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Extraction of Keratin from Human Hair with the Production of Biofertilizer

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KEYWORDS

Hair waste,
Keratin,
Fertilizer,
Seeds.

ABSTRACT:

Keratin has been attracting interest due to its stability against enzymatic degradation thereby allowing more predictable degradation profile for tissue regeneration. While the efficacy of keratin has been demonstrated indifferent models, there has been no systematic study to investigate and compare the different routes of keratin extraction from human hair. Here, undertaken work was designed to optimize the parameters for the keratin extraction from human hair with production of biofertilizer. Keratin extraction from human hair, hair dissolved in NaOH and stirred at 400C. The aliquot was then centrifuged at pH of 2-3.5, following which the precipitated keratin was extracted, dried and pulverized. The conformation study of the extracted keratin was done by performing xanthoproteic test, quantification of protein by Lowry's method and separation of protein by SDS-PAGE. The keratin powder was compared with infected leaf by diffusion method. To prepare the foliar spray. However, to study the fertilizing property foliar spray from keratin, they are applied on seeds and recovered its biometric characteristics for 60 days. These results provide new insight into the extraction of keratin from human hair with implications for its use as an organic fertilizer.

1. Introduction

1.1 Human Hair:

Hair is one of the characteristic features of mammals and serves as a unique mini-organ. In humans, hair has different functions such as production against external factors; impact on social & sexual interactions; producing sebum, sweat, and pheromones; thermoregulation, and being a resource for stem cells (Bilgen Erdogan, 2017). Hair is a filamentous biomaterial consisting mainly of proteins in particular keratin. Hair consists of two distinct structures: follicle- the living part under the skin and hair shaft- a fully keratinized non-living part above the skin surface. The hair shaft consists of three layers: cortex, cuticle, and in certain cases medulla (Bilgen Erdogan,2017). The structure of human hair is well known, the medulla is a loosely packed, discarded region near the center of the hair surrounded by the cortex, which contains the major part of the fiber mass, mainly consisting of keratin proteins and structural lipids. The cortex is surrounded by the cuticle, a layer of dead overlapping cells forming a protective layer around the hair. The lower hair follicle, the hair matrix, and the hair bulb which is the active reproductive portion of the hair follicle. The matrix cells are localized to the lowermost portion of the follicle and surrounded all sides of the follicular papilla. The hair shaft and IRS are derived from the matrix cells. To produce the main structural components of hair, they also produce the hair keratins and their associated proteins. Keratin proteins can be divided into two families: type1(acidic) keratins and type2(basic-neutral) keratins. The keratin-associated protein is a large group of proteins constituting the keratin matrix. The matrix proteins are separated into three major subgroups according to their amino acid composition. Different hair

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and epithelial keratins are expressed in the various concentration layers of the hair follicle, with hair keratins found primarily in the cortex and hair cuticle (Bilgen Erdogan,2017).

Every year, about 30,000 tons of human hair is treated as waste and discarded as such (Jyoti Singh,2020) (Hanna Lee1,2, Kwantae Noh. Et al., 2014). Although human hair is biodegradable (Tapan Kumar Maity, Nripat Singh, 2022) but takes more than two years to biodegrade, which causes environmentally difficult disposal problems. It is generally found in municipal waste streams in almost all world cities. Villages and areas with low population density throw away hair in their surroundings. Then it slowly undergoes a decomposition process for several years. Therefore, from the view of the economy and environment protection process to use these resources. Currently, many natural proteins have been applied in many functional applications. A film based on human hair keratin was used as cell culture and tissue engineering substrates. A film made of human hair as a nail plate model studied drug permeation (Gayathri Unnikrishnan1, Vijayaraghavan Ramasamy,2020). Hair eventually returned to its constituent elements, such as carbon, nitrogen, and sulfur. Human hair is considered a waste in most of the world but on the other hand waste of human hair are used in many industries. For example, cosmetics, test material for hair care products, making cosmetic brushes, fertilizers, pharmaceuticals, fabrics, artwork, pollution control, and remediation, and oil-water separation and oil spill remediation. This study suggests that non-composted hair waste could be used as a nutrient source for container-grown plants.

