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CHEMICAL ANALYSIS OF HAIR PROTEIN DAMAGE CONTROL BY ESSENTIAL OILS USING ANALYTICAL TECHNIQUES

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ABSTRACT

Essential oils were evaluated quantitatively in reducing the protein loss of hair fibers caused by the different grooming treatments such as cleansing, bleaching and straightening of hair. The present study involved in the reduction of protein loss of hair fibers with and without the application of the essential oils. Coconut, Mustard and Nem oil was used in this research work. Hair fibers under study were cleansed 15 times with and without the application of oils. 10% solution of SELE was used for cleansing purpose. Percent decrease in protein loss in cleansing treatment with the application of essential oils like Coconut, Mustard and Neem oil was 77, 76 and 80 respectively. Bleaching of the hair fibers were conducted with the same oils and the results reveal that percent decrease in protein loss was 14.7, 17.46 and 20.06 with Coconut, Mustard and Neem oil respectively. Hair fibers were bleached twice. 6% Hydrogen peroxide was used for bleaching. Percent decrease in protein loss in the hair straightening after the application of coconut, mustard and Neem oil was 5.08, 11.80 and 20.46 respectively. 3% NaOH was used for hair straightening. The protein loss of cosmetically treated hair fibers with the application of essential oils were less as compared to that without the application of oils. It was found that the Neem oil is best among the oils which are used in research. The order of efficacy of the oils was Neem ≥ Cocunut ≥ Mustard oil.

Keyword: Coconut, Mustard and Nem oil.

INTRODUCTION

To enhance beauty, appearance and health of the hair special care is very important. There is a need of proper preventative measures to ensure that hair does not get chemically damaged. One of the most beautiful parts of the body is hair and reasonable care must be taken in accurate way to keep hair healthy and protect from outer atmospheric damaging factors. Different types of cosmetic formulations for cleansing, conditioning and grooming hairs are used by people in Pakistan. Generally locally available essential oils are used for hair conditioning and preventing hair damage. The use of homemade recipes is also a trend for hair grooming. Both permanent and temporary hair colors of various brands are easily available in market. Various chemical and thermal methods are used in Salons for styling and straightening of hair. By continuously applying the products used for grooming and hair styling a damaging effect on hairs is studied which in turn disturb the integrity of hair both physically and chemically [1].

Different types of treatments are included in the hair grooming. These are cleansing, washing, conditioning, straightening, bleaching and dyeing. Oils which are necessary for hair have natural fragrances. It is extracted from each and every part of a plant. These oils are volatile in nature. Usually plants are the natural source of liquid aroma compounds. Essential oils have mainly volatiles such as terpenoids, benzene derivatives, alcohols and derivatives of fatty acid [2]. The FDA and other authorities declared essential oils are mostly safe. Essential oils are extensively used in cosmetic formulations. Properties of oil are differently remarkable to other oil. Each and every individual chemical compound that can be present in oil contributes to the overall character of the essential oil.

MATERIALS AND METHOD

Samples are prepared to check the protein loss after the extensive cleansing, oxidative bleaching and hair straightening. A set of hair fibers was selected, cleansing treatment were performed with and without the application of oils. Hair samples without the application is called controlled samples and with oil is called treated samples. After the application of oils on hair surface, samples were left in open air for 10-12 minutes to get homogenous oil distribution. Each hair fibers were cleansed with 1ml of 10% SELE for 2-5 minutes and washed with 20ml of deionized water for 10-15 minutes. Hair samples were dried at room temperature over night, after drying samples were preserved in aluminum foil for further treatment. This procedure was repeated 15 times with controlled as well treated hair fibers.

After five treatments half hair fibers were preserved to study the hair damage. Same was done after ten and fifteen treatments.

All these steps are applied for the hair straightening and oxidative bleaching. Oxidative bleaching was done with 6% hydrogen peroxide. pH was maintained at 10. Bleaching was done twice. For the purpose of hair straightening 3% NaOH was used. Hair samples for hair straightening and bleaching were kept for 30 minutes in their respective reagents.

