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Extraction of keratin from *Pavo cristatus* (Peacock) feather using HPLC

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ABSTRACT

For the first time attempted to extract keratin from peacock feathers using HPLC. Our study was successful and an amount of 1.2 mg of keratin was extracted. Chromatogram of peacock feather, showed a retention time at 4.661mints which was close to the retention time of standard keratin at 5mints, and the concentration of the compound was calculated as area under the curve /100. From this study it may be concluded that, peacock feathers may also be considered as one of the feasible options for extraction of keratin.

Figures : 02 References : 20 Table : 01
KEY WORDS : Beta Keratin, Chromatogram, HPLC, Keratin, *Pavo cristatus,* Peacock feathers.

Introduction

Pavo cristatus, is the scientific name of the blue Indian pea fowl, which is the national bird of India. It belongs to the class Aves and the order *Galliformes.* The bird is tall and slender adorned with beautiful iridescent tail feathers. These feathers typically have an eye pattern in the centre, making the bird look stunningly attractive and appealing to the female birds. The colors and eye pattern displays are not just attractive to peahens, but also to human eye. These feathers are often collected and kept as items of decoration in many cultures because of their aesthetic value and the design aspects have been extensively studied by specific author[3]. These feathers are not exclusively valued for their aesthetics, but are also known to have medicinal properties. These feathers as “bhasma” (ash) are frequently used in Indian traditional medicinal system, Ayurveda. Many authors [14] have extensively studied the medicinal properties of peacock feathers. These feathers when prepared as ash or bhasma has been frequently used against snake bites. Additionally this ash can also be used to treat other diseases such as coughing, vomiting, and other respiratory disorders [10]. Usage of Peacock feathers to treat certain respiratory conditions, such as lung abscess has also been reported even in Tibetan and Chinese medicine[9]. Studies by certain authors [17] who have distinctly analysed the constituents of peacock feathers, suggest that the feathers contain thirteen different elements, of which mercury content was found to be greater than others. Analytical studies by various authors [13, 16] revealed that the major protein of peacock feather is the beta keratin, which is known to have quite a number of impressive biomedical properties.

Keratin is the main constituent of bird feathers, wool, hooves, horns *etc.* Keratin exists as alpha and beta keratin. It is the naturally occurring biopolymer of great importance both industrially and commercially. Ideally keratin is extracted from chicken feathers and human hairs, and not many studies have been reported about its extraction from peacock feathers. Peacock, which is the national bird of India, whose scientific name is *Pavo cristatus*, is frequently found in our campus of Telangana Mahila Viswavidyalayam Hyderabad. As feathers are the best source for keratins, we speculated that peacock feathers may also be ideal for keratin extraction. Selection of peacock feathers for keratin extraction was ideally for two reasons. One as a source of beta keratins and secondly these feathers have quite a number of medicinal properties which have been widely exploited in various systems of alternative medicine. Various studies have identified keratin as a best suitable natural biopolymer for diverse industrial and biomedical applications, and hence its extraction from different sources becomes significant.

Keratin, biofilms, keratin hydrogels, are commonly used for wound healing[5], regeneration of skin, in nerve regeneration[4] and also in pulp dentine regeneration [20].

Based upon the studies, it is quite evident that keratin in particularly, beta keratin of peacock feathers seems to possess a wide range of valuable biomedical traits, which are exclusive to these feathers and hence the present study was attempted to extract keratin from peacock feathers using HPLC method.

Material and Method

Peacocks are frequently seen in the campus of Telangana Mahila Viswavidyalayam, Koti, Hyderabad. It is quite fascinating to see these birds, in the campus of this university, which is located in the heart of the city. These birds, stroll around the campus, at different times, and occasionally drop their feathers as they move around (**Fig.1)**. These feathers were collected, preserved, over a period of time and were used to extract the main protein keratin using HPLC (High Performance Liquid Chromatography).The feathers were collected and weighed, which were approximately 15g. Later they were treated with a solution of 2-mercaptoethanol and urea to break down keratin.

HPLC Setup: Column: C18 reverse-phase column.

Mobile Phase: Gradient of water with 0.1% TFA and acetonitrile.

The sample was loaded in to the HPLC unit. HPLC set up consists of the following sections. A Reservoir, a pump, sample injector, columns, and detector. Solvent reservoir, is a glass reservoir, in which the mobile phase is placed and the unit has a pump which aspirates the mobile phase from the solvent reservoir and forces it through the system's column and detector. Depending upon column dimensions, particle size of the stationary phase, the flow rate and composition of the mobile phase, high operating pressures was generated. There was sample injector and columns between 50 and 300 mm long and had an internal diameter of between 2 and 5 mm. They were filled with a stationary phase with a particle size of 3–10 ìm. Detector was located at the end of the column and detects the analyte as they eluted from the chromatographic column. Finally, there was a data collection device, which collected the signals from the detector on chart recorders or electronic integrators. The computer integrated the response of the detector to each component and placed it into a chromatograph that is easy to read and interpret.

Results and Discussion

Our experimentation with peacock feathers was quite successful. After the successful run of HPLC, a chromatogram was obtained.