Hair waste should not be used as a single nutrient source for fast-growing plants because of the time needed for the degradation of the hair before releasing plant nutrients. Hair contains all the nutrients required for plant growth. Elements of proteins are nitrogen, oxygen, hydrogen, carbon, hydrogen, phosphorus, sulfur, and potassium thus macro-elements are needed for plant growth. The main chemical elements present in the hair are composed of 45% carbon, 28% oxygen, 15% nitrogen, 6.7% hydrogen, and 5.3% sulfur. Hair also contains water 12 to 15% of any traces of mineral elements (zinc, iron, silicon, calcium, cadmium, chromium, and copper). These elements can be brought to the base of the hair follicle by blood circulation and then contribute to building the hair shaft. The hair also contains lipid components (3% of its composition). They are produced in the hair bulb from sterols, fatty acids, and ceramides.

Mats made of human hair are used to protect plant roots from bad weather and insects. An American company called SmartGrow is working on this. The company imports tons of hair from India and China and processes it into circular mats. These mats are placed on the sandy surfaces of the potted plants. Thus, the insects cannot attack the roots of the plant beyond the mats.

India exports most of its Human hair to China, the United States, and Myanmar and is the largest exporter of human hair in the world. The top 3 exporters of human hair are India with 207,163 shipments followed by Indonesia with 27,138 and China at the 3rd spot with 18,383 shipments. In 2021, the worldwide market value stood at USD 3.71 billion.

Dr. Prasad estimated that 1 kilogram of human hair can give 200gram of melanin, 360 grams of keratin, and 300 milliliters of ionic liquid, which can be used as a fertilizer. “We have extracted the crude form of melanin. If we further refine it to make it free of sulfur, it can be more expensive and valuable”.

1.2 Keratin:

Hair is made up of a tough protein called keratin. The word kera is derived from the Greek word meaning horn. Keratin fibrous protein is found in healthy hairs. Keratin is one of a family of structural fibrous proteins also known as scleroprotein. Keratins are the proteins of epidermal and skeletal tissues which are insoluble in the usual protein solvents, not digested by trypsin or pepsin, and high in cystine content (Vidmar and Vodovnik, 2018) (By David R. Goddard and Leonor Michaelis, 1934). Most of the cortical cells are composed of a protein known as keratin. These keratins are characteristically found in the epithelial cells. Keratin, like all proteins, is made up of amino acids. Each protein contains a specific order of amino acids, much like each protein containing its string of deoxyribonucleic acid (DNA). In humans, keratin is encoded by 54 genes. At the molecular level, keratin is a helical protein. Two types of keratin fibers exist in hair: type 1 with acidic amino acid residues and type 2 with basic amino residues. In proteolysis, bacteria, and fungi can degcannous substrates proficiently due to their ability to

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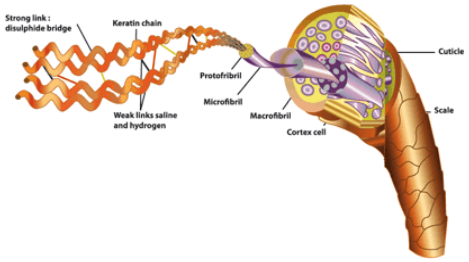
secrete extracellular keratinase enzyme into the medium. Keratinophilic microbes attack keratin substrates in or on soil; therefore, biodegradability takes place. Keratins are abundantly available as by-products mainly from slaughterhouses and poultry plants in the form of skin wastes hair, horns, hooves, feathers, and claws, etc., (McKittrick et al.,2012; Chilakamarry et al.,2021) (Gayathri Unnikrishnan1, Vijayaraghavan Ramasamy,2020) it is cause a deteriorating effect on human and environment. The main component of human hair is are keratotic protein, which is 65-95% of the total mass (Gayathri Unnikrishnan1, Vijayaraghavan Ramasamy,2020). There are two major protein groups: alpha keratin and beta keratin. Alpha-keratins, which are found in the hair, the skin, and the wool of mammals, and beta-keratins, which occur in birds and reptiles, consist of parallel sheets of a polypeptide chain. The keratin extracted from humans is found to be more biocompatible, less immune-stimulating when used in transplantation, and readily biodegradable. Amidst the substantial development that finds the use of keratin in various products like foods, catalysis, bone replacement, cosmetics, and fertilizers, still cumbersome keratin finds its way into landfills only due to a lack of efficient technology of contaminant-free extraction (Punam Sen, Arun.C .M., Divvyapriya.J 2021).

