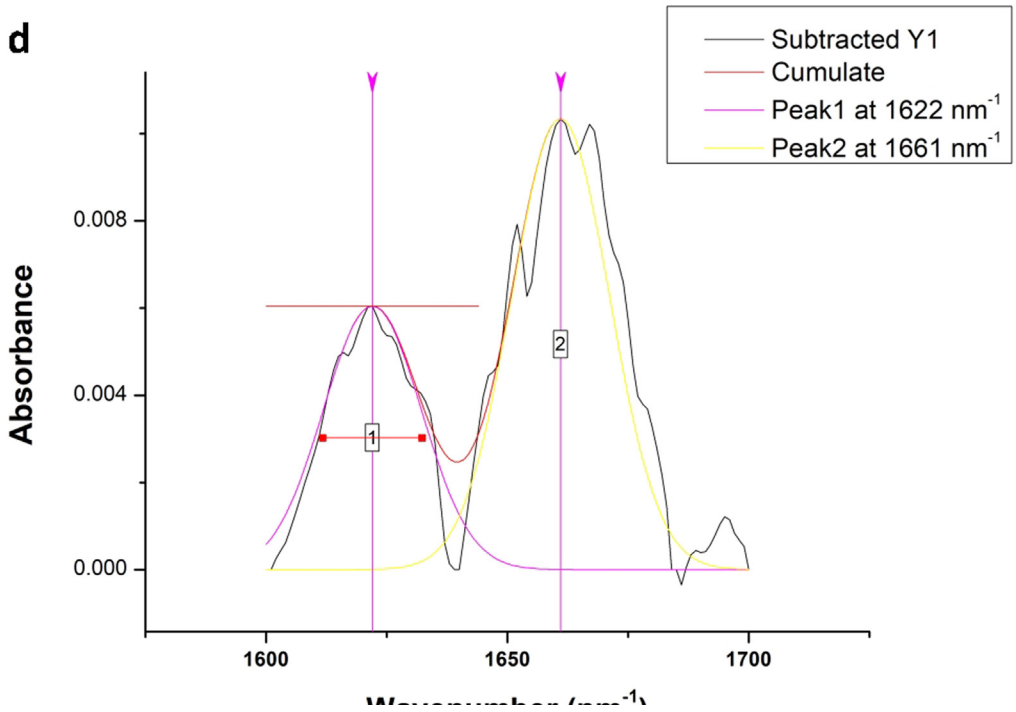


Fig. 3 (*continued*)

3.2.2. Antityrosinase activity

Tyrosinase enzyme inhibitors are used for treating skin hyperpigmentation such as melasma and diabetic dermopathy and in the cosmetic industry. Thus, the antityrosinase activity of the peptide samples was measured using the mushroom tyrosinase enzyme (Merck Sigma-Aldrich). The three feather peptide samples showed anti-tyrosinase activity. Except for the KER-102 peptide, the other two peptide samples exhibited lower EC_{50} values than the standard Kojic acid (Table 3). The antityrosinase activity of feather hydrolysate prepared using KER-102 keratinase was previously reported by our group [14]. The crude peptides demonstrated lower EC_{50} than the feather hydrolysate indicating that low-molecular-weight (<10 kDa) peptides are more effective in inhibiting the tyrosinase enzyme. Antityrosinase compounds are being

Table 1
Composition of secondary structures present in feather peptides.

Secondary Structures	% of secondary structures in peptide-6	% of secondary structures in peptide-102	% of secondary structures in peptide- KER102
α-helix	-	43.60	68.38
β-sheet	100	56.40	31.62
Random coil	-	-	-
β-turn	-	-	-

Table 2
Antioxidant activity of feather peptides.

Peptides	ABTS Assay (EC_{50} in μg/mL)	FRAP value (mM of Fe^{2+} for mg/mL sample)
Peptide-6	20.85 ± 3[a]	0.46 ± 0.004[a]
Peptide-102	75.0 ± 1.2[b]	0.17 ± 0.002[b]
Peptide-KER102	23.15 ± 2[a]	1.80 ± 0.05[c]
Ascorbic acid (Positive control)	3.0 ± 0.05[c]	11.60 ± 0.82[d]

Values with the same alphabet within a column are not statistically significant at a 5% level of significance according to Tukey's HSD test.

Table 3
Antityrosinase and ACE inhibitory activity of feather peptides.

Peptides	Antityrosinase activity (EC_{50} in μg/mL)	ACE inhibitory activity (EC_{50} in μg/mL)
Peptide-6	4.06 ± 0.65[a]	1995.6 ± 6.38[a]
Peptide-102	10.21 ± 0.82[b]	No activity
Peptide-KER102	842.0 ± 12.2[c]	1177.8 ± 20[b]
Kojic acid (Positive control)	27.04 ± 1.4[d]	-
Captopril (Positive control)	-	0.00458 ± 0.0[c]

Values with the same alphabet within a column are not statistically significant at a 5% level of significance according to Tukey's HSD test.

used as cosmetic ingredients and in the treatment of skin hyperpigmentation such as melasma and diabetic dermopathy [29]. Hence, these peptides may be a good ingredient in skincare products.