According to the chromatogram, a peak was recoded at 4.661 RT (retention time) (Table-I) which is very close to 5 RT which is the standard eluting time for Keratin. Therefore the peak obtained at 4.661 (**Fig. 2**) could be considered to be that of Keratin. The area under the peak was 1027147. The concentration of Keratin was calculated according to the formula

Concentration of the compound = Area under the peak /100 = 1027/100=1.2 mg/L. Feather weight was found to be 15 g, from which 1.2mg of keratin had been extracted. Though not very efficient method but some keratin was successfully extracted.

In the present study, we were very successful in extracting keratin, an important protein from the peacock feathers. This study may be first of its kind, where keratin was extracted from peacock feathers using the HPLC method. Keratin is a fibrous protein and is found as α keratin and beta keratin naturally occurring in animal's structures as feathers, hooves, horns and epidermal scales of reptiles [6 12]. Peacock feather is typically made up of β keratin, which is slightly different from α keratin. Beta keratin contains glycine-proline-rich and cysteine-proline-rich proteins and the secondary structure of Beta keratins, contains Beta pleated sheets along with alpha helices [11]. Beta keratins are tougher than alpha keratins. Beta keratins found in the feathers, have some unique features such as high water resistant ability, indigestible by enzymes, such as trypsin, pepsin in dilute acids and salt solutions [18, 19]. They can perfectly camouflage, and also act as excellent thermal insulators[15] and hence

TABLE -1 : Peak table of the Chromatogram

Peak Number	Name	Retention Time	Area	Area %	Relative Retention time
1	Keratin	2.009	254881	15.436	0.431
2		2.308	369164	22.357	0.495
3		4.661	1027147	62.206	1.000
			1651193	100.000	

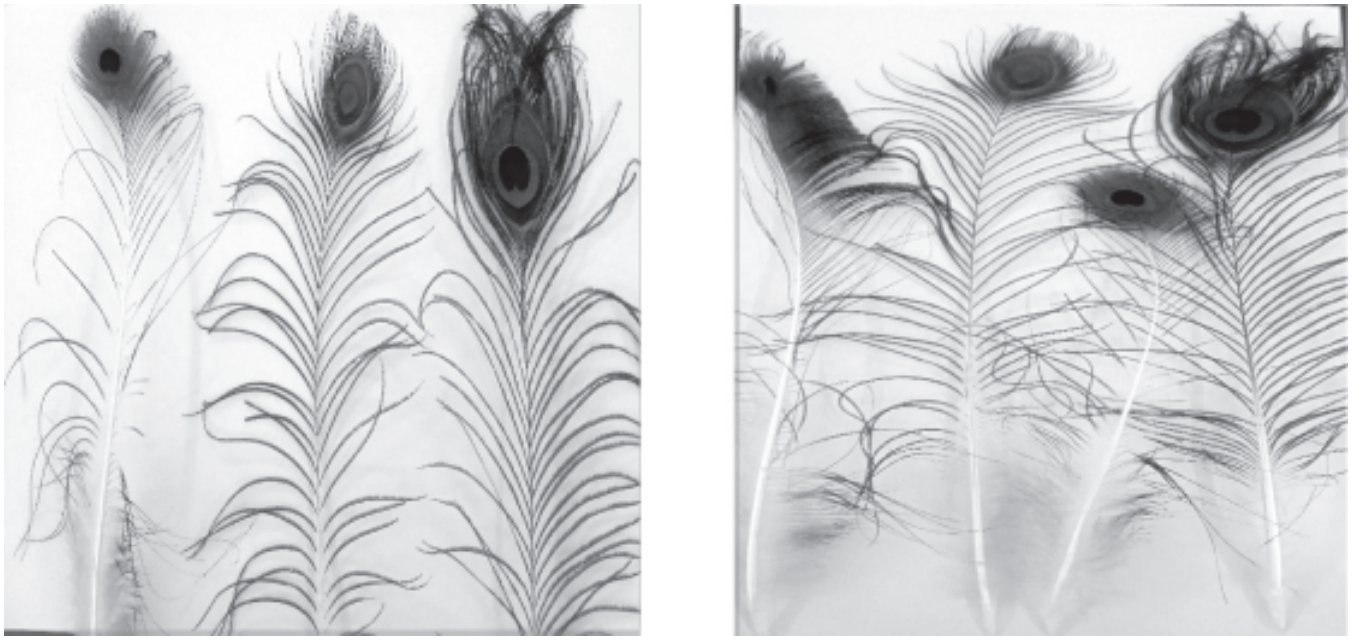

Fig. 1 : Feathers Collected from *Pavo cristatus*

make them a perfect fit for a bird's body. The complex arrangement of keratin fibres produces quite a number of mechanical properties and therefore capable of serving variety of functions, such as acting as diffusion barrier, averting any opponent attacks, opposing any kind of recurring stress and also importantly helping in deflecting in side movements as reported by certain authors [2]. More over keratin is a naturally occurring biopolymer coming next to chitin and has been widely studied for its tensile strength and various applications[1].