Keratin can be extracted using several methods such as chemical hydrolysis, enzymatic and microbial treatment, dissolution in ionic liquids, microwave technique, steam explosion technique, and thermal hydrolysis or superheated process. Keratin was extracted from human hair using agents namely, sodium sulfide, peracetic acid, urea, and thioglycolic acid. Keratin is an organic nitrogen fertilizer suitable for the nutrition of vegetables, flowers, and aquatic plants. As a major source of nitrogen, it is suitable for all crops with and increasingly demand nitrogen. In addition to nitrogen, it contains a small amount of other nutrients. The polyproteins in keratin can be converted into amino acids to serve as nitrogen sources for plant growth and then the microorganisms used in waste treatment will be another source of nutrients for plant growth. The keratin material is useful as a soil amendment providing organic and inorganic nutrients. The keratin material is also useful as a nutrient source in the bioremediation of toxic contaminants in soils and liquids. To convert keratin containing waste into biofertilizers.



In summary, keratin- containing waste is rich in amino acids serving as a valuable resource for the growth of plants. With proper treatment hair wastes which are rich in keratin can be converted into high- value biofertilizers to serve as nutrients for plants, reduce environmental pressure, and improving the quality of the soil, it's to play a role in sustainable agriculture.

1.3 Biofertilizer:

Organic farming is a form of agriculture that excludes the use of synthetic fertilizers and pesticides, plant growth regulators, and livestock feed additives, (Adetunji C, Makanjuola O.R, 2012) organic origins such as compost manure, green manure, and bone meal and places emphasis on techniques such as crop rotation and bone companion plating. The role of organic agriculture either farming processing, distribution, and consumption is to sustain and enhance the health of ecosystems and organisms (Adetunji C, Makanjuola O.R, 2012). Based on the type of microorganisms, the biofertilizer can also be classified as, bacterial biofertilizer: e.g. rhizobium, azospirillum, azotobacter and phosphobacteria; fungal biofertilizer: e.g. mycorrhiza; algal biofertilizer: e.g. blue-green algae and Azolla; and actinomycetes biofertilizer: e.g. Frankia. Biofertilizer is a substance that contains living microorganisms that, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey, 2003) (Vessey, J.K. 2003) (Packialakshmi & Aliya, 2014). Plant growing promotes rhizobacteria as biofertilizers. This definition separates biofertilizers from organic fertilizers. Increase crop yield by 20-30%, replace chemical nitrogen and phosphorus by 25%, and stimulate plant growth, using biofertilizers provides these three benefits. The latter contains organic compounds which directly or by their decay, increase soil fertility. Likewise, the term biofertilizer should not be used interchangeably with the terms, green manure,

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manure, intercrop, or organic supplemented chemical fertilizer. Not all plant growth-promoting rhizobacteria (PGPR) can be considered biofertilizers, Bacteria that promote plant growth by control of deleterious organisms are biopesticides, but not biofertilizers. Similarly, bacteria can enhance plant growth by producing phytohormones and are regarded as bio-enhancers, not biofertilizers. Interestingly, some PGPR can promote growth by acting as both biofertilizer and biopesticide or bioenhancer (Japan Atomic Industrial Forum (JAIF), 2006). Rhizobium is a genus of gram-negative soil bacteria that fix nitrogen. Rhizobium specious form an endosymbiotic nitrogen-fixing association with the roots of legumes and other flowering plants. The production and use of biofertilizer are proposed, to improve the yield of crops by using root nodule bacteria (rhizobia), mycorrhizal fungi, and other microorganisms that can increase the availability of plant nutrients from the soils. For this purpose, the most effective microorganisms for each specific crop will be identified, for example, by measuring N_2 fixation activity by using nitrogen-15 isotope as a tracer and using other methods too. Ionizing radiation is used to the carriers of rhizobia and other biofertilizer microorganisms (Japan Atomic Industrial Forum (JAIF), 2006). An, biofertilizer, is used as nitrogen to boosting fast growth, dark green leaves, phosphorus for strong root growth, and potassium for maintaining plants in full health and obtaining splendid blooms of calcium, sulfur, and magnesium.