3.2.3. ACE inhibitory activity

ACE inhibitors are widely used in the treatment of hypertension. Among the three samples, peptide-6 and peptide-KER102 exhibited ACE inhibitory activity. The EC_{50} values of the peptides and standard captopril are presented in Table 3. The ACE inhibitory activity of feather hydrolysates has been reported previously [4]. Similarly, peptide-6 and peptide-KER102 also exhibited ACE inhibitory activity. Interestingly, the peptide-102 did not show ACE inhibitory activity. Peptide-102 was obtained by hydrolysing the feather with the microbial strain *Bacillus* sp. RCM-SSR-102. Peptide-KER102 was obtained by hydrolysing the feather with KER-102 keratinase purified from *Bacillus* sp. RCM-SSR-102. This indicates that hydrolysis of a feather by whole microbial cells or by a purified enzyme produces different peptide profiles. This observation is also indirectly supported by SDS-PAGE, wherein hydrolysates showed different protein profiles. The difference in the secondary structures may be another reason for the feather peptides showing different bioactivities.

3.2.4. Lipoxygenases (LOX) inhibitory activity

Lipoxygenases are a group of oxidative enzymes with a non-heme iron atom in their active site, which are involved in the regulation of inflammatory responses through the generation of pro-inflammatory mediators. The crude feather peptides were screened for LOX inhibitory activity by using the LOX Inhibitor Screening Assay Kit (Cayman, USA). The enzyme used in this assay kit is 15-lipooxygenase. All three crude peptides showed lipoxygenase inhibitory activity. The enzyme-hydrolyzed peptides showed lowest EC_{50}. Nordihydroguaiaretic acid (NDGA), was used as a positive control (Table 4). Lipoxygenase (LOX) enzymes are involved in inflammatory reactions and induce prostaglandin formation. Inhibitors of these enzymes can be used for the development of an analgesic drug [30]. The peptides revealed positive results for lipoxygenase-15 inhibitory activity. Inhibition of LOX enzyme by an octapeptide obtained from casein has been reported [31].

Table 4
Lipoxygenase and xanthine oxidase inhibitory activity.

Peptides	LOX inhibition (EC_{50} in μg/mL)	XO inhibition (EC_{50} in mg/mL)
Peptide-6	297.2 ± 14.2[a]	3.75 ± 0.05[a]
Peptide-102	174.16 ± 8.6[b]	1.12 ± 0.02[b]
Peptide-KER102	95.40 ± 6.2[c]	2.6 ± 0.1[c]
Nordihydroguaiaretic acid (Positive control)	0.4832 ± 0.06[d]	-
Allopurinol (Positive control)	-	0.077 ± 0.004[d]

Values with the same alphabet within a column are not statistically significant at a 5% level of significance according to Tukey's HSD test.

3.2.5. Xanthine oxidase (XO) inhibitory activity

XO catalyzes the oxidation of hypoxanthine to xanthine, and it can further catalyze the oxidation of xanthine to uric acid. XO inhibitors are being used for treating gout [32]. The crude feather peptides were screened for XO inhibitory activity. All the peptides showed inhibition against XO. The EC_{50} values of the peptides and standard (allopurinol) are presented in Table 4. The peptides could inhibit XO effectively as indicated by their low EC_{50} value. XO inhibitory peptides have been reported in the literature, for instance, Yu et al. [33] have reported a novel tetrapeptide, EEAK from tuna protein, which exhibited XO inhibitory activity.

4. Conclusions

The present study revealed that microorganisms/enzymes can be used to produce feather hydrolysate having different protein or peptide profiles. This implies that there is a possibility to generate a variety of BPs from feather keratin if hydrolysis is performed by using different bacterial species and extracted enzymes. It was also observed that peptides have diverse beneficial bioactivities such as antioxidant, antityrosinase, ACE inhibitory, LOX inhibitory, and XO inhibitory actvities. The antioxidant, antityrosinase, and ACE inhibitory activities of feather hydrolysate have been reported earlier. To the best of our knowledge, this is the first report on lipoxygenases and XO inhibitory activity of feather peptides. Further research on the effect of various experimental conditions such as time, pH, and temperature on the production of protein or peptide profile as well as on the identification of the purified BPs is required to explore the insight of the therapeutical potential, pharmaceutical, and other health beneficial properties.

Author contributions

- Study conceptualization and design: SS Roy, P Kshetri
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- Analysis and interpretation of results: P Kshetri, TS Singh, R Chongtham, K Tamreihao, HN Singh, MN Singh
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Conflict of interest

The authors declare no conflict of interest.

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