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# "Formulation and Evaluation Herbal Hair Dye by QbD Approach"

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***Absract***

Now-a-days, graying of hair has become one of the most common problems for both men and women; this is due to the adoption of unhealthy lifestyle, pollution, stress, poor diet, hormonal changes and usage of detergents. The number of pigment granules naturally begin to decrease as a person ages. The person usually begins to gray between twenty-eight and forty years of age. The reason for this is that the melanocytes begin to slow down and produce less melanin. This is part of the natural aging process in humans. There are two types of melanin which is responsible for the coloring for the hair these are 1.eumelanine 2. Pheomelanine. In recent years there is increasing demand for synthetic and natural dye to overcome problems related to graying of hair. Considering the harmful effect of synthetic dye such as temporary skin irritation and allergy, hair breakage, skin discoloration, hypersensitivity reaction, and unexpected hair color as well as problem. The application of synthetic dyes with the increase in the usage of hazardous chemicals in the process of manufacturing. Hence an attempt has been made to review the use of natural products obtained from plant sources to replace the synthetic dyes. The Composition of organic hair dye consisting of Henna (Lawsonia inermis), Indigo (Indigofera tinctoria) amla powder (Emblica officinalis). Camellia Sinensis (Tea Powder), Loha Bhasma, Mandur Bhasma or churna is ferric oxide/hematite or red iron oxide with chemical formula Fe2O3) powders are blended and mixed with water and the mixture to obtain a dye The plant samples have been tested for the presence of PPD which is very harmful chemical substances used in synthetic hair dye A supportive analytical data has been obtained by using TLC and colorimetric test. Further this study is directed for coloring of hair, by applying each and every plant material and also the mixture of formulated dye. Also to check the color retention property and color intensity of a hair by washing the hair with synthetic shampoo as well as the herbal shampoo this has shown that there is no hair damage or scalp irritation. Hence this formulation proves to be a key alternative for the synthetic hair.

**Keywords**: **Natural and synthetic Hair Dye, Lawsonia inermis, Indigofera tinctoria, Camellia Sinensis, Loha Bhasma, Mandur Bhasma.**

## Introduction

Now-a-days, graying of hair has become one of the most common problems for both men and women; this is due to the adoption of unhealthy lifestyle, "A hormonal imbalance, Hyperthyroidism and Hypothyroidism, malnutrition, pernicious anemia, nutritional deficiency, using electric dryers and concentrated hair dyes, as well as genetic disorders, chemotherapy and radiation, are all factors. At times, a few gray hairs can develop in children as young as 8 years and then it is generally progressive with increasing age." "First is a faulty diet and second, mental worries. Lack of some B Vitamins, iron, copper and iodine in the daily diet are said to be contributory factors. Mental stress also produces extraordinary tension in the skin of the scalp, which interferes with the supply of nutrition necessary for good hair health [1]. Although graying of hair is a natural phenomenon associated with ageing, there has been a significant occurrence of premature graying specially in women and men also, attributable probably to stress and use of synthetic shampoos. Loss of color in hair is due to varied reasons like genetic influence, effect of environmental factors, use of alcoholic preparation, etc(2) Though permanent synthetic hair dyes are available in varied color and ranges retain natural luster, they have the chief disadvantage of producing hypersensitive reactions in some individuals. Some hair dyes marketed as natural dye, contain 1- 3% of phenylenediamine which is a synthetic hair dye and stain the skin and clothes during use(3) The number of pigment granules naturally begin to decrease as a person ages. The person usually begins to gray between twenty-eight and forty years of age. The reason for this is that the melanocytes begin to slow down and produce less melanin. This is part of the natural aging process in humans. In recent years there is increasing demand for synthetic and natural dye to overcome problems related to graying of hair. Considering the harmful effect of synthetic dye such as temporary skin irritation and allergy, hair breakage, skin discoloration and unexpected hair color as well as problem with excess use of herbal dye such as competition to food grain crops, environment and mislead to excess use of natural adulterated formulation with synthetic chemicals. To avoid this genuine problem in use of synthetic as well as natural hair dye it is need to think a holistic approach for natural remedies and to study root cause analysis for graying of hairs. Based on composition or ingredients, hair colors can be classified as:

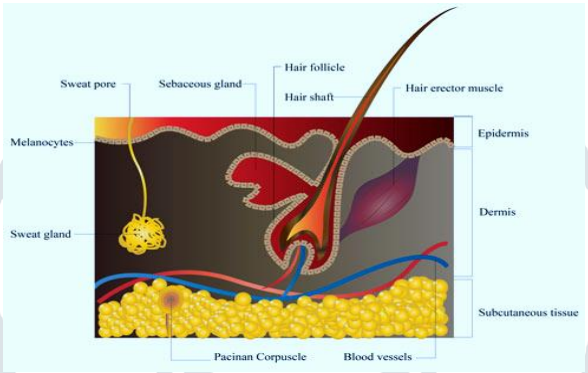
Chemical – These contain various chemicals that are used to prepare the artificial colors. While these have long color retention period and offer a large variety of colors, it caused both short term and long term damages and also causes various type of allergies like boils, itches, burning sensation, patches etc. Organic – They are the most abused marketing strategy especially as people confuse it with natural or herbal.

In actual sense, it conveys the way the ingredients have been treated before being formulated into hair color. However, According to laws in few countries, a brand requires proper certification to label their product as Organic. However, an Organic product may not be completely safe as for example it can have 90% of harmful chemicals and just 10% of organic products but still be marketed as organic product to motivate people to buy it.

Herbal – These are derived from plants and herbs. Although herbal products are supposed to be 100% made of plants and thus completely safe, companies on the other hand add few herbs to their chemical composition as a USP to market their product

Natural – These products are not to have any artificial or synthetic ingredients. Thus it can have some herbal substance as well as some naturally processed chemical. Companies mislead public with the world 'nature' also by adding few natural components to their high chemical composition[4] In comparison to natural hair dyes, synthetic hair dyes are reported to cause skin and other skin related diseases. The manufacturing process is hazardous to health of the people involved in the process and its applications leads to environmental pollution and also causes potential side effects to the consumers of the product. It is to be noted however, that some serious illnesses or emotional conditions may also cause the hair to gray[5]. Hair get black by using either synthetic hair dye or natural hair dye but if we are using the synthetic hair dye it will be dangerous or harmful to body if using the natural dye it will affect the production of crop means the more of the land get acquired to fulfill the requiremen of the people and there for it may the huge chances the adulteration of the natural crop according to the WHO it says that prevention is better than cure.

**PHYSIOLOGY OF HAIR (6 7 8)**



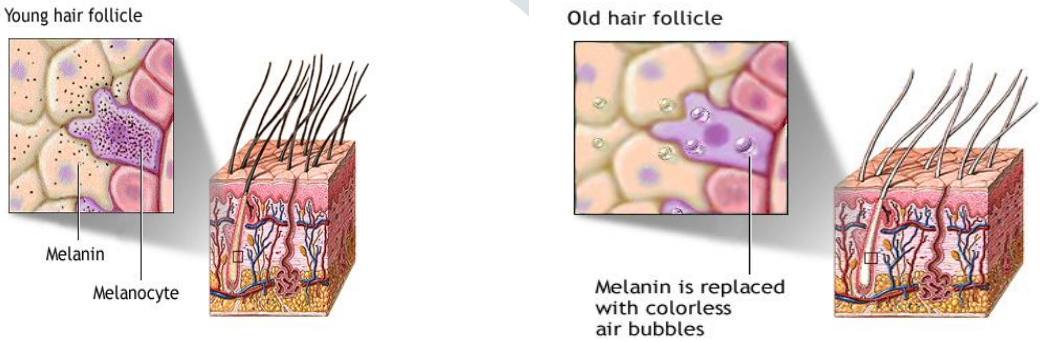

**Physiology of hair**

The different layers of the visible part of hair (shaft) are due to the alterations in the morphology and structure of matrix cells of the bulb. In the matrix cells takes place protein synthesis -especially Keratin synthesis- which contributes to the strength and endurance of the hair shaft and to nail configuration as well. Keratin also lies in the skin. Keratin is a group of proteins that contain sulphur and is being produced in the keratogen zone of the root. Hair consists of proteins (65% - 95%), lipids (1% - 9%), trace elements, polysaccharides and water.

**I) Lanugo hairs-** In the 3rd month of pregnancy, very smooth, soft and colourless hairs cover all the embryo's body and remains till few weeks before birth.

**II) Vellums hairs-** Vellums hairs are fine, soft and barely pigmented hairs, usually 1 or 2 cm. Due to the small quantity of pigment, they are nearly invisible. They emerge from hair follicles and usually have no sebaceous glands, so they don't get oily.

**III) Terminal hairs-** They are the distinguishable hairs of body and head, bigger in diameter and length than vellus hairs. They are responsive to hormonal influence and the hair follicles they come from have sebaceous glands. In men who experience androgenetic alopecia, the most common type of male hair loss, a percentage of the terminal hairs progressively weakens and converts into vellus hairs




**Physiology of hair**

Hair color is caused by a pigment (melanin) that is produced by the hair follicle. With aging, the follicle produces less melanin; Melanin is the natural substance that gives color (pigment) to hair, skin, and the iris· Hair is actually dead material when it leaves it's root otherwise it would hurt very much when your hairdresser works with his scissor. Most people know about this fact, but did you know about other facts on a normal scalp there are about 100150 thousand hair fibers. A blonde head of hair has usually much more fibers than red or dark haired heads. Hair consists mainly of keratin, which is also responsible for the elasticity of fingernails. A single hair has a thickness of 0.020.04mm, so that 2050 hair fibers next to each other make one millimetre. Hair is strong as a wire of iron. It rips after applying a force equivalent to 60kg, after it stretched itself for about 70%. The root of a hair fiber sticks in a bag in the skin. The fiber is pushed out of this bag about 0.35mm per day,

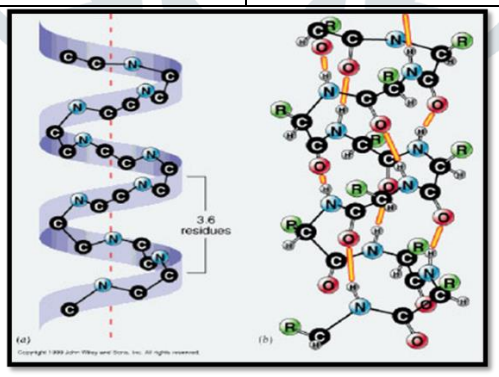
making an average growth rate of 1cm, or half of an inch, per month. The growth rate is however very much related to the individual person, his/her age, his/her diet etc Healthy hair has an average lifetime of 26 years. After a rest period of three months the single hair fallout and a new fiber starts to grow out of the bag. The lifetime depends on circumstances and person, too. The lifetime of hair is responsible for the maximum of hair length you can have. Waist length hair takes about 6 years to grow out from a short haircut, periodic trims included. If your hair has a lifecycle of 2 years, you will never achieve a nice waist length mane. Baby hair begins to grow around the third month after conception within the womb of the mother. {Trichocysts} are first formed. They develop into hair follicles as the fetus grows, and then become downy hairs several centimeters long when the baby is born. The total number of hairs is determined before the baby is born. After that, the number of hairs never increases. It just decreases. The number of hairs greatly varies for each person, from sixty thousand to one hundred and fifty thousand, and makes up one of natural characteristics of each person when they are born. We should take good care of our hair. Human beings have about one million and four hundred thousand hairs on their body, with about four hundred and fifty thousand of them to be found above the neck! Approximately we have 100 thousands of hair strands and these strands will increase its length for about half inch every month and keep growing its length for around two to six years and then lead to a relaxing stage.