Evaluation of Hair Damage

To check out the hair for surface damage it was necessary to cut each hair sample into small pieces of desired size. Hair fibers were suspended in 10ml of deionized water in a narrow mouthed 50ml flask. Each flask was corked and shaken at the speed of 200rpm. For most of the studies the shaking time of hair samples were four hours. Selection of the shaking time period was based on the observation, specifically on chemically damaged/altered hair. This gives suggestion that the said time period not only provide an adequate quantity of protein concentration in the solution for further analysis but also that a major amount (approximately 50% or greater) of the total material lost by cuticle during shaking in any given 24 hour period was recovered within the first four hours of shaking [3]. At the end of the shaking period, the water solutions were observed and concluded that the solution are visibly turbid. These solutions comprise of cuticular material in a very fine suspension. However the hair tended to mat together at the bottom of the flask. The suspended or solubilized protein was separated out. Further solublization of this protein was carried out under alkaline conditions and analyzed for protein concentration.

Estimation of Hair Protein

Lowery Method was used to analyze the protein concentration in any given sample having hair protein recovered during shaking. This is one of the most extensively applied methodologies for the measurements of protein in biological samples. It is modified to our suitability and need. For the solubilization of the protein fragments, each sample was well shaken by hand. 1ml of the turbid solution containing protein was pipetted directly from the flask. The separated solution was added to the test tube containing 0.1ml of 5 N NaOH. The contents of the tube were mixed well. Mixed samples were allowed to sit at room temperature for the time periods of 30minutes for the purpose of solubilize the suspended protein. At the end of the incubation period, 3ml of Cu-carbonate solution was added. Cu-carbonate solution was prepared fresh every day by mixing 1ml each of $CuSO_4$ solution (1%w/v) and potassium

tartrate solution (2%w/v) with 20ml of Na_2CO_3 solution (10%w/v). The tubes containing Cu-carbonate treated with alkaline protein solution was incubated at room temperature for 15 minutes. At the end of the incubation period, 9ml of Folin-phenol solution, prepared by diluting the 5ml of 2N Folin phenol reagent (sigma complex) with 50ml of distilled water was added and mixed well immediately. The samples were further incubated at room temperature for 40 minutes. At the end of the incubation period, the absorbance of each sample was determined in a spectrophotometer at the wavelength of 750nm.

RESULT AND DISCUSSION

Protein loss by cleansing treatment

This is the most important measurement relevant to protein damage. It is well known that repeated cleansing and shampooing of hair make the hair surface more vulnerable to damage. Hair cuticle is composed of exocuticle and endocuticle. Exocuticle is highly crosslinked and is not swollen by water as compared to endocuticle which can be easily swollen and damaged. When hair is repeatedly washed and cleansed, swelling of endocuticle causes the lifting of surface layer by bending. This damaged cuticle can be easily broken [4]. The protein loss resulting from repeated shampooing can be measured by colorimetry. When normal hairs are repeatedly washed and shampooed, this makes the cuticle weaker and weaker resulting in the damage to the protein cells. The protein loss can be measured by UV-VS Spectroscopy. However when hairs are properly protected before any treatment with condition agents, this protein loss can be decreased. Oils are good conditioner and make a protective hydrophobic layer at the hair surface and also penetrate inside the hair. This protective covering on hair decrease the damage to the hair caused by repeated washing and abrasive damage. This process helps in decreasing the protein loss.

The difference in the protein loss with and without oil application to the hairs before cleansing treatment, determines the effectiveness of given oil sample in the reduction of damage to the hairs. In this study we have used three different oils in order to see their effect in protecting and preserving hair. I used Coconut oil, Mustard oil and Neem oil because these oils are traditionally used in Pakistan due to their healthy effect on hairs. Neem oil has been used due to its antiseptic properties. However it is important to determine quantitatively the amount of protein loss during hair cleansing treatment after application of oil. Bovine serum albumin was used as standard for purpose of calibration. Comparison of colorimetric results of samples with standard gives the information about the protein quantity in each sample.

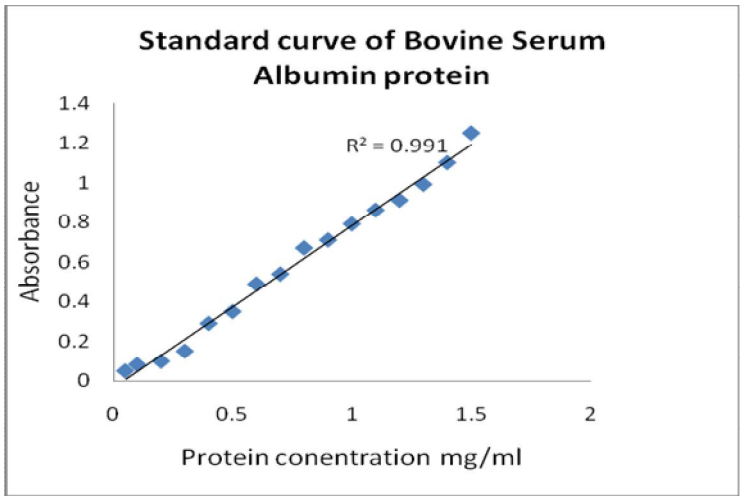

Figure: 1. Standard curve of Bovine Serum Albumin (BSA) protein.