The N_2-fixing biofertilizer contains microorganisms such as rhizobium, actinobacteria, azotobacter, and azospirillum. They help in transforming nitrogen into organic compounds. Rhizobium is an important N_2-fixing bacteria. Rhizobium lives in symbiotic association with leguminous plants, specifically in their root nodules, It traps the atmospheric nitrogen and converts it into ammonia that enhances the growth of the plant.

Biofertilizers are important for sustainable agriculture by providing nutrients to enhance the growth speed of the plant and production. Certain microorganisms, hair keratin can be converted into biofertilizers which contain nutrients for plant growth and microorganisms important for the soil ecosystem. So, the hair waste which is rich in keratin can be converted into high-value fertilizers to serve as nutrients for plants, reduce environmental pressure and improve the quality of the soil for sustainable agriculture.

1.4 Rhizobium:

Rhizobium biofertilizer is a substance that contains living microorganisms and is applied to plant surfaces, seeds, or soil. Here, the rhizobium bacteria colonize the rhizosphere or the interior of the plant to promote growth by enhancing the supply or nutrient availability to the host plant. Rhizobia are special bacteria that can live in the soil or nodule formed on the roots of legumes. It is a biofertilizer that contains Acetobacter bacteria which can colonize the plant roots and fix atmospheric nitrogen. It is especially beneficial for sugarcane plantations as it activates the soil biologically and stimulates plant growth. So, the rhizobium is used as a biofertilizer.

The role of rhizobium is known to form colonies on the root surface stimulating biological nitrogen fixation and providing nitrogen to the leguminous crops and hence considered as a significant process for improving yield and soil fertility.

Overall, the utilization of Rhizobium-derived products for plant growth enhancement offers an eco-friendly and sustainable solution for modern agriculture that promotes soil health, reduces water pollution, and enhances food quality. However, more research is needed to fully understand the mechanisms underlying Rhizobium-mediated plant growth enhancement and to optimize the use of these bacteria in agricultural settings.

Rhizobium- *Arachis hypogaea*

In this, a paper we used the Rhizobium strain used for biofertilizer. The strain was isolated from *Arachis hypogaea* (peanut).

3.METHODOLOGY

3.1 Materials required:

The materials and laboratory equipment used for this research work included Conical flasks, Distilled water,

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Hot air over, Laboratory reagents, Whatman filter paper, Microcentrifuge, Microfuge tubes, p^H meter, Conical flask, Petri dishes, Laboratory chemicals, Laboratory autoclave, Sterile cotton plugs, Spectrophotometer, Laminar air flow chamber, Incubator.

3.2 Sample collection:

Waste human hair was collected from local barber shops. Human hair samples were washed and rinsed thoroughly thrice with distilled water to remove all dirt and then drained. After drying it completely in an oven at 100^0C for 2 hours, it was used as raw material for further studies. (Tapan Kumar Maity, Nripat Singh, 2022)

3.3 Extraction of keratin from human hair:

The de-lipidized hair fibers were dissolved in 1M NaOH solution and mixed at 40^0C for a period of 1-2 hours. After 1-2 hours, the mixture was filtered by using Whatman filter paper. The aliquot was centrifuged for 10 minutes at 5000rpm and the supernatant recovered and the pH of the solution was bought to 2-3.5 to precipitate out the keratin from the hydrolysate, using 1N HCL. The solution was kept undisturbed for 2 hours to precipitate. Keratin was filtered from the precipitate and dried at 45^0C for 2 hours, following which flakes of keratin were obtained. keratin is recovered and pulverized. (Tapan Kumar Maity, Nripat Singh, 2022)

3.4 Quantification of protein by Lowry's method:

Different dilutions of BSA solutions are prepared by making stock BSA solution (1mg/ ml) and water in the test tube as given in the table. 0.2ml of BSA working standard in 5 test tubes and makeup to 1ml using distilled water. The test tube with 1ml distilled water serves as blank. Add 4.5 ml of Reagent A and incubate for 10 minutes. After incubation add 0.5 ml of Reagent B and incubate for 30 minutes. Zero the colorimeter with blank and take the optical density at 680 nm. Plot the absorbance against protein concentration to get a standard calibration curve. Check the absorbance of the unknown sample and determine the concentration of the unknown sample using the standard curve plotted.