**HAIR CHEMISTRY AND STRUCTURE** [9][10][11]

We will begin by defining the hair. Hair is composed primarily of proteins (88%). These proteins are of a hard fibrous type known as keratin. Keratin protein is comprised of what we call "polypeptide chains." The word, polypeptide, comes from the Greek word "poly" meaning "many" and "peptos" meaning "digested" or "broken down". In essence, if we break down protein, we have individual amino acids. Many (poly) amino acids joined together form a "polypeptide chain". Two amino acids are joined together by a "peptide bond", and the correct number of amino acids placed in their correct order will form a specific protein; i.e. keratin, insulin, collagen and so The "alpha helix" is the descriptive term given to the polypeptide chain that forms the keratin protein found in human hair. Its structure is a coiled coil. The amino acids link together to form the coil and there are approximately 3.6 amino acids per turn of the helix (coil). Each amino acid is connected together by a "peptide bond". The peptide bond is located between the carbon atom of one amino acid extending to bond with the nitrogen atom of the next amino acid. The best hair vitamins are typically great sources of amino acids. There are various elements found in the hair and they are used to make amino acids, keratin, melanin, and protein. The average composition of normal hair is composed of 45.2 % carbon, 27.9% oxygen, 6.6% hydrogen, 15.1% nitrogen and 5.2a% sulphur. The keratin found in hair is called "hard" keratin. This
type of keratin does not dissolve in water and is quite resilient. So what is keratin made from? Keratin is an important, insoluble protein and it is made from eighteen amino acids. The most abundant of these amino acids is cystine which gives hair much of its strength.

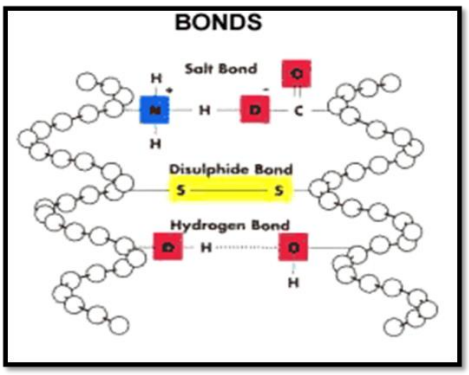
**Various Amino Acid Present in Hair**

| | |
|---|---|
| Cysteine | Aspartic acid |
| Serine | Alanine |
| Glutamic acid | Proline |
| Threonine | Isoleucine |
| Glycine | Tyrosine |
| Leucine | Phenylalanine |
| Valine | Histidine |
| Arginine | Methionine |




**The Alpha Helices Coil**

In the organization of a single hair, three "alpha helices" are twisted together to form a "protofibril". This is actually the first fibril structure of the hair. Nine protofibrils are then bundled in a circle around two or more to form an elevens ran ded cable known as the "microfibril" These microfibrils are embedded in an amphorous unorganized protein matrix of high sulfur content, and hundreds of such microfibrils are cemented into an irregular fibrous bundle called a "macrofibril". These macrofibrils are grouped together to form the cortex (or the main body) layers of the hair fiber. Packed dead cells surround these structures and are known as the cuticular layers of the hair. In the centerof these structures lies the medullary canal, which is actually apart of the excretory system and houses any foreign debris, heavy metal· synthetics and medications that are thrown off by the body and eventually released through the canal. Bonding in Keratin Protein When the hair is in its normal unstretched state. It is referred to as A of alpha keratin. The original configuration of the hair is held in place by the bonding found in the cortex layers of the hair. As we stated earlier, keratin protein begins with an alpha helix building into protofibrils, microfibrils, macrofibrils, then cortex layers. The bonds in the hair are located within each and every alpha helix.




**The Hydrogen Bond**

The first bond we will discuss is the hydrogen bond. This bond is located between the coils of the alpha helix and is responsible for the ability of the hair to be stretched elasticity) and return back to its original shape. The hydrogen bonds allow us to change the shape of the hair temporarily with the aid of water. These bonds are electrolytically controlled and are the most readily broken down and the most readily reformed. These bonds are responsible for approximately 35% of the strength of the hair and 50% of the hair's elasticity (some would argue up to 99.9% of the hair's elasticity).

**The Salt Bond**

The salt bond is also an ionic (electrolytically controlled) bond formed by the electron transfer from the side chain of a basic amino group (an amino acid with an 00Cgroup) to the side chain of an acidic amino acid, i.e. NH3+. (This is two positive and negative charges attracting one another.) This occurs in a position paralleled to the axis line of the rotation of the helix of the hair. The salt bond is responsible for approximately 35% of the strength of the hair and 50% of the hair's elasticity.

**Cystine Bond**

The cystine bond also known as the disulfide bond, sulfur bond, or just S bond is formed by cross links between cystine residues (amino acids) of the main polypeptide chains. This bond is perpendicular to the axis of the hair and between the polypeptide chains Because of its position in the hair; it is responsible for the hair's toughness or abrasion resistance. (It actually holds the hair fibers together.) These crosslink are frequent in the hair fiber, with maximum of frequency of one cystine bond every four turns of the alpha helix. This is what enables us to permanent wave the hair.

**The Sugar Bond**

The sugar bond is formed between the side chain of an amino acid having an OH group and an acidic amino group. This bond is also formed perpendicular to the axis of the hair. Because of its position, it gives the hair toughness but little strength (5%). Some moisture is contributed to the hair as a byproduct of this bonding.

**Chemistry of Hair Dye [12][13][14]**

In humans, all the different hair colors are due to just two types of pigment (melanin) called eumelanins and pheomelanins (European spelling, phaeomelanin). Eumelanins are the dark brown and black pigments while pheomelanins are the red and blonde pigments. The different colors of hair in different people are due to a combination of these two different basic biochemical structures. By mixing the two types together in different concentrations the many different shades of hair color are made. A study that analyzed the amount of eumelanin and pheomelanin in human hair suggested that; black hair contains approximately 99% eumelanin and 1% pheomelanin, brown and blond hair contain 95% eumelanin and 5% pheomelanin; and red hair contains 67% eumelanin and 33% pheomelanin Although people with dark hair may still produce the yellow - orange pheomelanin[12], A wide variety of dyes, dressings, and conditioners are available to men and women to enhance the color of hair or to alter its condition, providing the "feel good" factor. Natural hair dyes such as henna and mineral salts are still used, but hair dyeing increasingly involves careful chemical manipulation of the chemistry of hair fibers through bleaching or enhancement of natural colors. Available hair dyes include Minerals such as lead acetate (<1% aqueous), lead sulfide (kohl); silver nitrate; salts of bismuth, copper, and cobalt (commonly called "gradual" colorants) Vegetable materials such as henna (flowers and leaves of Lawsonia interims that contain acidic naphthoquinone, chamomile, and indigo)

**Essential Ingredients of Oxidative Permanent and Semi permanent Dyeing Systems**

- Surfactant
- Solvent
- Alkalizing Agent
- Oxidant
- Dye

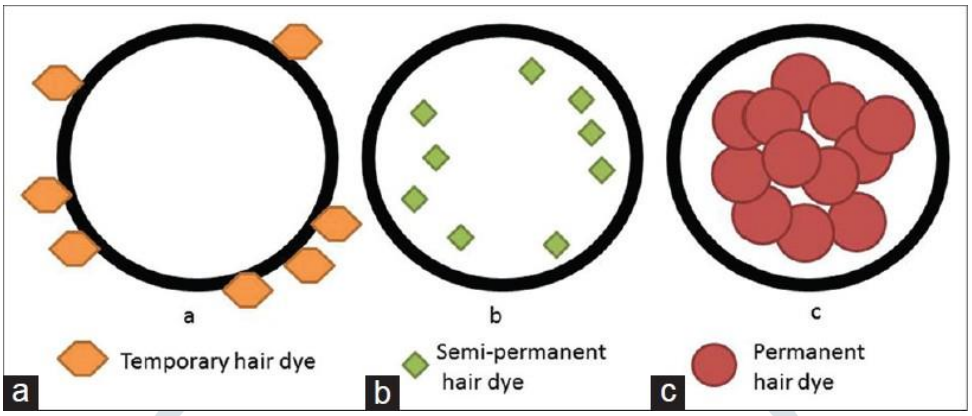
In general, hair dyes are classified into four categories according to how long the color remains in your hair:

1. **Gradual coloring**, normally used to color grey hair, uses metallic dyes such as salts of lead, bismuth, or silver. These dyes cause a chemical reaction on the cuticle (the outermost layer of the hair) and then build up on the hair shaft. Particles from the dye often cause hair to become stiff, dull, and brittle. Using gradual colouring, it usually takes a few weeks for hair to reach the desired colour.

2. **Temporary coloring** uses water soluble dyes (dyes that dissolve in water) made up of large molecules that normally can't penetrate the hair shaft. Instead, they are temporarily deposited on the surface of your hair and eventually wash off with shampoo and water. Usually, this happens after just one shampoo. But , unless your hair has previously been chemically treated, which can cause the dye to penetrate beyond the surface of the hair shaft.

3. **Semi-permanent coloring** uses mostly synthetic dyes that have a lower molecular weight (the mass of a singular molecule) than temporary dyes. This allows the colour to remain on the hair shaft longer. Semi-permanent colouring usually washes off after 6-8 shampoos. The smaller-sized particles in these dyes can penetrate freely into the hair shaft, increasing the likelihood of breakage. They are a good option if you're looking for a temporary change, or you just can't commit to a colour!

4. **Permanent coloring** uses synthetic dyes and accounts for the majority of hair dye sales. The color results from a chemical reaction that occurs between the hair shaft and the synthetic chemicals in the dye. An ammonia and hydrogen peroxide solution is often used to help the hair dye molecules penetrate the hair shaft by causing the shaft to swell up. However, the solution has a very strong odour and can irritate the scalp.




**Types of Dyes**

of the colorant into/to the hair structure, bleaching or otherwise masking the natural melanin colors or alteration of the structure of the hair shaft, allowing deep penetration of the colorant. The hair cuticle provides a barrier to the absorption of hair dyes Henna is the oldest and most widely used vegetable dye utilized in hair coloring. A temporary chestnut color is produced in blond or auburn hair by applying a paste of henna flowers and leaves ground in hot water immediately before use. (The dye is unstable in aqueous solution**.**) The addition of indigo achieves darker blue-black shades; extracts of walnut shell or logwood enhance brown coloration, Mineral dyes of the mineral dyes, only lead acetate is commercially available In the United States, the FDA permits maximal concentrations of ppd in the Synthetic Formulation.

**Oxidative dyes.**

Oxidative hair dyeing systems involve the use of more toxic reagents. They are multistep processes leading to semi permanent or permanent coloration, according to the extent of bleaching involved. Surfactants and solvents influence the penetration of the active constituents; alkalizing agents determine pH. The resulting hair colorations are more stable against normal wearing processes than semi permanent preparations and involve an initial oxidation reaction, a coupling reaction, and production of a color reaction with dyeing of the hair fiber. The process requires a primary intermediate, a *m* -coupler or secondary intermediate (color modifiers), and hydrogen peroxide.

Hydrogen peroxide is commonly used as the oxidizing agent; it has the capacity to bleach melanin but it initiates the first coupling reaction and the ultimate development of the color. Initial oxidation of primary intermediates (e.g., *p* -aminophenol, *p* -phenylenediamine) by hydrogen peroxide is followed by coupling with an agent like resourcinol, phenols, *m* -aminophenols, or *m* -phenylenediamines. Further oxidation of this secondary intermediate leads to the formation of colored indamines, indolanilines, and indophenols. As a general rule, the higher the electron-donating capacity of the coupling agent (especially unsubstituted carbocyclic *m* -couplers), the higher the absorbance maximum of the indo-dye formed. In the presence of couplers, di and monoimines react to produce indo-dyes. Many of the organic aromatic amines used in hair dyes are strong sensitizers and oxidative dyes should be used with extreme caution. *P* -phenylenediamine and its derivatives, commonly employed in permanent or semi permanent hair colorings, are strong sensitizing agents and may damage the hair. Although a large number of possible combinations of primary intermediates and couplers leading to the production of exotic hair colors is possible, the cost of conducting regulatory toxicological evaluation is prohibitive in developing many interesting colors.