Studies are conducted to determine if the measurement of hair protein loss can be used to evaluate the hair damage due to cleansing, bleaching and hair straightening treatments. For this purpose, hair tresses of virgin and undamaged hair were used. Hair samples were prepared and shampooed with SLES.

Two batches of hair samples each weighing 500 mg were used to study the effect of each essential oil in reducing hair damage. Cleansing treatment was done using Sodium lauryl ether sulphate which is active ingredient in shampoos. A 10% solution of SLES was prepared and hair samples were cleansed gently without extensive rubbing. Then hairs are washed with distilled water for 5 minutes and dried in air. Cleansing treatment is repeated five times each after hair drying. After cleansing treatments, damaged hair protein is extracted and measured quantitatively using colorimetric method of protein determination. Protein amount in mg/ml is measured by comparison of colorimetric results with standard curve of bovine serum albumin. Protein loss measurements for Coconut oil are represented in figure 2. Coconut oil has been traditionally known for its healthy effects on hair. However, quantitative analysis of the reduction in protein loss by coconut oil is necessary. Comparison of Protein loss with and without coconut oil application for five cleansing treatment shows a considerable reduction in protein loss. About 61 percent of hair protein is preserved if hairs are treated with coconut oil before washing and cleansing. This is quite significance. As the number of cleansing treatments is increased, amount of protein loss is also increased for hairs without any conditioning prior to cleansing. However this damage is effectively reduced in case of hairs which are pre-treated with coconut oil.

Most commonly used oil for hairs in Pakistani culture is mustard oil. Comparison of mustard oil with coconut oil for its effectiveness in reducing protein damage clearly shows that coconut oil is more effective in reducing protein damage. Coconut oil applied before washing performed better than mustard oil is due to high penetration ability of coconut oil.