3.5 SDS-PAGE:

The molecular weight of the keratin was estimated by the Sodium Dodecyl Sulfate- Polyacrylamide Agarose Gel Electrophoresis (SDS-PAGE) technique.

To do estimate the molecular weight of proteins on the SDS-PAGE, proteins of known molecular weight need to be run simultaneously on the gel. A mixture of these proteins is called protein standard or protein molecular weight markers. 10% Separating gel: Distilled water - 4.0ml, 1.5M Tris HCl - 2.5ml, 30% Acrylamide - 3.2ml, 10% SDS - 0.1ml, 10% APS - 0.1ml, TEMED - 0.008ml. Pour separating in-between the glass plates and allow it to solidify for an hour. Ensure no air bubbles get trapped in between. Allow the gel to polymerize for 30 mins. After polymerization prepare the stacking gel as follows: 5% Stacking gel: Distilled water - 3.4ml, 0.5M Tris HCl - 0.63ml, 30% Acrylamide - 0.83ml, 10% SDS - 0.05ml, 10% APS - 0.05ml, TEMED - 0.004ml. Place all microcentrifuge tubes containing samples for SDS-PAGE into a water bath for 15 minutes. Load all samples into gel lanes starting with the molecular weight standards. Sample loading volumes should be dependent on a gel. Load 100 µL of staining dye then all the loading samples range from 50 µL. Connect both the anode and the cathode and set the voltage on the electrophoresis power supply to a constant voltage of 150V. Allow the gel to electrophorese for 3 hours and proceed with the desired direction method.

3.6 Xanthoproteic test:

Take 0.2g of sample dissolved with 1ml of distilled water, were treated with few drops of concentrated Nitric acid. Then add 1ml of 40% of NaOH. The formation of orange color indicates the presence of protein.

3.7 Identify the microorganisms from the infected leaf:

Take an infected leaf and isolate the microorganism from the leaf sample. The infected leaf is washed with distilled water, then diluted 10-fold serial dilution.

3.7.1 Isolation of microorganisms:

To isolate the microorganism, colonies from the infected leaf a series of steps were followed. At first, the liquid sample was diluted using 5-fold serial dilution method. After that, each diluted sample was spread on a Petri plate using the spread plate technique. Mixtures were identified after 24 hours of incubation at 37^0C. Finally, they were inoculated in an LB broth medium to obtain a pure culture.

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3.7.2 Serial dilution:

Six test tubes were thoroughly washed with detergent, and 9 ml of distilled water was added to each of the test tubes, wrapped, and autoclaved at 121^0C. After autoclaving, they were brought inside a laminar airflow cabinet. Now, 1ml of water sample was added to the first test tube and mixed thoroughly with the distilled water to make a 10ml diluted solution, 1ml of solution from the first test tube was pipetted out and added to the second test tube and mixed thoroughly. Again, 1ml of solution from the second test tube was added to the third one, repeat the process till the last dilution.

3.8 Inoculation into Nutrient broth:

Three test tubes and 5ml of Nutrient broth was prepared and autoclaved at 121^0C. The broth was poured into three test tubes. A discrete colony from each of the streak plates was picked up using sterilized inoculated into each pf the three autoclaved test tubes inside the laminar air flow cabinet.

The test tubes were incubated at 37^0C on a shaker for 24 hours. After that, the culture was observed on the test tubes that are identified by the turbidity of the broth.

3.8.1 Sample preparation:

The keratin (100mg/ml) powder sample was dissolved with DMSO (Dimethyl Sulfoxide).