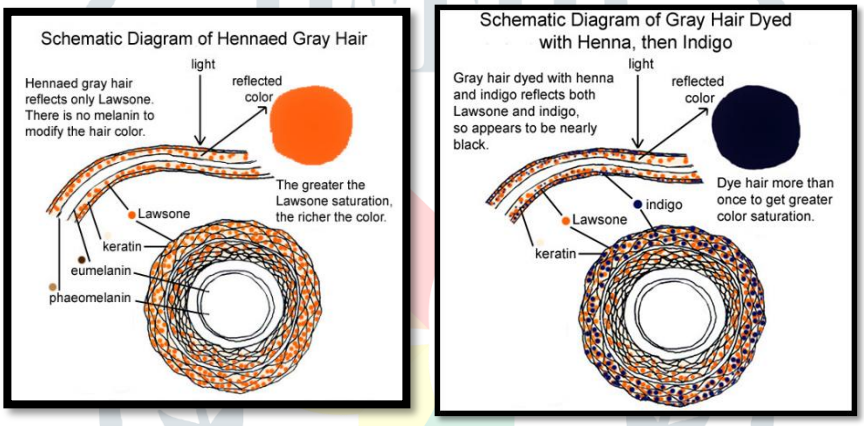
**Natural dyes plant used as hair colorant**

| **Hair Colorant (General Name)** | **Hair Colorant(Chemical Name)** |
|---|---|
| Heena | Lawsonia inermis |
| Indigo | Indigofera tinctoria |
| Mandur Bhasma | ferric oxide or red iron oxide |
| Amla Powder | Emblica officinalis |

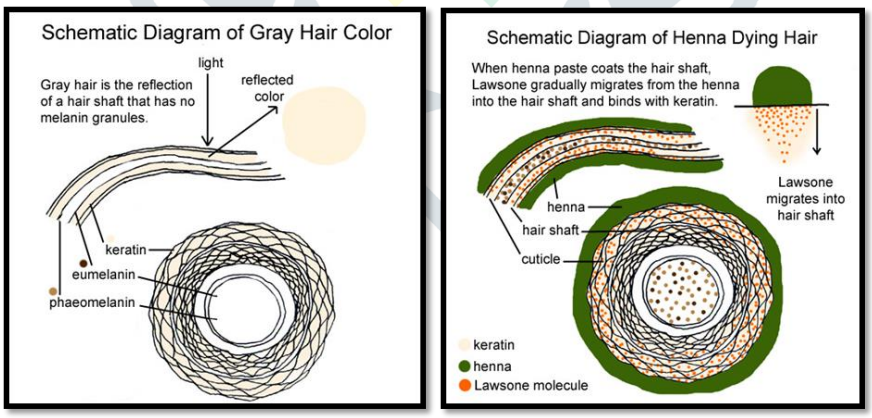
| | |
|---|---|
| Tea powder | Camellia sinensis |
| Loha Bhasma | Iorn |

**HAIR DYE CHEMISTRY & ITS INTERACTION**[15 16]

When you dye gray hair with henna, the Lawson molecules will penetrate the keratin in the hair shaft and dye it orange. If you dye your hair with indigo immediately afterward, the indigo molecules will penetrate the keratin in the outer layers of the hair shaft with indigo molecules which will oxidize to dark blue. Powdered indigo leaves prepared for hair will react with the acidic henna to blacken the orange. Your hair will be dyed black as the result of this interaction. In Turkey, white woolen yarn was dyed rich, long-lasting black by dying it once with henna, and overlying that with indigo. Your hair is made of keratin, just as is wool If you use pure body art quality henna that has been tested at an independent laboratory and certified to have lawsone at 2.3% or above, and leave the paste in your hair for several hours, the keratin hair shaft will become saturated with dye. If you use high quality indigo immediately after body art quality, laboratory certified high dye content henna; you have the best chance of dying your gray hair a deep, natural-looking black color. If the indigo has been lab tested and proven to be a neutral PH, your hair will be sleek, shiny, healthy and perfectly black! You can get these from Mehendi. After you complete the dye process, it will take two days for the dyes to naturally darken to peak color. If you do not get satisfactory color saturation the first time, repeat the dye process until you build up the color you want. Henna is beneficial for your hair, you can dye as often as you like! However, once you've gotten your perfect color, you probably only need to keep up the roots. The indigo molecules are slightly larger than Lawsone molecules, so they stain the outer layers of the hair shaft. Some people find the indigo color fades slightly over several weeks, and the henna begins to show through. You can refresh the color by dying your hair with a mix of mostly indigo, and a little henna.




**Hair dye chemistry & its interaction**

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**QUALITY BY DESIGN IMPLEMENTATION:**

The first step in implementing Quality by Design system understands the cause and effect relationship between the raw material attributes, process conditions and the critical quality attributes of the final product by employing design of experiments (DOE). There are several designs of experiments that are available in any commercial software that a formulator can use. Examples:- factorial designs, Taguchi designs, mixture designs, response surface designs, etc. And within each design there several options available for the formulator to suit the needs and goals of the experiments. Examples: Blocking, confounding, fractional factorial, 3 factorials, Placket-Burmann designs, Latin Squares design, etc. After the designed experiments are executed, the results are analyzed and studied to identify the cause and effect relationships between input parameters and responses. The next step in implementing QbD is scaling up the experiments either to the manufacturing level or intermediate level. In this processes, one can use prior knowledge to run fractional designs that will eliminate the need to run several large-scale experiments. The last step in implementation of QbD is defining the control strategies for raw materials and manufacturing process parameters. The implementation of control strategy inherently addresses the implementation of design space. If certain inputs, such as excipient particle size or drug crystal surface area, are related to the performance of the final product, then it is logical, in QbD, to control the particle size or surface area to the ranges dictated by the experiments. Once the control strategies are identified, manufacturer should procure, install, commission and validate the control systems to implement QbD[25].

**1. Elements of Quality by Design:**

Various elements of quality by design as described in ICH Q8 (R2) include Target product profile, Identification of quality attributes, Risk assessment to identify process/product risk, Design space development and Control strategies

**2. Identifying a Quality Target Product Profile (QTPP):**

The quality target product profile (QTPP) is a summary of the quality characteristics or attributes of a drug product that ideally will be achieved and thereby ensure the safety and efficacy of a drug product. The QTPP forms the basis of design for the development of the product. It is both prospective, that is, it describes the goals for the development team, and dynamic, that is, the QTPP may be updated or revised at various stages of development as new information is obtained during the development process.

**3. Identification of Critical Quality Attributes (CQA):**

Pharmaceutical development consists of product and process design and development. The TPP provides the basis for the ideal dosage form. A critical quality attribute is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. CQAs are generally associated with raw materials (drug substance, excipients), intermediates (in-process materials), and drug product. Drug product CQAs are the properties that are important for product performance, that is, the desired quality, safety, and efficacy.

**4. Quality Attributes Important to the Performance of the Drug Product:**

From a clinical perspective, safety and efficacy (product performance) is of prime importance. For example for an oral CR product, it is important to consider attributes that are potentially critical for performance. These may be drug dissolution/release, potency, polymer concentration, polymer viscosity, glass transition temperature (Tg) of composite, etc., or any other attribute that can either be substituted for drug release or clinical performance

**5. Quality Risk Assessment:**

A key objective of risk assessment in pharmaceutical development is to identify which material attributes and process parameters affect the drug product CQAs, that is, to understand and predict sources of variability in the manufacturing process so that an appropriate control strategy can be implemented to ensure that the CQAs are within the desired requirements.

**6. Critical Process Parameters:**

A critical process parameter (CPP) is any measurable input (input material attribute or operating Parameter) or output (process state variable or output material attribute) of a process step that must be controlled to achieve the desired product quality and process consistency For example the following are the process parameters and material attributes related to wet granulation process:

- ✓ Attributes related drug substance: Amount, Form, Particle size, Moisture content and Bulk density.
- ✓ Attributes related to excipients: Excipient amount, Excipient particle size and Excipient density.
- ✓ Blend uniformity, Granule size distribution, Agglomerate size, Moisture,Bulk density and Flow properties.

A parameter is critical when a realistic change in that parameter can cause the product to fail to meet the TPQP. Thus, whether a parameter is critical or not depends on how large of a change one is willing to consider. Thus the first step in classifying parameters is to define the range of interest which we call the potential operating space (POS). The POS is the region between the maximum and minimum value of interest for each process parameter. Criteria for identifying critical and non-critical parameters are that a parameter is non-critical when there is no trend to failure within the POS and there is no evidence of interactions within the proven acceptable range (PAR), which is the range of experimental observations that lead to acceptable quality.

**7. Design Space:**

Design Space is defined as the multidimensional combination of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post approval change process. Design space is proposed by the applicant and is subject to regulatory assessment and approval.

**8. Control Strategy:**

A control strategy normally include input material controls, process controls and monitoring, design space around individual or multiple unit operations, and/or final product specifications used to ensure consistent quality. The finished drug products are tested for quality by assessing if they meet specifications. In addition, manufacturers are usually expected to conduct extensive in process tests, such as blend uniformity or tablet hardness.

**Methods and material**

The following plants were used in the study Henna :(Lawsonia inermis Linn) Tea powder:(Camellia sinesis) Indigo:(Indigofera tinctoria Linn) amla (Emblic Myrobalan, Indian Gooseberry), lohabhasma, mandur churn.

**Henna**

The botanical name is Lawsonia inermis which is the only species of the genus Lawsonia and belongs to the family Lythraceae. The leaves of this plant posess a red dye molecule called lawsone (2- Hydroxy – 1yl- naphtha quinone), which has the ability to bond with protein. The other components like Lawsone 1, 4 – naphtha quinone; 2- methoxy- 3- methyl- 1, 4 - naphtha quinone; flavonoids, coumarins and phenolic acids; 5-10% gallic acid and tannin. Henna balances the pH of the scalp preventing premature hair fall and graying of hair. Henna is a tall shrub or small tree, standing 1.8 to 7.6 m tall (6 to 25 ft). It is glabrous and multi-branched, with spine-tipped branch lets. The leaves grow opposite each other on the stem. They are glabrous, sub-sessile, elliptical, and lanceolate (long and wider in the middle; average dimensions are (1.5–5.0 cm x 0.5–2 cm or 0.6–2 in x 0.2–0.8 in), acuminate (tapering to a long point), and have depressed veins on the dorsal surface. Henna flowers have four sepals and a 2 mm (0.079 in) calyx tube, with 3 mm (0.12 in) spread lobes. Its petals are obvate, with white or red stamens found in pairs on the rim of the calyx tube. The ovary is four-celled, 5 mm (0.20 in) long, and erect. Henna fruits are small, brownish capsules, 4–8 mm (0.16–0.31 in) in diameter, with 32–49 seeds per fruit, and open irregularly into four splits.