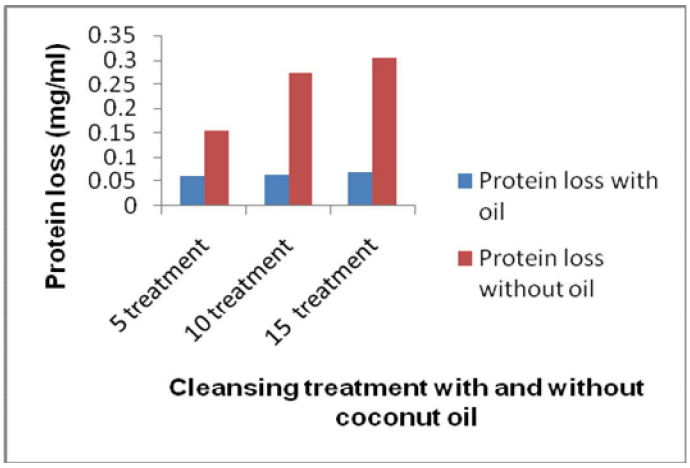

Figure: 2. Protein loss for cleansing treatment with and without coconut oil

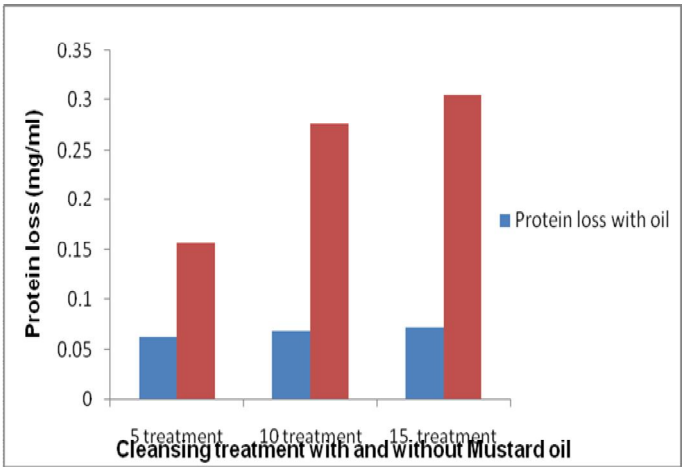

Figure: 3. Protein loss for cleansing treatment with and without Mustard oil

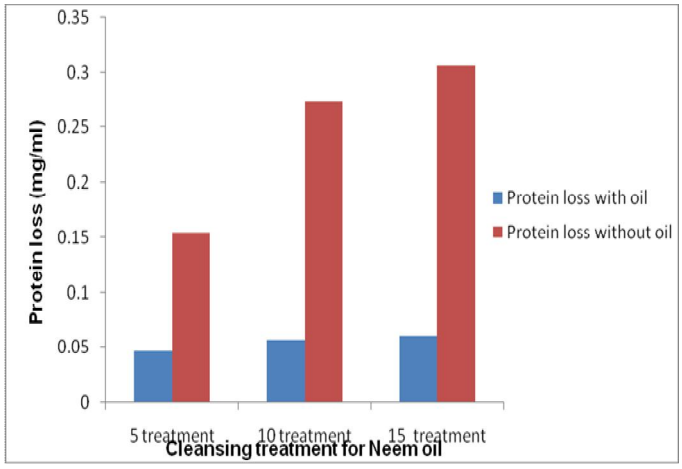

Figure: 4. Protein loss for cleansing treatment with and without Neem oil

Neem oil contain high level of long chain fatty acids (Oleic, linoleic and phenols and can be a good source of hair conditioning and anti-oxidant. Long chain fatty acids and their esters exhibit adhesion to hair fiber due to its outer lipophilic surface. These oils are also rich in sulfur contents and might have dermatological effects as discussed in a recent report claiming anti-lice properties of neem oil. Neem oil has also been investigated as a biological pesticide. Results from the colorimetric study of protein loss by application of neem oil are shown in Figure 4. These clearly shows that Neem oil is most effective when compared to Mustard and coconut oil in reducing protein loss. This is due to higher adhesion and absorption of neem oil to the hairs which in turn reduce the swelling of cuticle and protect the hair protein. At the surface of the hairs oil forms a protective hydrophobic layer which reduces the water penetration and swelling of the cuticle. All the factors reduce the surface damage and protein loss from hair. Neem oil is composed of oleic acid, linoleic acid and other unsaturated fatty acids. These fatty acids are very effective in forming hydrophobic layer on hair cuticle.

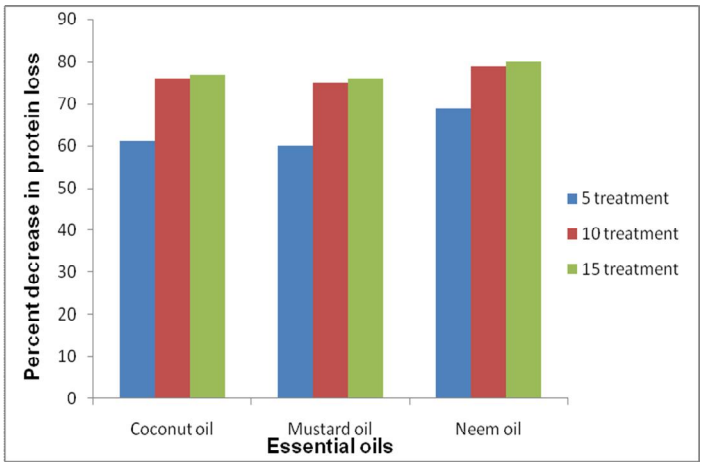

Figure: 5. Comparison of essential oils for percent decrease in protein loss

Protein loss by bleaching treatment

The most important objective of hair bleaching is to lighten the natural colour of hair and this purpose is mainly achieved by the process of oxidation. Through partial or total decolourisation of hair colour pigments melanin by the reaction with an oxidizing agent, the purpose of bleaching is easily achieved. Hair bleaching formulations generally consist of the solution up to 6% of hydrogen peroxide and ammonia to give final pH around 10, and thickeners. If extensive bleaching is required “bleaching boosters” (usually ammonium and potassium per sulphates) are added to the peroxides.