3.8.2 Antibiotic activity:

Four Mueller Hinten agar plates were prepared and swabs are autoclaved and poured MHA into Petri dishes. It was allowed to solidify. After polymerization, plate with the bacterial suspension or by streaking the swab three times over the entire agar surface. Allow the inoculated agar surface to dry for 3 to 5 min. Place the antibiotic discs (Penicillin) onto inoculated agar medium at adequate distance of 2 cm or more using sterile forceps. Gently press each disk onto the agar to provide uniform contact. Incubate the plate in an inverted position at 37^0C for 16-18 hours. Observe the incubated plates for the presence of inhibitory zones around the discs and measure the zone by using a millimeter scale. At the same time conduct the well diffusion method.

3.8.3 Antimicrobial test:

Four Mueller Hinten agar plates were prepared and the swab are autoclaved and poured MHA into Petri dishes. It was allowed to solidify. After polymerization, plate with the microbes suspension or by streaking the swab three times over the entire agar surface. Allow the inoculated agar surface to dry for 3 to 5 min. Place the antibiotic discs (Penicillin) onto inoculated agar medium at an adequate distance of 2 cm or more using sterile forceps. Gently press each disk onto the agar to provide uniform contact. Incubate the plate in an inverted position at 37^0C for 16-18 hours. Observe the incubated plates for the presence of inhibitory zones around the discs and measure the zone by using a millimeter scale. At the same time conduct the well diffusion method.

3.9 Rhizobium strain:

Rhizobium is used as a strain for biofertilizers. The strain was collected from *Arachis hypogaea*.

3.10 Formulation of liquid biofertilizer inoculants:

The strains used for liquid biofertilizer formulation were Rhizobium. The Nutrient broth are prepared and sterilized broth was inoculated with the respective strain and incubated at 48 hours. The culture was centrifuged at 10,000rpm for 10mins then collected the supernatant. The supernatant was equally divided there were a total of two types of liquid biofertilizer. One is supernatant only (without keratin), second one is supernatant + keratin powder (with keratin; 5ml supernatant: 1% of keratin).

3.11 Source of seeds:

The *Coriandrum Sativum L.* (Coriander)seeds used for this experiment were obtained from a nearby shop.The family name of coriander is Umbelliferae/ Apiaceae. It is also known as Chinese parsley, dhania, or cilantro. It can be grown on loamy soil and can also grow on heavy black soil (Da Costa, R.S., et.al, 2019). This plant has been have some properties that are, Anti-pasmodic, Carminative, Anti-microbial, Anti-fungal, Diuretic, and Anti-oxidant. The plant is used in Culinary, Gas, Bloating, Belching, Hiccups, Diarrhea, Indigestion, Anodyne, Modulating blood sugar, High blood pressure, and Optimizing cholesterol levels. All parts are edible. But, the fresh leaves and the dried seeds (which are both a herb and a spice) are the parts most traditionally used in cooking. Raw coriander leaves are 92% water, 4% carbohydrates, 2% protein, and less than 1% fat. The nutritional profile of coriander seeds is different from that of fresh stems or leaves. In a 100-gram reference amount, leaves are particularly rich in vitamin A, vitamin

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C, and vitamin K with moderate content of dietary minerals. Although seeds generally have lower vitamin content, they do provide significant amounts of dietary fiber, calcium, selenium, iron, magnesium, and manganese. Excessive use of inorganic fertilizers results in salt accumulation in the soil and forces the plant to spend more energy taking the water from the soil, and may result in low yield or the complete wilting of a plant (Ismail, M.M., et al.,2017). It is a cool season crop.