**Indigo**

Indigofera tinctoria, also called true indigo, is a species of plant from the bean family that was one of the original sources of indigo dye. It has been naturalized to tropical and temperate Asia, as well as parts of Africa, but its native habitat is unknown since it has been in cultivation worldwide for many centuries. Today most dye is synthetic, but natural dye from. tinctoria is still available, marketed as natural coloring. The plant is also widely grown as a soil-improving groundcover True indigo is a shrub one to two meters high. It may be an annual, biennial, or perennial, depending on the climate in which it is grown. It has light green pinnate leaves and of pink or violet flowers. The plant is a legume, so it is rotated into fields to improve the soil in the same way that other legume crops such as alfalfa and beans are. Dye is obtained from the processing of the plant's leaves. They are soaked in water and fermented in order to convert the glycoside indican naturally present in the plant to the blue dye indigotin. The name indigo comes from the Roman term indicum, which means a product of India. This is somewhat of a misnomer since the plant is grown in many areas of the world, including Asia, Java, Japan, and Central America. Another ancient term for the dye is nil from which the Arabic term for blue, al-nil, is derived. The English word aniline comes from the same source. The dye can be extracted from several plants, but historically the indigo plant was the most commonly used because it is was more widely available. It belongs to the legume family and over three hundred species have been identified. Indigo tinctoria and. suifruticosa are the most common. In ancient times, indigo was a precious commodity because plant leaves contain only about small amount of the dye (about 2-4%). Therefore, a large number of plants are required to produce a significant quantity of dye. Indigo plantations were founded in many parts of the world to ensure a controlled supply

**Amla Powder**

Amla has been used for centuries as a beauty and health regimen, in textiles and leather tanning, and in ayurvedic medicine. The fruits are made into pickles and preserves. Amla Powder is an Ayurvedic herb made from the Indian gooseberry (Emblica officinalis). It can boost your henna hair color. When added to the mix, it can darken henna and prevent indigo from fading, creating cooler hair colors. Amla also can help enhance the dye uptake for problematic roots. You can apply a full treatment of amla to boost your hair's volume and help bring back waves/curls lost from multiple henna treatments. It can also be used as a treatment by itself to add body to your hair The botanical name is Emblica officinalis or Phyllanthus emblica L. (Emblic Myrobalan, Indian Gooseberry) It has anti-inflammatory, antibacterial and antioxidant properties that can help promote the growth of healthy, lustrous hair. Amla powder is from the dried and ground fruit of the Indian Gooseberry tree,. Amla contains natural tannins, vitamin C, ascorbic acid, and acts as a natural astringent. Amla has been used for tanning and dying hides as well as a cosmetic and folk remedy. Amla grows in central and southern India, Burma, and Ceylon. Amla is an excellent hair volumizer and curl enhancer, and facial scrub and toner It will tone down the red and create a deeper, browner tone in henna or henna/indigo hair treatments. Use as often as you like for healthy hair, scalp, skin, Non drying is considered safe for those with chemical sensitivities. Amla powder may be added to henna and indigo paste to tone down or cool down the reddish color and create a richer, deeper brown color on hair.

**Camellia Sinensis :( Tea Powder)**

Is a species of evergreen shrub or small tree whose leaves and leaf buds are used to produce tea. It is of the genus Camellia of flowering plants in the family Theaceae. Common names include "tea plant", "tea shrub", and "tea tree" black tea are also great source of rich color. Though you could use either of these things to add shine and depth to different brunette shades, this is not recommended over blonde hair. There is not enough pigment to saturate the cuticle of blonde hairs to make it brown, so it will look grayish and drab However, if falls in the brunette category, super strong black tea emulsions will add warmth to the color and their acidic nature will tighten the cuticle layer, smoothing strands to add intense shine and lessen tangles. More opaque blondes or light brown ladies have the lucky option of lightening many shades quickly with one natural ingredient: citrus. But the nice, subtle lightener that isn't too acidic is chamomile tea.

**Loha Bhasma**

It is an Ayurvedic medicine prepared from Iron. It is used in Ayurvedic treatment prevent the premature graying of hair

**Mandur Bhasma**

Mandur Bhasma is an Ayurvedic preparation which is used as medicine and also as ingredient of various medicines. It is prepared from iron rust or Mandur by complex Ayurvedic procdure. Mandor is ferric oxide/hematite or red iron oxide with chemical formula $Fe_2O_3$. It is purified and calcinated to get Mandur bhasma.

**Collection and identification of plant materials**

The plant materials were collected from the Aurade Herbal shop from latur, Mandur Bhasma was purchased from Yogesh Pharmacy from Nanded and authentication was done by botanist Dr. Swami in Institute of Dayanand Science College, Latur.

**Collection of Unpigmented Hair**

The human hair was collected Samples of gray hair were obtained from sixty-five-year-old women and were colorized by herbal hair dye procedure and used for study

**Preliminary preparation of natural hair colorants**

One gram each of henna, indigo, amla, mandur churna, loha bhasma, Tea powder was taken separately and added 200mg. A smooth paste was made with water separately. The pastes were kept aside for 1 h for imbibitions. The hair was kept in the above pastes for 1h and then washed with water and observed for its coloring.

**Selection of Suitable Combination with Henna**

One gram of henna was mixed with same proportion of indigo, tea, amla, mandur chrna,loha bhasma water was added to that in order to make a smooth paste. The paste was kept aside for 1 h for imbibition. The hair was kept in above paste for 1 hour. After that it was washed with water and observed for its colouring.

**Selection of Suitable Combination of Henna and Indigo**

Henna was mixed with indigo in different ratios from 1:1 to 1:5, mixed with water to make smooth paste and processed similarly as above to observe coloring

**Effect of pH on the selected combination on Henna and Indigo**

1:3 Ratios of henna and indigo were selected because it produced altered color and effect of pH was studied. To the paste of above ratio, of was added 1% ferric chloride solution in order to obtain the pH of 6, 7, 8, 9. Further process was same for observing its effect.

**Formulation and Evaluation of Ayurvedic Herbal Hair Dyes**

Different grades (1-,6) were assigned to colours ranging from jet black to blonde using an experimental color grade scale. White hair was used as a control for the in vitro study. Various dye formulations viz $SF_1$, SF2, SF3, SF4, SF5 and $SF_{6.....}$ SF11 were prepared using different ratios of Mehendi: Nili: Amla: Loha bhasma (Table 1, 2, 3). The herbal powders were weighed accurately, mixed geometrically followed by mixing in a polybag.

**Evaluation of Herbal Hair Dyes – In Vitro Studies**

The prepared hair dye formulations were studied for dyeing efficiency in vitro, on human white hair strands. Dyeing efficiency was determined in terms of colour grade and colour lasting capacity. Each herbal hair dye was applied to 0.2 - 0.5 gm of white human hair in vitro in a paste form. The dyed hair sample was washed with tap water after the period of 2 h; a second coat of dye was applied after 24 h of first application, kept for 2 h and then washed with tap water without aid of shampoo.

**Table No1: Color Intensity of Herbal Hair Dye and Mktd Formulation**

| Product code | Ratio of Heena:Indigo:Mandur Churna:Lohabhasma:Amla:Tea | Color Grade |
|---|---|---|
| SF1 | 2:1:0.5:0.2:0.2:0.1 | Light Brown |
| SF2 | 2:1.5:0.5:0.2:0.2:0.2 | medium brown |
| SF3 | 1:3:1.5:0.2:0.2:0.2 | Off Black |
| SF4 | 0.5:1.5:2:0.2:0.2:0.1 | Faint Brown |
| SF5 | 2:2:3:0.2:0.2:0.3 | Medium blonde |
| SF6 | 1:0.5:0.5:0.2:0.2:0.3 | Red |
| SF7 | 0.5:0.5:0.5;0.2:0.2:0.1 | Dark blonde |
| SF8 | 0.5:2:1:0.2:0.2:0.2 | Black |
| SF9 | 3:0:1:0.2:0.2:0.2 | Brown |
| SF10 | 1.5:3.5:0:0.2:0.2:0.2 | Marie gold Blonde |
| SF11 | 2:2:1:0.2:0.2:0.2 | Dark Brown |
| Mkted | | Jet black |

A color grade of Hair initially it shows the dark color as per the number given for each color after applying the shampoo whether it is herbal shampoo or synthetic shampoo the color intensity get gradually decreases The initial number is 1 after washing with shampoo number get increases while the color intensity decreases the following color grade diagram shown below:


**Table No 2: Color Grade after Washing with Synthetic Shampoo**

| Product Code | Color Grade (Day 1) | Color Grade after No shampoo washes | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 |
| SF1 | 2 Brown | 2 | 2 | 3 | 3 | 4 | 5 |
| SF2 | 3 Blonde | 3 | 3 | 4 | 4 | 5 | 5 |
| SF3 | 2 Off Black | 2 | 3 | 3 | 3 | 4 | 4 |
| SF4 | 5 Light Brown | 5 | 6 | 7 | 7 | 7 | 7 |
| SF5 | 3 Medium Blonde | 3 | 3 | 4 | 4 | 5 | 5 |
| SF6 | 2 Red | 2 | 2 | 3 | 3 | 4 | 4 |
| SF7 | 3 Blonde | 3 | 3 | 3 | 4 | 5 | 5 |
| SF8 | 1 Black | 1 | 1 | 2 | 3 | 3 | 4 |
| SF9 | 1 Dark red | 1 | 1 | 2 | 2 | 3 | 4 |
| SF10 | 2 Marie Gold Blonde | 2 | 2 | 3 | 4 | 4 | 5 |
| SF11 | 1 Darkest Brown | 1 | 1 | 2 | 2 | 3 | 4 |
| Mkted | 1 Jet black | 1 | 1 | 1 | 2 | 3 | 4 |

The hair strands were observed on 1st day and alternate days after subsequent shampoo Colour grade: Hair colour was graded as 1 to 5 by comparing with experimental colour washes till 12 shampoo washes for following (Table 1, 2) grade scale. The observations were reported after 2, 4, 6, 8, 10, 12; shampoo washes Retention capacity / Colour lasting capacity: Retention capacity of herbal hair dye can be defined as ability of hair dye to retain or last particular hair colour on white /blonde human hair for definite period of time. In present investigation, the hair was washed with mild shampoo on alternate days using tap water till 12 shampoo washes (32 days) and retention capacity was determined in terms of number of shampoo washes that a colour can withstand.

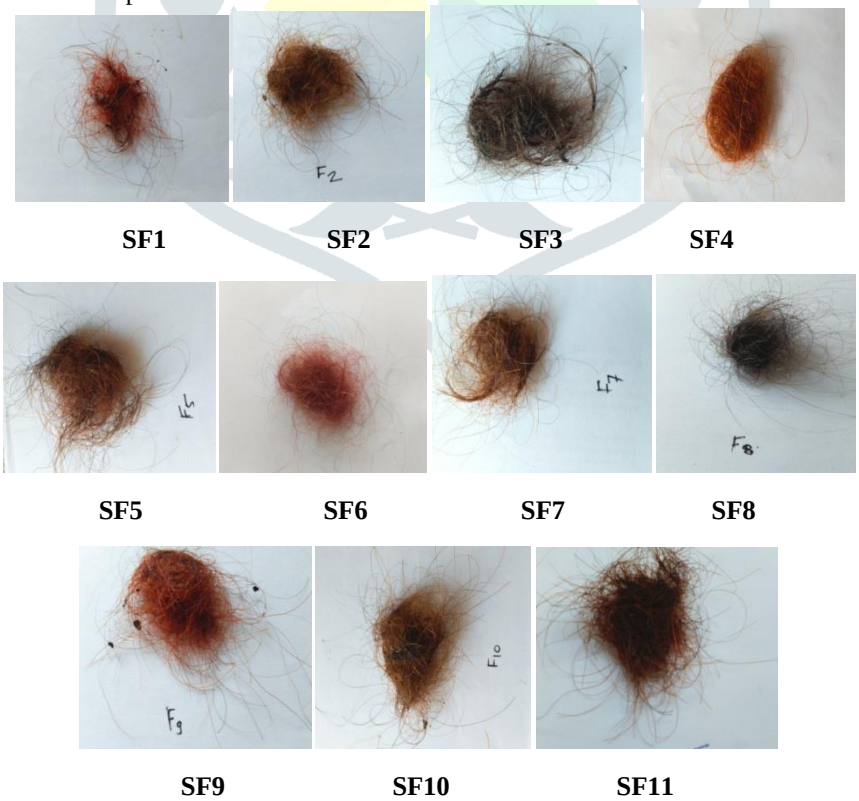



**Fig 1: Color Combination by using Different Composition of ingredients.**

**Table No 3: Color Grade after Washing with Herbal Shampoo**

| Product Code | Color Grade (Day 1) | Color Grade after No shampoo washes | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 |
| SF1 | 2 Brown | 2 | 2 | 2 | 3 | 3 | 4 |
| SF2 | 3 Blonde | 3 | 3 | 3 | 4 | 4 | 4 |
| SF3 | 2 Off Black | 2 | 2 | 2 | 3 | 3 | 4 |
| SF4 | 5 Light Brown | 5 | 5 | 6 | 6 | 7 | 7 |
| SF5 | 3 Medium Blonde | 3 | 3 | 3 | 4 | 4 | 5 |
| SF6 | 2 Red | 2 | 2 | 2 | 3 | 3 | 4 |
| SF7 | 3 Blonde | 3 | 3 | 3 | 4 | 4 | 5 |
| SF8 | 1 Black | 1 | 1 | 1 | 2 | 2 | 3 |
| SF9 | 1 Dark red | 1 | 1 | 1 | 2 | 2 | 3 |
| SF10 | 2 Marie Gold Blonde | 2 | 2 | 3 | 3 | 3 | 4 |
| SF11 | 1 Darkest Brown | 1 | 1 | 2 | 2 | 3 | 3 |
| Mkted | 1 Jet black | 0 | 0 | 0 | 0 | 1 | 1 |