The bleaching process consists of two steps. A fast dissolution steps in which melanin pigment granules disperse and dissolve. The second step is much slower also called the

decolourisation step. As a consequence of the decolourisation of the melanin pigments, a secondary side effect also takes place whereby a side reaction changes the properties of hair keratin protein producing oxidative or “bleaching” damage. The damage is caused by the oxidative cleavage of disulphide bonds or cross links to form cysteic acid [5-7]. Severe bleaching also reduces the concentration of free sulphydryl groups and to small degree degrades other amino acid residues such as tyrosine, threonine, and methionine. During the bleaching process with H_2O_2, the reactivity of melanin with regard to H_2O_2 is high and this causes a large colour change with the increase in the concentration of H_2O_2 As a result fiber structure becomes weak with lower degree cross linking and overall its hydrophilic nature is increased, due to anionic site formation, e.g. cysteic acid residues. In particular the fiber feels more (weak) brittle, is more vulnerable to breakage, become more porous and hence will absorb larger amount of water. Hair protein damage caused by extensive bleaching is far higher and irreversible than caused by any other grooming treatment. With the 6% H_2O_2 treatment, it is quite clear to see that the cuticles of hair are lifted after undergoing the treatment. The damage may be caused by the oxidation process during the bleaching treatment [8, 9]. The hair damage becomes more serious when the concentration of hydrogen peroxide is increased. It is obvious that the lifting of the cuticle cells is more severe when the hair sample is bleached with 9% H_2O_2. Under the influence of 12% H_2O_2 treatment, the scales in the hair sample surface are diminished and they cannot be clearly defined. The cuticles cells are lifted more seriously when compared with those samples bleached with 6% H_2O_2 and 9% H_2O_2 [10]. Since the bleaching compositions are working at pH 10, side reaction as hydrolysis of the peptide and amide bonds and formation of new crosslinkage as lanthionine or lysinoalanine are possible. The formation of new crosslinkages will decrease the solubility of the hair proteins. The oxidative scission of disulphide bonds may lead to the decrease of crosslinkage density of the protein leading to a decrease in the tensile properties.

As in case of other grooming treatments where protein loss can be reduce to a significant level by application of essential oil to hair, protein loss caused by bleaching is very difficult to decrease. As has been shown by figure 5 percent decrease in protein loss by bleaching hair after oil treatment is very small for coconut oil. However results for reduction in protein loss for Mustard oil and Neem oil are better than coconut oil. Mustard oil protects hair and decrease in loss for about 14 and 17 percent for first and second bleach respectively. Neem oil is even better than Mustard oil and decrease the protein loss by bleaching by 18 and 20 percent for first and second bleach respectively. These results showed clearly that if Neem oil

is applied to hairs beforebleaching hairs with hydrogen per oxide then protein loss can be decreased to a significant level.