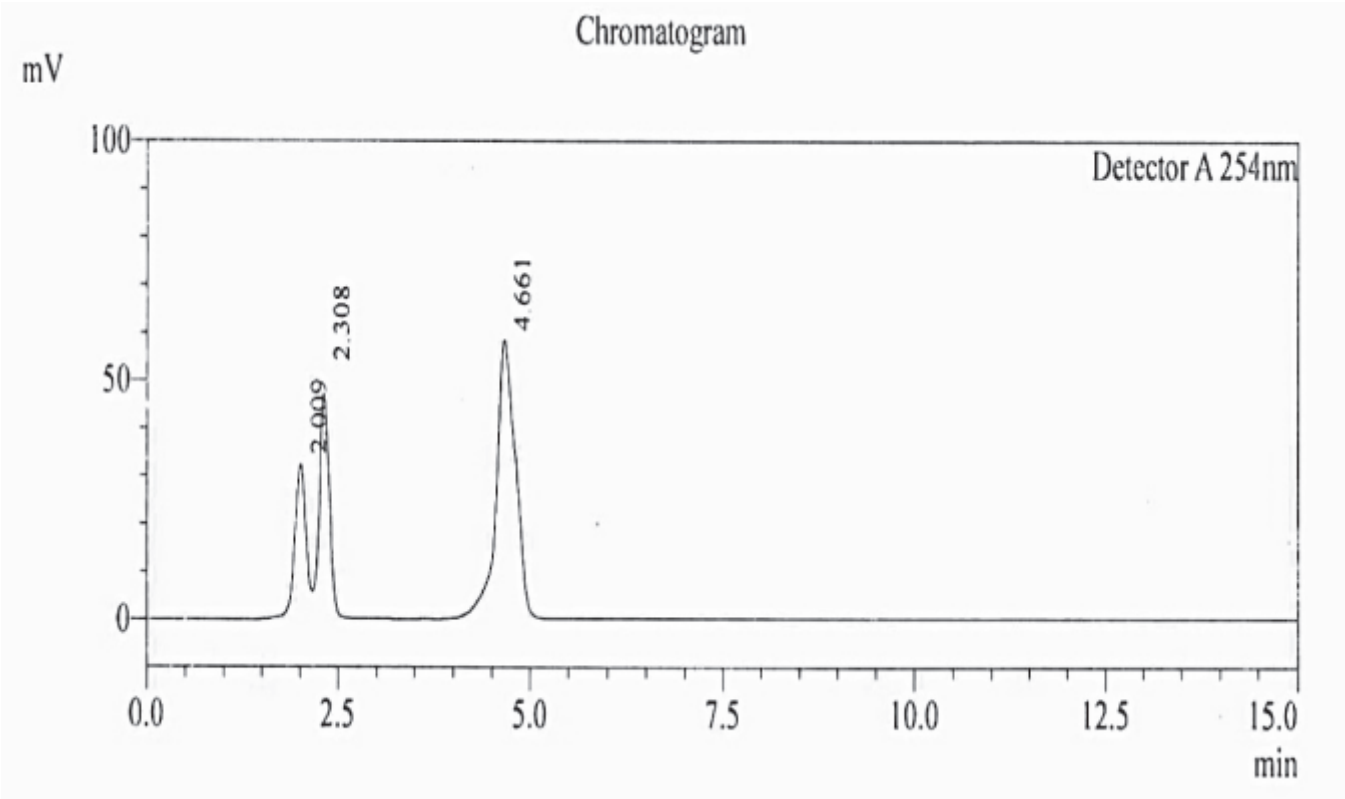

Fig.2 : Chromatogram of Keratin extracted from Peacock feathers,
X-Axis (Horizontal): Retention Time in minutes, Y-Axis (Vertical): Detector Response in mV (milli volts)

Beta Keratin has better mechanical properties, tensile strength and also has the ability of reversible adhesion through Vander Waal's forces[1] and so makes it best suited for aerodynamic body of the birds. Keratin fibres are frequently used as biofilms, as bone morphogenic protein carrier (BMP), for ocular surface construction and also for tissue scaf folding in tissue engineering [7]. Apart from these applications, specific and exclusive uses of beta keratins have been reported from the studies conducted by certain authors[8].Their studies, reported hair based keratins (Beta keratins) as more suitable to incorporate bioactive molecules and to act as suitable molecules for controlled release of biomolecules. This property is of great importance and has been well exploited as drug delivery agents in biological systems. These studies distinctly highlight the importance of beta keratin which can be extracted from feathers and hair. Identifying the value of beta keratins, the present study, was attempted and a considerable amount of keratin from peacock feathers, more specifically the beta keratin, was extracted. Though keratin can be extracted from other animal structures and beta keratin from feathers, and hairs extraction from peacock feathers makes this study unique and distinct.

Though the present method may not be the best approach, for extraction, as the yield of keratin was quite low, in future better methods may be developed for simple dissolution of peacock feather followed by keratin extraction.

Conclusion

Beta keratin can be exclusively extracted from bird feathers, and therefore peacock feathers may also be considered as a viable source for the extraction of keratin, which is one of the highly valued biopolymer.

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