4. RESULTS

4.1 Hair sample:

Figure 1: Human Hair

4.2 Keratin extraction

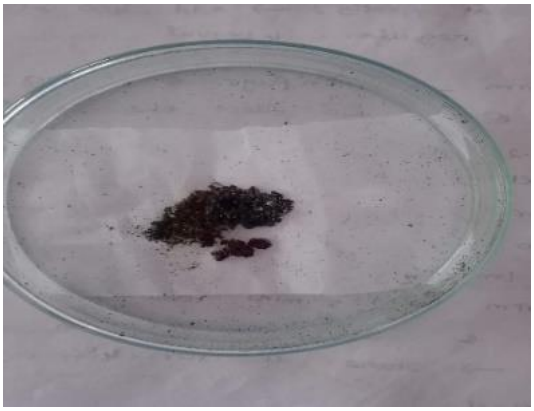
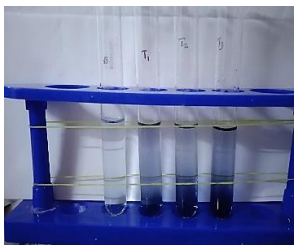


Figure 2 The keratin are extracted from human hair under the four process.-1)De-lipidization 2) Solubilization 3) Dialysis and 4) Drying

4.2 Biochemical test:

Protein Estimation:

The protein was concentration of keratin was estimated by following the method of Lowry et al(1951).



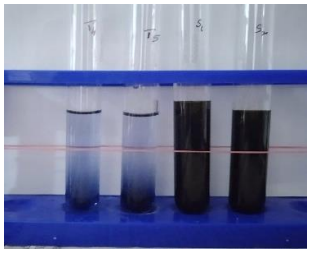


Figure 3: Quantification of protein by Lowry's method

	Vol. of BSA (ml)	Vol. of Distilled water (ml)	Vol. Reagent A (ml)	Incubation for 10 minutes at Room temperature	Vol. of Reagent B (ml)	Vol. of Folin phenol (ml)	Incubation for 30 minutes at Room temperature	OD at 680nm

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Blank	-	1	4.5		0.5	0.2		0
S1	0.2	0.8	4.5		0.5	0.2		0.2020
S2	0.4	0.6	4.5		0.5	0.2		0.2064
S3	0.6	0.4	4.5		0.5	0.2		0.3977
S4	0.8	0.2	4.5		0.5	0.2		0.4204
S5	1	-	4.5		0.5	0.2		0.4210
T1	0.5	0.5	4.5		0.5	0.2		3.2636
T2	1	-	4.5		0.5	0.2		3.1739

4.3 SDS-PAGE:

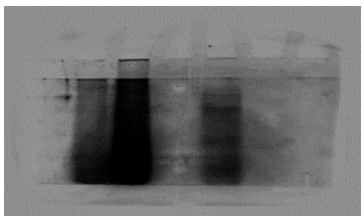


Figure 4: SDS-PAGE of standard protein molecular weight marker and hair keratin showing band.

4.4 Xanthoproteic test:

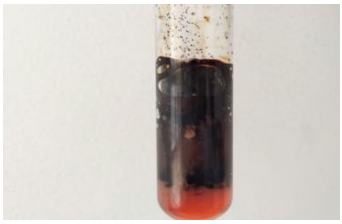


Figure 5: Sam 1(0.2g)

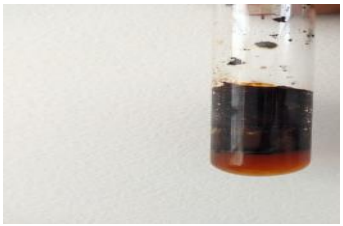
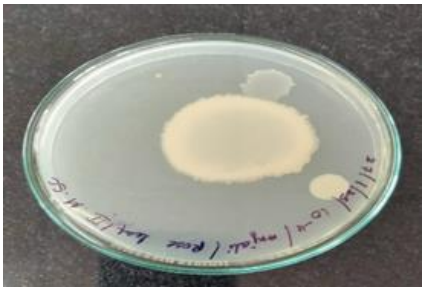
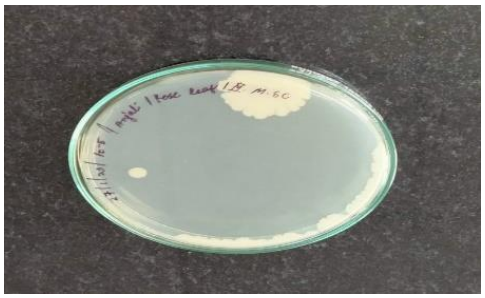


Figure 6: Sam 2(0.2g)