**Table No 4: Different Combination of Three Ingredients**

| Formulation | Henna | Indigo | Mandur churna | Result |
|---|---|---|---|---|
| F1 | - | - | - | Brown |
| F2 | - | - | + | Light brown |
| F3 | - | + | + | black |
| F4 | + | + | + | Dark brown |
| F5 | - | - | 0 | blonde |
| F6 | 0 | - | + | Light blonde |
| F7 | 0 | 0 | - | Light brown |
| F8 | + | 0 | - | Red |
| F9 | - | 0 | + | Marie gold |
| F10 | + | + | 0 | Dark brown |
| F11 | 0 | + | - | black |
| F12 | - | + | 0 | Off black |

**Table No 5: comparison of Different Herbal and Synthetic shampoo and Water**

| Product code | Color grade after washes with water | Color grade after washes with Ayurveda shampoo | Color grade after washes with synthetic shampoo |
|---|---|---|---|
| SF1 | 5 | 6 | 7 |
| SF2 | 8 | 9 | 10 |
| SF3 | 2 | 4 | 4 |
| SF4 | 6 | 7 | 7 |
| SF5 | 9 | 10 | 10 |
| SF6 | 16 | 17 | 18 |
| SF7 | 8 | 9 | 9 |
| SF8 | 1 | 3 | 4 |
| SF9 | 14 | 16 | 17 |
| SF10 | 10 | 12 | 13 |
| SF11 | 4 | 6 | 6 |
| Marketed | 0 | 1 | 3 |

**Table No 6: Comparison of Color Retention Property of Herbal Hair Dye Formulation and Marketed Formulation**

| Product Code | Ratio Of Heena:Indigo:Mandu Churna:Lohabhasma:Amla:Tea | Color Grade | Color Grade After No Of Shampoo Washes | | | | | | Retention Property Of No Of Shampoo Washes |
|---|---|---|---|---|---|---|---|---|---|
| | | | **2** | **4** | **6** | **8** | **10** | **12** | |
| SF8 | 0.2:2:1:0.2:0.2:0.2 | 1BLACK | **1** | **1** | **2** | **3** | **3** | **4** | 10-12 |
| MKTED | | JET BLACK | **0** | **0** | **0** | **1** | **2** | **3** | 10-12 |

**Table No 7: Comparison of Physicochemical Property of herbal powder and marketed**

| **Test** | **Ayurvedic Herbal Hair Dye** | **Mkted herbal product** |
|---|---|---|
| Appearance | Fine Powder | Crysatl |
| Color | Brown | Dark Brown |
| Odor | Characteristic | Characteristic |
| PH | 06 | 09 |
| Particle size | 800-200µ | 80-160µ |
| Angle of repose | 24-28 ° | 30 - 34° |
| Bulk Density | 0.388 g/cc | 0.380 g/cc |
| Tapped Density | 0.495 g/cc | 0.482 g/cc |

**Table No 8 : Different marketed formulation**

| **Brand** | **Composition** | **Marketed by** |
|---|---|---|
| Godrej indica hair dye | Henna, Hibiscus, PPD – NLT 20% in powder form and NMT 2.5% on dilution | Godrej Hair Care Institute |
| L'Oreal color natural | Amla, Shikakai, Reetha, Mehendi, PPD- NLT 25% in powder form and NMT 2.5% in dilute form | CavinKare Private Ltd. |
| Garner hair color | Bhringraj, Amla, Methi, Henna and Hibiscus, Colorant and Softener PPD – NLT 10% in powder form and NMT 3% on dilution | Garner hair color Private Ltd. |
| Hennakali mehandi | Hydrogen peroxide, Diaminophenol, Ammonia, Resorcinol and PPD | Godrej Consumer Products Ltd. |

Final Equation in Terms of Actual Factors:

Colour Intensity = +3.20426+1.06400* Henna -1.71200* Indigo -1.42593* Mandur churna -0.30400* Henna^2 +0.33600* Indigo^2 +0.64815* Mandur churna^2

**Table No.9: actual factorial Box-Behenken design for the 3 independent variables brown col**

| **Factor** | **Name** | **Level** | **Low Level** | **High Level** | **Std. Dev.** | **Coding** |
|---|---|---|---|---|---|---|
| A | Henna | 1.75 | 0.50 | 3.00 | 0.000 | Actual |
| B | Indigo | 2.25 | 1.00 | 3.50 | 0.000 | Actual |
| C | Mandur Curan | 1.10 | 0.20 | 2.00 | 0.000 | Actual |

| | | Factor 1 | Factor 2 | Factor 3 | Response 1 | Response 2 | Response 3 |
|---|---|---|---|---|---|---|---|
| Std | Run | A:Henna | B:Indigo | C:Mandur churna | Colour Intensity | Colour Retention Capacity (1st Washing) | Colour Retention capacity ( 12th Washing) |
| | | gm | gm | gm | | | |
| 1 | 10 | 0.5 | 1 | 1.1 | 2 | 2 | 5 |
| 2 | 16 | 3 | 1 | 1.1 | 1 | 1 | 4 |
| 3 | 8 | 0.5 | 3.5 | 1.1 | 1 | 1 | 4 |
| 4 | 11 | 3 | 3.5 | 1.1 | 1 | 1 | 5 |
| 5 | 12 | 0.5 | 2.25 | 0.2 | 1 | 1 | 5 |
| 6 | 13 | 3 | 2.25 | 0.2 | 1 | 1 | 5 |
| 7 | 4 | 0.5 | 2.25 | 2 | 1 | 1 | 4 |
| 8 | 9 | 3 | 2.25 | 2 | 2 | 2 | 4 |
| 9 | 2 | 1.75 | 1 | 0.2 | 3 | 3 | 5 |
| 10 | 15 | 1.75 | 3.5 | 0.2 | 2 | 2 | 6 |
| 11 | 6 | 1.75 | 1 | 2 | 2 | 2 | 6 |
| 12 | 3 | 1.75 | 3.5 | 2 | 2 | 2 | 5 |
| 13 | 14 | 1.75 | 2.25 | 1.1 | 1 | 1 | 4 |
| 14 | 7 | 1.75 | 2.25 | 1.1 | 1 | 1 | 4 |
| 15 | 17 | 1.75 | 2.25 | 1.1 | 1 | 1 | 5 |
| 16 | 5 | 1.75 | 2.25 | 1.1 | 2 | 2 | 4 |
| 17 | 1 | 1.75 | 2.25 | 1.1 | 1 | 1 | 5 |

**Table No.10: actual factorial Box-Behenken design for the 3 independent variables black color**

**Final Equation in Terms of Actual Factors:**

**Colour Intensity= +3.20426 +1.06400 * Henna -1.71200* Indigo -1.42593 * Mandur churna -0.30400* Henna^2 +0.33600* Indigo^2 +0.64815* Mandur churna^2Formulation Few Herbal Hair Dye Products of Indian Market.**

**Open Patch Test**

Sensitizing the potential of formulation is to be tested. Hence a small quantity has been applied on the forearm[22] to check for any local reaction like irritation and erythrema within three hours of application.

**Primary Skin Irritation Test** [23 24]

Four healthy female Wister albino rats, weighed 200-250gm were selected for study. Each rat was caged individually food and water given during the test period 24hrs prior to the test. The hair from the back of each rat of 1cm2 was shaved on the side of the spine to expose sufficiently large test areas, which could accommodate three test sites were cleaned with surgical spirit. 1ml quantity of formulations HF1, HF2, HF3 was applied over the respective test sites of one side of the spine. The test sites were observed for erythema and edema for 48hrs after application (Uno et al, 1991)

**Application of Test Formulations**

Female wistar albino rats, 200-250gm, were used for hair growth studies. They were placed in cages and kept in (23°C±10, 60% ±10 RH) standard environmental conditions, fed with standard diet and allowed free access to drinking water for two days. All animal experiments were carried out in accordance with guidelines of CPCSEA and the study was approved by the Institutional Animal Ethical committee (379/01/ab/CPCSEA). The rats were divided into 5 groups of 6 rats each 6cm$^2$ area of dorsal portion of all the rats shaved area to remove all hair. Group I was kept as control, where there was no drug treatment. Group II was treated as standard, where 1ml of (2% Minoxidil ethanolic solution) was applied over the shaved area, once a day. The animals of remaining groups were given application of 1ml of formulation HF1, HF2 and HF3 respectively, once a day. This treatment was continued for 30 days (Adhirajan et al, 2001).

**Predictive sensitivity Testing**: [ 25 26]

The ascertainment of the irritant and sensitizing potential of formulation is prophetic or predictive sensitivity testing. Under this test the ingredients of the formulation and the formulation as a whole were tested for their irritation and sensitivity[25] This testing was conducted on 8 female volunteers hence, human ethical committee approval was obtained (No. is UCPSC/s/2006-01) The formulation was applied to 1 cm$^2$ area of cotton gauze. The gauze was backed with adhesive tape in order to hold it in position. Then 8 female volunteers were asked to apply this patch to the inner surface of forearm. The purpose of selection of this site for application was to enable the volunteer to readily remove the patch if any irritation is encountered. The volunteers were asked to remove patch immediately, if they feel itching or burning. Further they asked to keep the patch site dry, avoid rubbing or scratching test site. The patch was kept in position for 24 hours. After 24 h, the patch was removed. The test site was observed up to 40 min from the time of removal. This time interval is necessary so that the skin may recover from the effects of pressure of certain patch test substance

**Colorimetric Filter Paper Test** [27 28]

This method is based on colorimetric assay in order to detect PPD in a given sample. The test consisted of preparation of control solution, test solutions as well as a developing reagent solution. The control solution was prepared using 0.5 mg of PPD in 10 ml of ammonium hydroxide. In the same way, all test solutions were prepared taking 0.5 mg of the study samples in 10 ml of ammonium hydroxide. The developing reagent solution consisted of 1 g of vanillin in 15 ml of isopropyl alcohol. The study test solution was diluted with isopropyl alcohol in the ratio of 1:1 (v/v). A drop of the diluted test sample was placed on a piece of filter paper. After a minute, a drop of developing reagent solution was added onto the filter paper. The filter paper was allowed to dry off completely. The development of yellow colour on the filter paper indicated presence of PPD in the study sample

**Thin Layer Chromatography** [29]

Another qualitative test in order to confirm the presence of PPD in the study samples was using the traditional approach of separation thin Layer Chromatography. The method is based on extraction of the chemical component (PPD) into 96% ethanol. PPD if present in the study samples will be extracted into the solution very quickly. Following this step, one – dimensional thin layer chromatography was performed. To identify the coloring material present in the study sample. Silica chromatography plates were cut into suitable sizes and were placed for activation into oven at 100°C for 45 minutes. Mobile phase designed for the active separation of the constituents was acetone – chloroform - toluene in the ratio of 35:25:40 (v/v). The mobile phase was kept for saturation into a chamber for around 1 hour. In the mean while all study samples were extracted into 96% ethanol, also the standard PPD was extracted in the same way that acted as control for detection of PPD in other study samples the activated silica plates were spotted with the standard and study samples and placed into the saturated chamber containing. Mobile phase kept at room temperature and left to develop. The plates were removed, dried and inspected visually with reference to the standard plate for the presence of PPD in the study samples. Usually the darker the color obtained, higher is the concentration of PPD present in the marketed hair color.

## RESULTS AND DISCUSSION

**Effect of application of plant material and also the formulated organic dye**

Each powdered plant material has been applied to the hair by mixing in water and the contact time has been1.30 hr. Among the seven plant sources lawsonia Inermis, Indigofera tinctoria, loha bhasma, Embilica officianalis, Camellia Sinensis and mandur bhasma have shown the dying effect on the hair without damaging the property of hair.