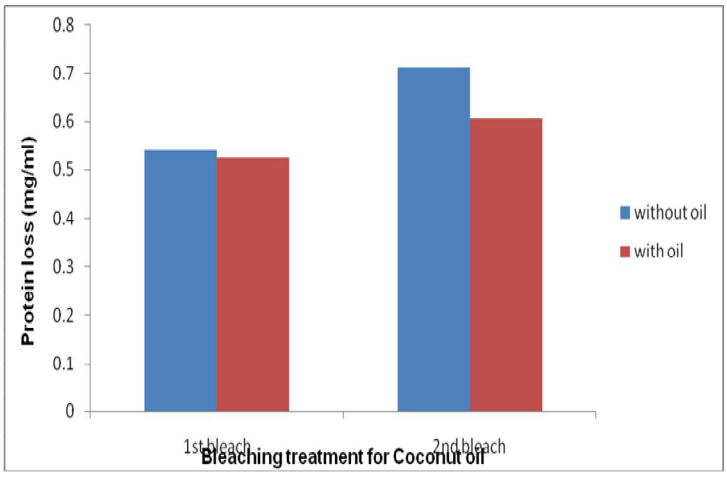

Figure: 5. Bleaching treatment with the application of coconut oil

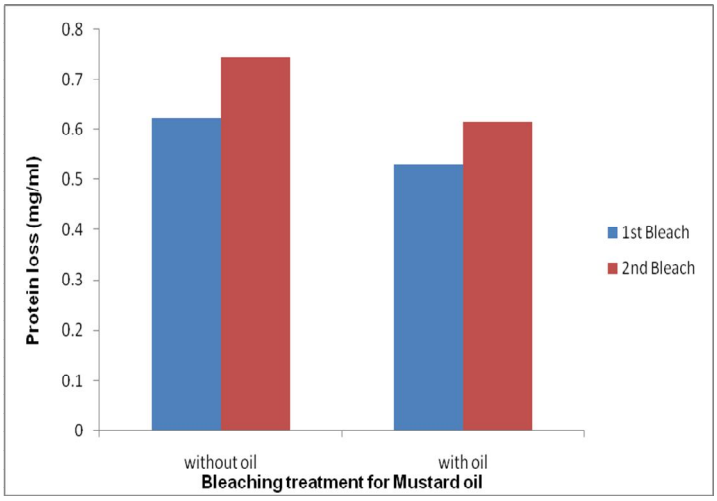

Figure: 6. Bleaching treatment with the application of mustard oil

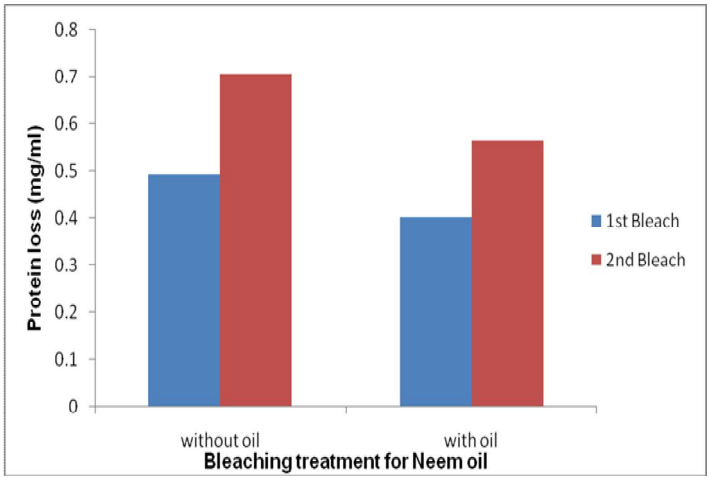

Figure: 7. Bleaching treatments with the application of Neem oil

Protein Loss by Hair Straightening

Hair straightening is meant for rebounding of hair protein. Hair straightening formulations designed for most of the African type hair employ strong bases such as sodium hydroxide as the active ingredient. The process involves the fission of disulphide bond by hydrolysis or nucleophilic substitution of sulphur by hydroxide ion. Then disulfide bonds are again formed resulting in the straightening of the hair protein. Straightening can also cause damage to the stable peptide bond. Damage caused by straightening is also irreversible as it results in the reduction of disulfide bond and rate of new bond formation is not as efficient resulting in the weakening of the hairs. Also if hairs are treated with conditioning agents before treatment with sodium hydroxide then efficiency of hair straightening is decreased although hair damage is also decreased. As shown by the results in and Figure 8, there is only a small decrease in protein loss by the application of essential oils. Only Neem oil cause most effective decrease in protein loss.

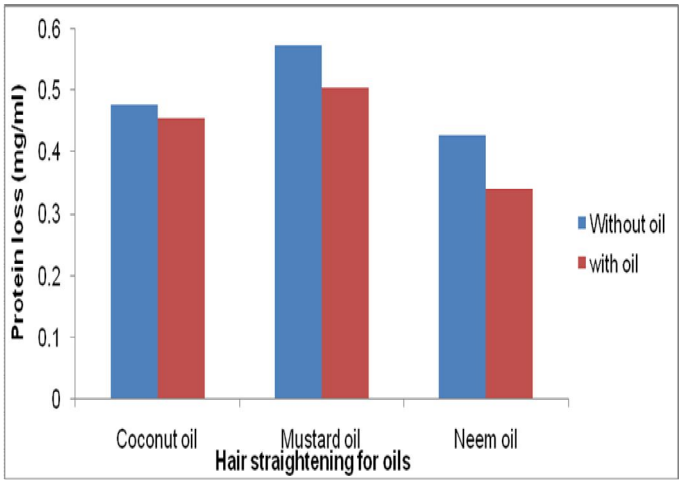

Figure: 8. Hair straightening with the application of essential oils

CONCLUSION

This research work focuses on the efficacy of the different essential oils such as Coconut, Mustard and Neem oils. Study revealed that the mechanical and chemical deterioration of hair fiber which results in the production of the split ends. Surface damage to human hair is because of the various damaging chemical treatments such as cleansing, bleaching and straightening. Protein is lost because of the different damaging treatments. To prevent the damage caused by the cosmetic treatments the application of the essential oils such as Coconut, Mustard and Neem oil is desirable. These oils are rich in log chain fatty acid. Oils are also rich in sulphur contents. Essential oils have the greater penetration power. There is need to reduce the damage and improve the manageability. So the application of the oils

before washing, bleaching and straightening of hair fiber reduces the protein damage. The colorimetric procedure used in this research gives quantitative measurements of protein lost during shaking of hair samples in the simple medium like water. Neem oil helps more in the reduction of protein loss as compare to the other two oils.

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