4.5 Identified the microorganism from infected leaf:

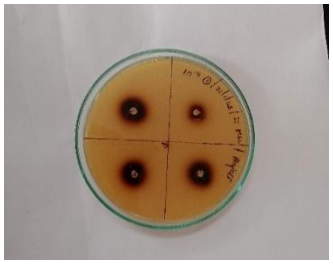


Plating 1:10^{-4}



Plating 2: 10^{-5}

4.6 Antibiotic properties:



Plating 3: $10^{-4(1)}$

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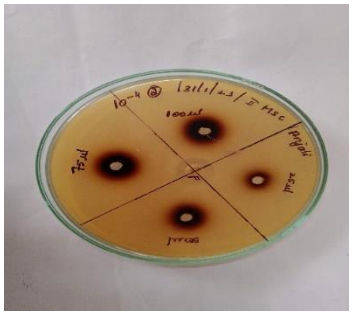
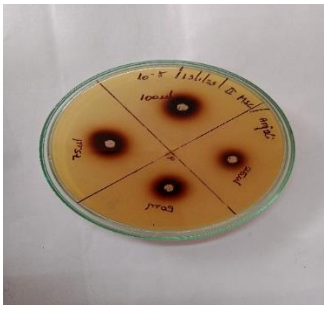


Plating 4: $10^{-4(2)}$

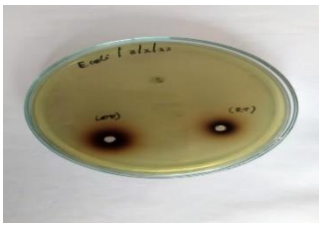


Plating 5:10^{-5}

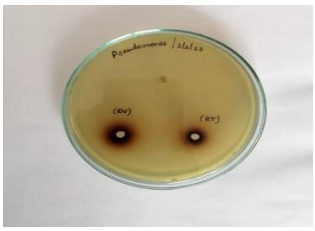
Zone of inhibition:

Microorganism	Disc	ZOI				
		Well Diffusion				Disc Diffusion
		25	50	75	100	
$10^{-4(1)}$	Penicillin	1.4	1.8	1.8	2	0
$10^{-4(2)}$	Penicillin	1.2	1.6	1.6	1.7	1.5
10^{-5}	Penicillin	1.2	1.5	1.8	1.9	0

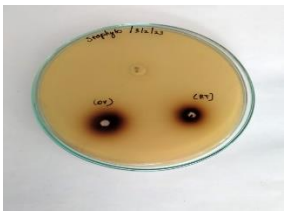
4.7 Antimicrobial property:



Plating 6: *E.Coli*



Plating 7: *Pseudomonas*



Plating 8: *Staphylococcus*

Zone of inhibition:

Microorganism	Disc	ZOI		
		Disc	Oven (Keratin+ DMSO)	Room Temperature (Keratin+ DMSO)

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E.Coli	Penicillin	0	1.2	0.9
Pseudomonas	Penicillin	7	1.1	0.9
Staphylococcus	Penicillin	0	1	0.9

Seed:

Figure 7: *Coriander Sativum L*

Figure 8: DAY 7 (Control- Water only)

Figure 9: DAY 7 (Supernatant only)

Figure 10: DAY 7 (Supernatant + keratin)

5. CONCLUSION

In summary, the solubility of human hair and the yield of keratin are dependent on the destruction of the cuticle layer and the fracture of a disulfide bond. The extraction rate of keratin was 70% is reported. Out of 20g of hair subjected to extraction, 5g of keratin was extracted. The sample was compared with infected Damask rose leaf (*Rose damasceneL*). Keratin are functioning against microorganisms from an infected leaf. This review represents the effect of organic fertilizers added to soil having physiological changes such as plant height, plant weight, fresh leaves weight, and the number of leaves of plant growth. It also includes the comparison of the different fertilizers like water(control),

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Rhizobium(supernatant) only, and *Rhizobium* (supernatant + keratin). In addition to that morphological studies of coriander plants having the effect of selected organic fertilizers are also included. Overall, all three organic fertilizers are proven as potential, cheap, and rich sources of nutrients and phenolic compounds.

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