Figures-1 shows the effect of herbal hair dye on hair with different combinations of plant material and observes the color of each combination on hair. in that the different color combination was obtained as shown in fig 1. SF3, SF8, SF11 were found to produce off black to dark brown color initially. And such a combination was selected to further study.

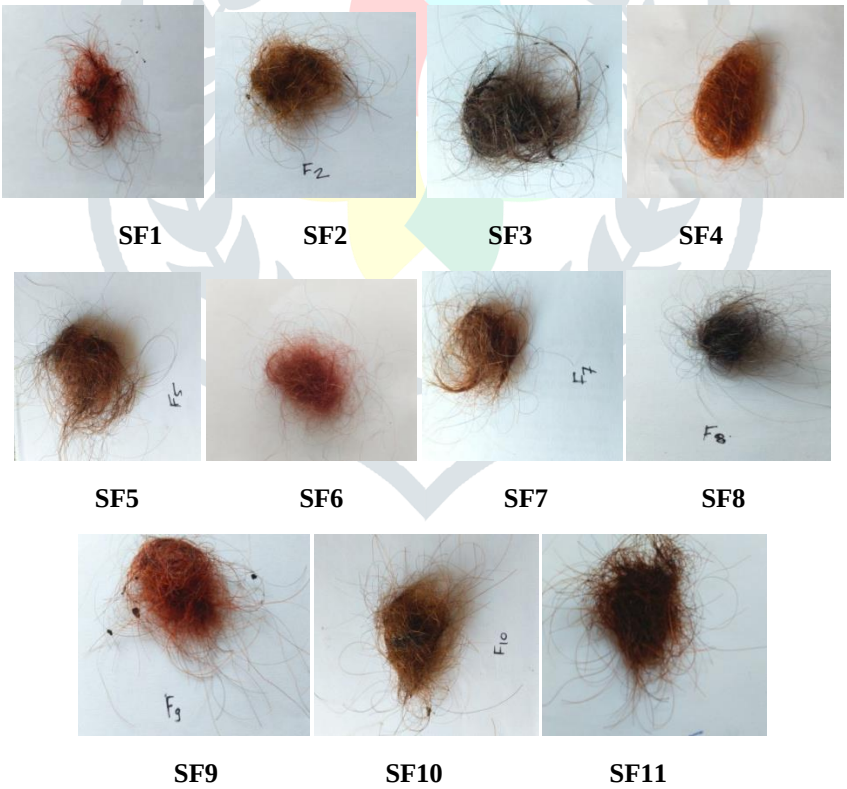



**Fig-1Color Combination by using Different Composition of Ingredients.**

1st wash 2nd wash 3rd wash 4th wash 5thwash

6th wash 7th wash 8th wash 9th wash 10th wash

**Fig-2 washing of hair 10 times with synthetic shampoo on Herbal Formulation**

1st wash 2nd wash 3rd wash 4th wash 5th wash

6th wash 7th wash 8th wash 9th wash 10th wash

**Fig- 3 washing of hair 10 times with Herbal shampoo on Herbal Formulation**

**The result of such combination in after washing with synthetic shampoo**

Compositions SF3, SF8, SF11 were found to produce off black to dark brown color initially (grades1) and fades gradually to grade 5, 6 (chestnut – medium brown) after 10 to 12 Synthetic shampoo washes (Figure 2 , Table 2). ). Dyes 'SF3 and SF8' produce grades 2 and 3 while dye 'SF11' showed grade 3 and4 after 6 and 8 washes and grade 1(darkest brown)

**The result of such combination in after washing with Herbal shampoo**

The Same Composition Washed by Ayurvedic Shampoo the results show in (fig 3 tables 3) the result show is different as compare to washes with synthetic shampoo the color retention property and color intensity get increases after 10 -12 shampoo washes the composition SF3,SF8 Initially Grade 1- 4 and stand with Grade 2 - 3 After 10 Shampoo washes same thing was happened with composition SF11 the color grade initially 1 and stand with 3,4 after 10 shampoo washes Herbal Powder composition using (0.5:2) ratio of Madayanti : Nili ; (1:1) ratio of amla : loha bhasma and additional ingredient 1 gm Mandur bhasma powder was added and characterized dyeing properties shows after 2 times application on white hair in order to get better results viz darker colour (grade1/2) and better retention of color after (10 -12 shampoo washes.

Control 1st wash 2nd wash 3rd wash 4th wash

5th wash 6th wash 7th wash 8th wash 9th wash 10th wash

**Fig-4 washing of Hair 10 times by synthetic shampoo on marketed formulation**

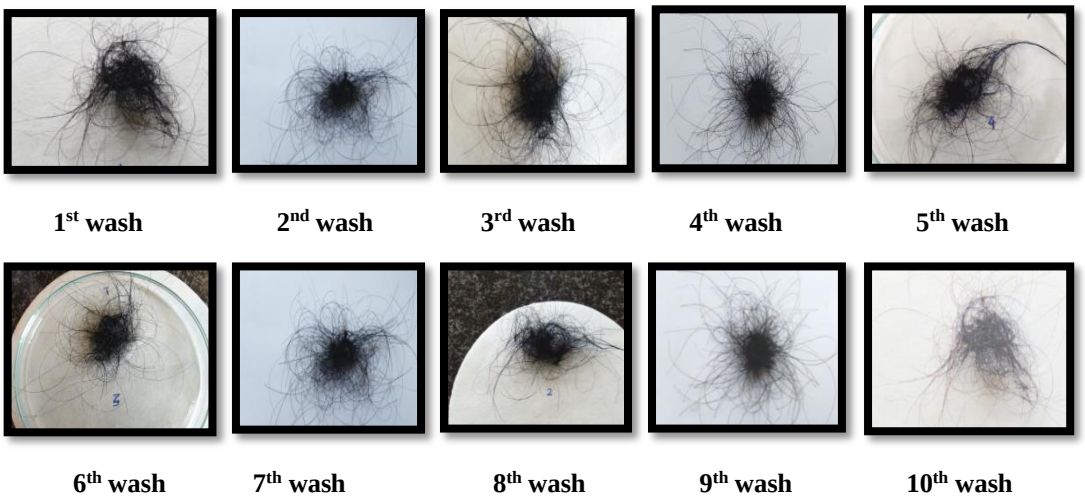



**Fig-5 washing of Hair 10 times by Herbal shampoo on marketed formulation**

**Comparison of Herbal hair formulation with Marketed Dye M1- In Vitro Evaluation**

Herbal formulation was compared with marketed dye M1 for colour grade, retention capacity and color intensity the herbal formulation dyed the hair strands off black when tested in vitro and showed retention capacity and color intensity of at least 10-12 shampoo washes either synthetic or herbal shampoo washes. The herbal formulation fade to grade 4 (burgundy color) after 10 washes, however marketed dye M1 initially gave jet black colour after 10 shampoo washes stand with light brown colour brown color (grade 6). The retention capacity of marketed dye M1 was found to be only 6-8 shampoo washes, little greater than Herbal formulation.

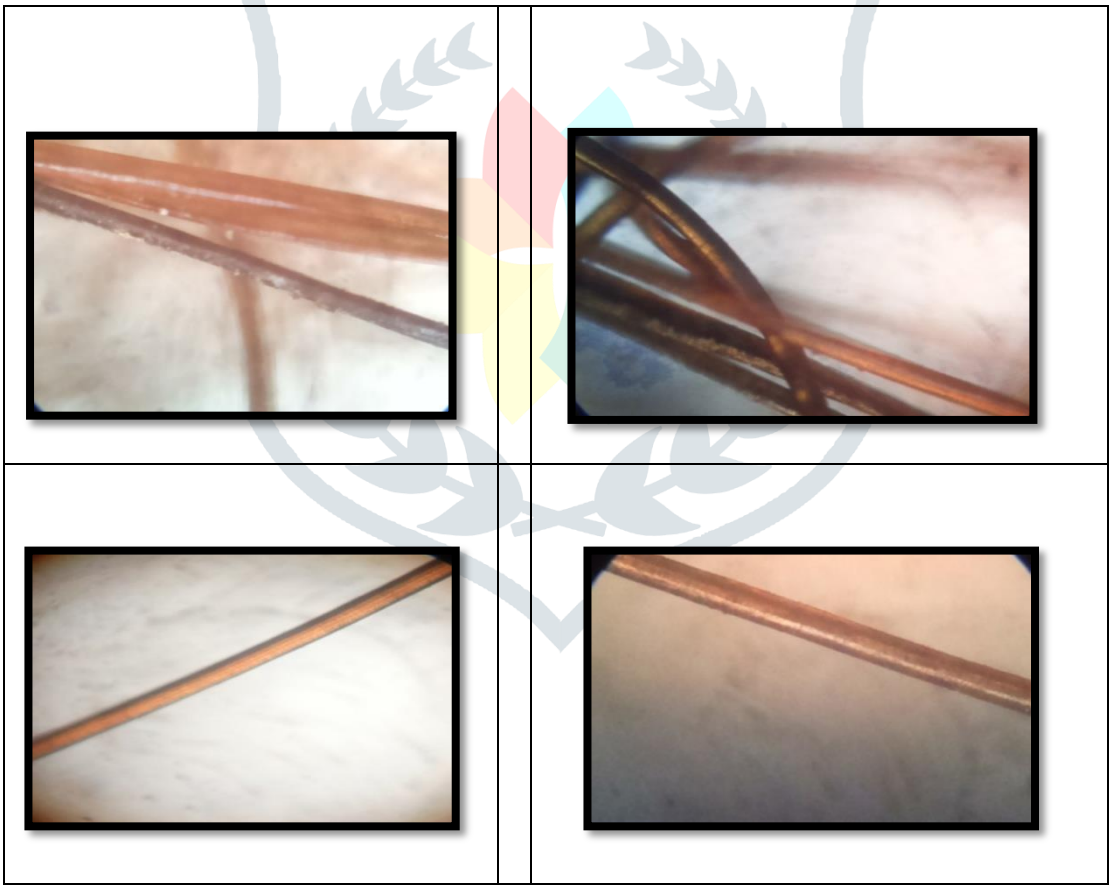


**Fig-6 Microscopic evaluation of hair under the microscope of Herbal Hair Formulation**

In Comparison of microscopic photograph (Figure 6) further confirmed the darker grade (off black) produced Herbal dye in the medulla and cortex region of hair region as compared to marketed dye M1 the herbal formulation was safe and there is no damage of cortex and medulla region of hair as shown in fig 6.

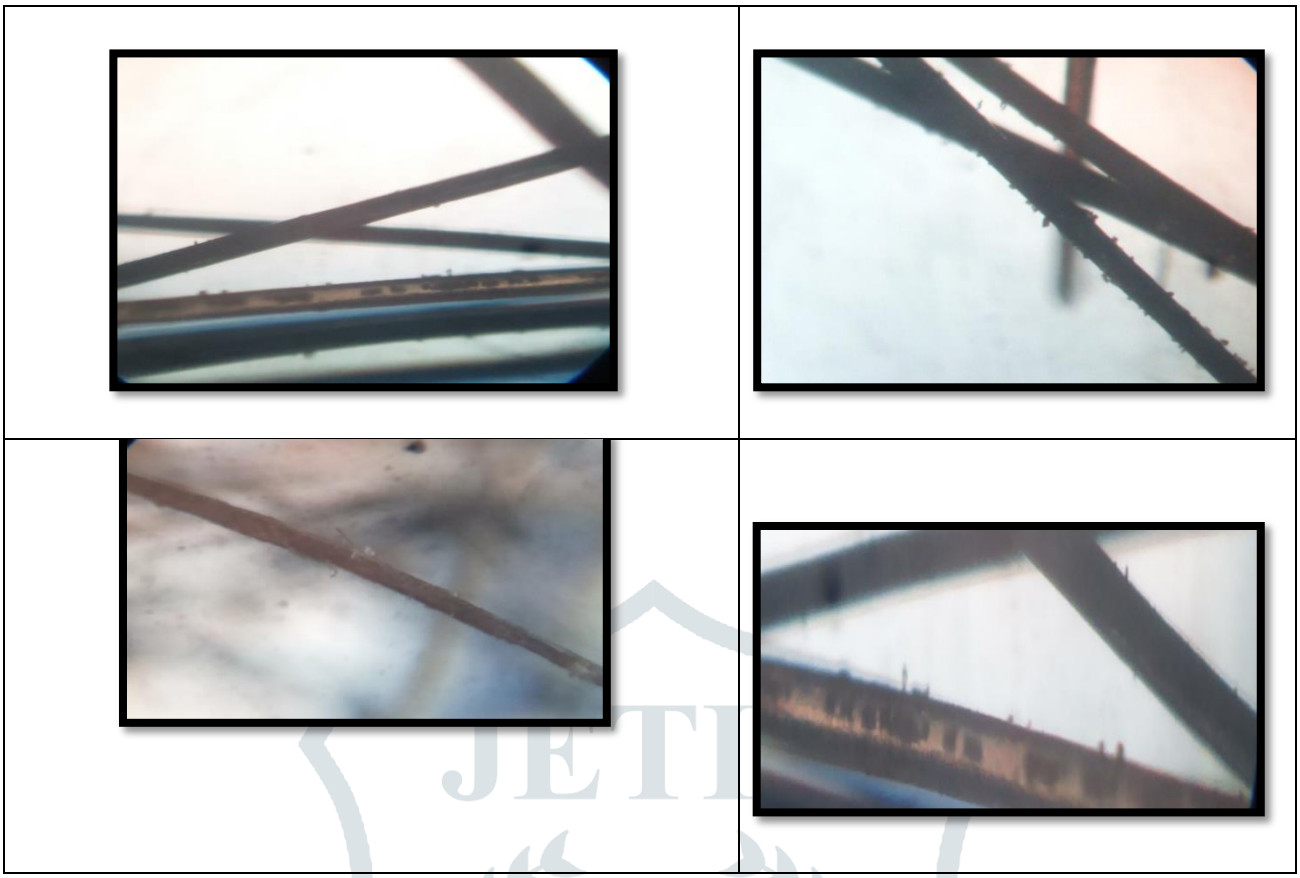


**Fig- 7 Microscopic evaluation of hair under the microscope of Marketed Formulation**

The marketed formulation M1 shows the microscopy the medulla region of the hair and cortex region of hair get damaged after repeated application on hair of marketed formulation when the herbal formulation get apply on hair with 2 -3 times there is no side effect and no damage the medulla and cortex region of the hair and as shown in microscopic observation of herbal hair formulation on hair.

Thus the desired off black colour can be obtained by selecting the proper proportions of Mehendi, Nilini, Loha Bhasma, Amla And Mandur Bhasma and Black tea powder. Both the formulations were compared with respect to various physicochemical properties in (Table 4) Increased dyeing effect and better retention capacity and color intensity Have been developed.

**Herbal Hair dye formulation may be attributed to following property.**

The various constituents present in these herbs such as coloring principles lawsone (mehendi), indigotin (nili), tannins and minerals in combination with metallic dye loha bhasma produce desired burgundy to off black colour. Dyeing action of madayanti may be attributed to reaction of lawsone (thiol group) with keratin of hair[11]. It was also suggested that dyeing action is because of acceleration of blood circulation, activation of dermal papilla and increased nutrition to the hair follicle[12]. Indigofera, one of the oldest and widely used coloring agents in the world[13] contains 'Indigotin' that gives violet colour to hair. Kesharanjan action of nili results into krishnakesha and it is described in Nighantu period as neelkeshi and krishnavyanjanakesi. Dried fruits of amla have been used in different shampoo and oil preparations[8], for promotion of hair growth and prevention of hair greying- classic sign of pitta dosha. Amla fruit is tridoshashamaka, especially pittashamaka and is effective against manifestations of pitta on hair. Amla alleviates pitta dosha due to its sheeta and madhura properties and thereby promotes pigmentation of hair.

Loha bhasma is a microfine powder of iron oxide containing Fe, $Fe_2O_3$, $Fe_3O_4$[15] and interaction of iron oxide with fine amla powder (ascorbic acid) produce fused black particles (chelates) capable of dyeing hair. Thus modifiers amla and loha bhasma in 1:1 ratio in a formulation enhance penetration of black particles as well as lawsone and indigotin, deep into the medulla region, thereby increasing colour intensity and retention property.Further tannins present in large amounts in amla create affinity between adjective dyes and hair[16] Microscopic evaluation facilitates the observation of colour grade, intensity and penetration of a dye. Dyeing efficiency of Herbal dye and marketed dye M1 was also compared using photomicrograph of herbal hair dye and M1 dyed hair strands to Grade 2 color and increased penetration was observed in case of Herbal dye when compared Analysis of hair coloring products among which 5 products, samples 1 to 5 indicated the presence of PPD in their label claim showed positive results. All 100 % natural henna powders, samples 6 to 10 are absolutely free from PPD and have only natural coloring materials in them because they showed negative results for presence of PPD Thus, it was verified that these henna powders are actually 100% natural and do not contain any chemical coloring material in them for imparting color on their application to hair

**The result of colorimetric test**

A drop of the diluted test sample was placed on a piece of filter paper. After a minute, a drop of developing reagent solution was added onto the filter paper. The filter paper was allowed to dry off completely. The development of yellow color on the filter paper indicated presence of PPD in the study sample (Figures 3 and 4)[20].

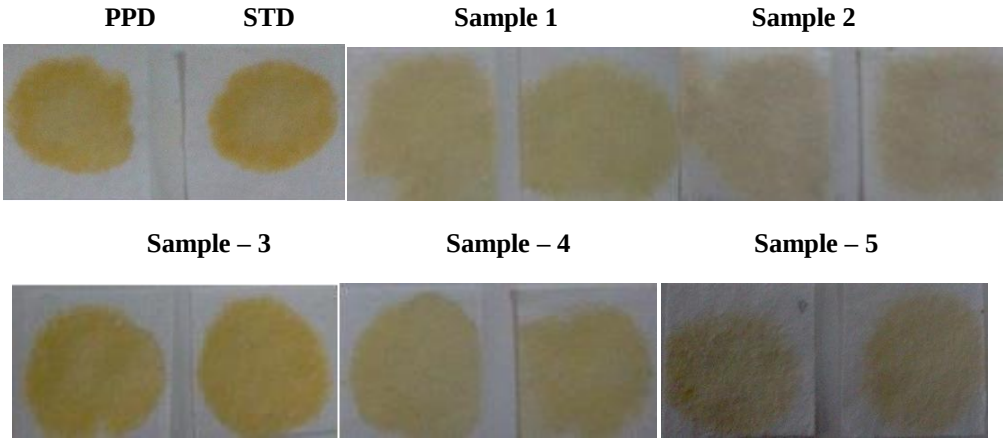



**Fig-8: Synthetic Hair dyes**

**Results of qualitative colorimetric filter paper test for detection of paraphenylenediamine in products containing PPD**

**100% Natural Henna Powders**

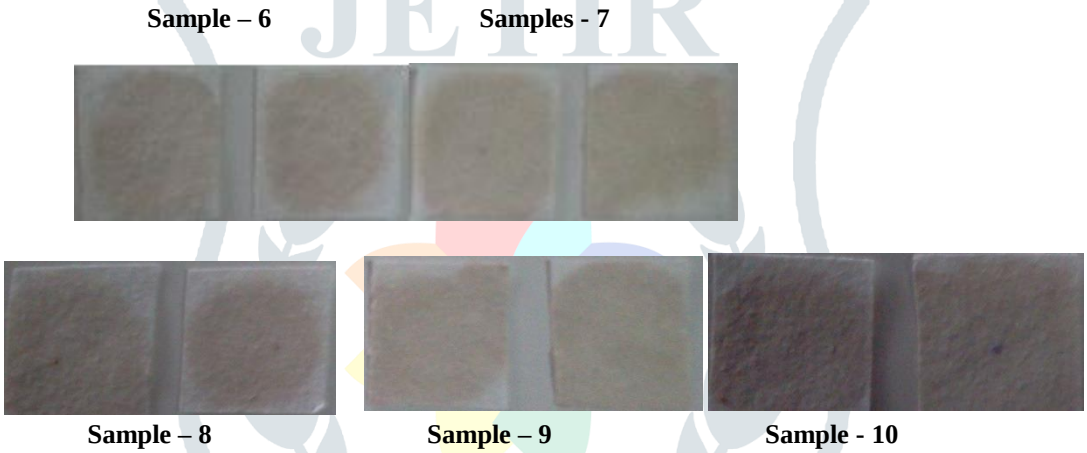



**Fig -9: Results of qualitative colorimetric filter paper test for detection of paraphenylenediamine in natural henna products**

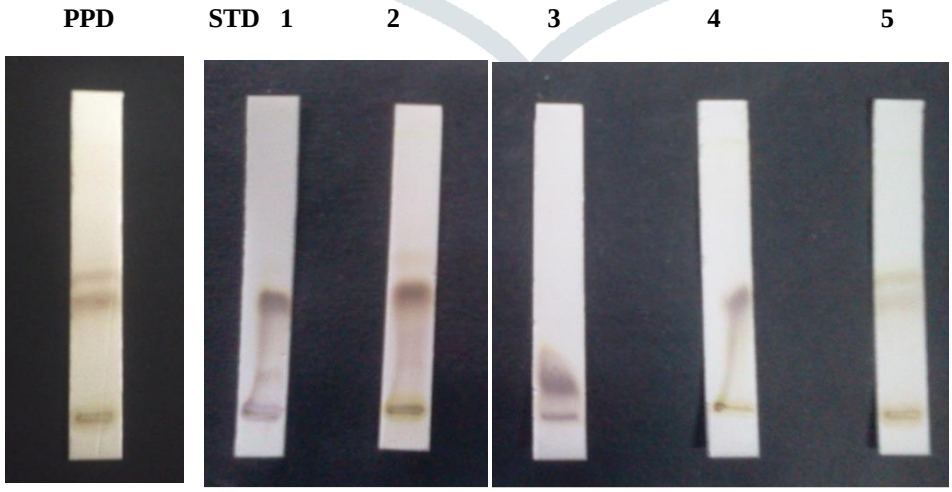



**Fig10: Results of qualitative Thin Layer Chromatography for detection of paraphenylenediamine in products containing PPD**

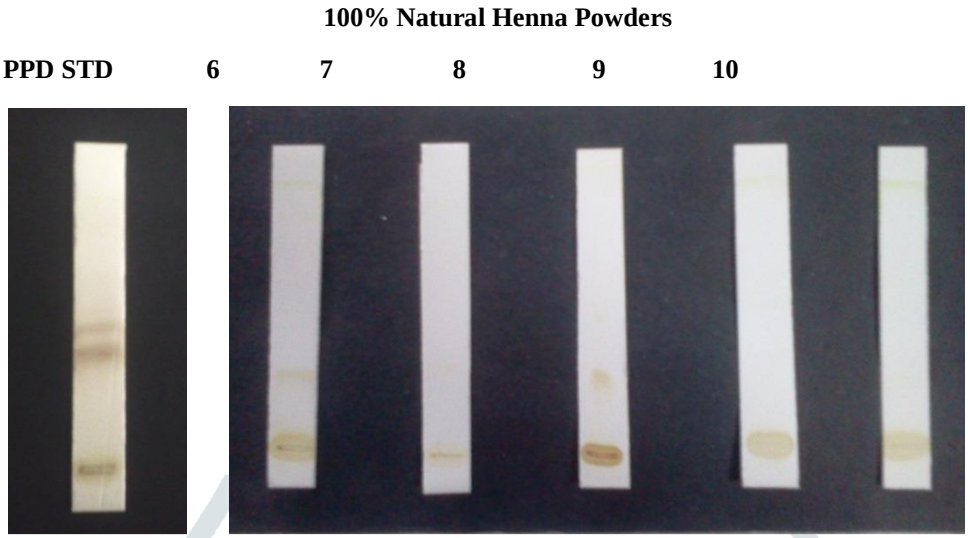



**Fig-11: Results of qualitative Thin Layer Chromatography for detection of paraphenylenediamine in natural henna products**

The natural henna powders were found to be free from this chemical. Also visual inspection of TLC plates helped us to depict the presence of the chemical in black henna and synthetic hair dyes depending upon the intensity of the band developed on the TLC plate. Concluding, the commercially available black henna and hair dye which contain PPD is a known trigger of contact allergies and various adverse reactions and one of the main sensitizing agents associated with permanent and semi-permanent dyes.

**Result and discussion of Predictive sensitivity Testing and open patch testing:**

The patch was kept in position for 2-3 hours. After 3 h, the patch was removed. The test site was observed up to 40 min from the time of removal. This time interval is necessary so that the skin may recover from the effects of pressure of certain patch test substance there is no complain of any female volunteer for itching and burning sensesion on her forearm

**Result and discussion of primary irritation testing**

1gm quantity of formulations HF1, HF2, HF3 was applied over the respective test sites of one side of the spine. The test sites were not observed for erythema and edema or any kind of the allergic type of reaction for 48hrs after application.

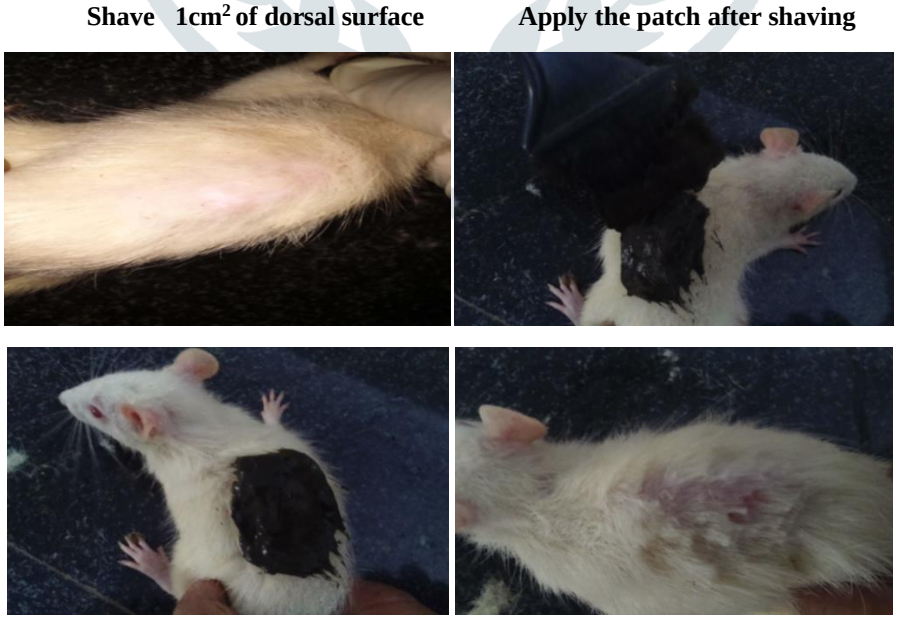



**Fig-12: Apply the patch on albino rats No Edema, Erythema Occur**

**Result and discussion of Predictive sensitivity Testing and open patch testing:**

The patch was kept in position for 2-3 hours. After 3 h, the patch was removed. The test site was observed up to 40 min from the time of removal. This time interval is necessary so that the skin may recover from the effects of pressure of certain patch test substances there is no complaint of any female volunteer for itching and burning sensation on her forearm.

**Design of Experiment:**

**Table No 11:- 3 Factorial design with upper, middle & lower limits of all factors Statistical Optimization technique**

| 3 factors | 3 Levels | | |
|---|---|---|---|
| | -1 | 1 | +1 |
| Henna | 0.5 | 1.5 | 3 |
| Indigo | 1 | 2.5 | 3.5 |
| Mandur churn | 0.2 | 1.5 | 2 |

Design Expert, Version: 9.0.51, stat- Ease, software was used for formulation and evaluation of herbal hair dye (HHD) developed by using Box Behnken experimental design.

**Table No.12: Coded full factorial Box-Behnken design for the 3 independent variables**

| | | Factor 1 | Factor 2 | Factor 3 | Response 1 | Response 2 | Response 3 |
|---|---|---|---|---|---|---|---|
| Std | Run | A:Henna | B:Indigo | C:Mandur churna | Colour Intensity | Colour Retention Capacity (1st Washing) | Colour Retention capacity ( 12th Washing) |
| | | gm | gm | gm | | | |
| 1 | 10 | -1.000 | -1.000 | 0.000 | 2 | 2 | 5 |
| 2 | 16 | 1.000 | -1.000 | 0.000 | 1 | 1 | 4 |
| 3 | 8 | -1.000 | 1.000 | 0.000 | 1 | 1 | 4 |
| 4 | 11 | 1.000 | 1.000 | 0.000 | 1 | 1 | 5 |
| 5 | 12 | -1.000 | 0.000 | -1.000 | 1 | 1 | 5 |
| 6 | 13 | 1.000 | 0.000 | -1.000 | 1 | 1 | 5 |
| 7 | 4 | -1.000 | 0.000 | 1.000 | 1 | 1 | 4 |
| 8 | 9 | 1.000 | 0.000 | 1.000 | 2 | 2 | 4 |
| 9 | 2 | 0.000 | -1.000 | -1.000 | 3 | 3 | 5 |
| 10 | 15 | 0.000 | 1.000 | -1.000 | 2 | 2 | 6 |
| 11 | 6 | 0.000 | -1.000 | 1.000 | 2 | 2 | 6 |
| 12 | 3 | 0.000 | 1.000 | 1.000 | 2 | 2 | 5 |
| 13 | 14 | 0.000 | 0.000 | 0.000 | 1 | 1 | 4 |
| 14 | 7 | 0.000 | 0.000 | 0.000 | 1 | 1 | 4 |
| 15 | 17 | 0.000 | 0.000 | 0.000 | 1 | 1 | 5 |
| 16 | 5 | 0.000 | 0.000 | 0.000 | 2 | 2 | 4 |
| 17 | 1 | 0.000 | 0.000 | 0.000 | 1 | 1 | 5 |

The independent variables selected were Sodium Alginate, Citric acid, Sodium Bicarbonate and the dependent variables selected were the floating lag time and Swelling index. In the Box Behnken experimental design, in this design, three factors was evaluated and experimental trials were performed in all 17 possible combinations.

**Data Analysis**

The model parameters obtained from the analysis of variance (ANOVA) for the responses of all the formulations are shown in tables. These parameters were used to construct the models that describe the effect of the independent variables on the responses. Different batches of formulations within the experimental design were prepared to color intensity and color retention capacity The F values for the responses color intensity and color retention capacity were found to be 1.46 and 3.3746 respectively, which indicate that the models are significant. The values of Prob >F less than 0.05 for all the responses are indicating that the models are significant. The response of model terms A, B, C, D, A^2, C^2 and D^2 for floating lag time and A, B, C, D, BC, A^2 and B^2 for were found to be significant. The F value of lack of fit for floating lag time and swelling index was found to be 1.46 and 0.3746 respectively which implies that the lack of fit is significant. Similarly 'R-squared' value was also calculated for all responses and found to be closer to the ideal value High 'R- squared' value signifies that the model terms are highly correlated to each other leading to a poor model. In contrast to this 'R- squared' value obtained in the present model is close to zero, which indicates a good model. In all the cases 'Pred R squared' values are in reasonable agreement with the 'Adj R squared' values. In all the cases 'Adeq Precision' values are in the range of 10.10 – 10.345 indicating an adequate signal and that the model can be used to navigate the design space. The VIF (variance inflation factor) values for the all models were found to be near to One indicating a good estimation of coefficient. The application of response surface methodology yielded the following regression equations which give an empirical relationship between the logarithmic values of floating lag time and swelling index. Test variables in coded units.

**Final Equation in Terms of Coded Factors**

Colour Intensity = +1.979E-016* A-0.25* B+3.643E-018* C-0.48 * A^2+0.52* B^2+0.53* C^2

**Table No13: Analysis of variance (ANOVA) of SF8 formulation on washing**

**Analysis of variance (ANOVA) of SF8 formulation on color intensity**

| Analysis of variance table [Partial sum of squares - Type III] | | | | | | |
|---|---|---|---|---|---|---|
| **Std. Dev.** | **0.50** | | **R-Squared** | | | **0.5910** |
| | **Sum of** | | **Mean** | **F** | **p-value** | |
| **Mean** | 1.47 | | Adj R-Squared | | | 0.3457 |
| **C.V. %** | 34.34 | | Pred R-Squared | | | -0.3231 |
| **Model** | 3.69 | 6 | 0.61 | 2.41 | 0.1052 | significant |
| **PRESS** | 8.25 | | Adeq Precision | | | 4.629 |
| ***B-Indigo*** | *0.50* | *1* | *0.50* | *1.96* | *0.1917* | |
| ***C-Mandur churna*** | *4.441E-016* | *1* | *4.441E-016* | *1.742E-015* | *1.0000* | |
| ***A^2*** | *0.95* | *1* | *0.95* | *3.73* | *0.0824* | |
| ***B^2*** | *1.16* | *1* | *1.16* | *4.55* | *0.0587* | |
| ***C^2*** | *1.16* | *1* | *1.16* | *4.55* | *0.0587* | |
| **Residual** | 2.55 | 10 | 0.25 | | | |
| ***Lack of Fit*** | *1.75* | *6* | *0.29* | *1.46* | *0.3726* | *significant* |
| ***Pure Error*** | *0.80* | *4* | *0.20* | | | |
| **Cor Total** | 6.24 | 16 | | | | |

**Model coefficients of thermally F9 formulations estimated by multiple regression on color retention capacity (significance of regression coefficients) :**

**Table No.14: Model coefficients of SF8 formulations estimated by multiple regressions on color intensity**

| | Coefficient | | Standard | 95% CI | 95% CI | |
|---|---|---|---|---|---|---|
| Factor | **Estimate** | **df** | **Error** | **Low** | **High** | **VIF** |
| **Intercept** | 1.20 | 1 | 0.23 | 0.70 | 1.70 | |
| **A-Henna** | 1.979E-016 | 1 | 0.18 | -0.40 | 0.40 | 1.00 |
| **B-Indigo** | -0.25 | 1 | 0.18 | -0.65 | 0.15 | 1.00 |
| **C-Mandur churna** | 3.643E-018 | 1 | 0.18 | -0.40 | 0.40 | 1.00 |
| **A^2** | -0.48 | 1 | 0.25 | -1.02 | 0.073 | 1.01 |
| **B^2** | 0.52 | 1 | 0.25 | -0.023 | 1.07 | 1.01 |
| **C^2** | 0.53 | 1 | 0.25 | -0.023 | 1.07 | 1.01 |

The contour and response surface plots for the responses of all formulation factors are shown in Figure...To. In contour and response plots, the response surface is established as a function of two factors at a time, holding all other factors at fixed levels which are more helpful in understanding both the main and the interaction effects of these two factors. Contour and surface plots were drawn for color intensity and color retention capacity.

**Response surface methodology:**

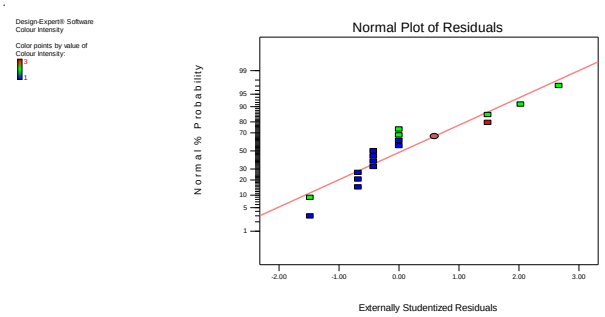



**Figure No.13: Normal Plot of residual graph Normal %Probability vs Externally Studentized Residuals.**

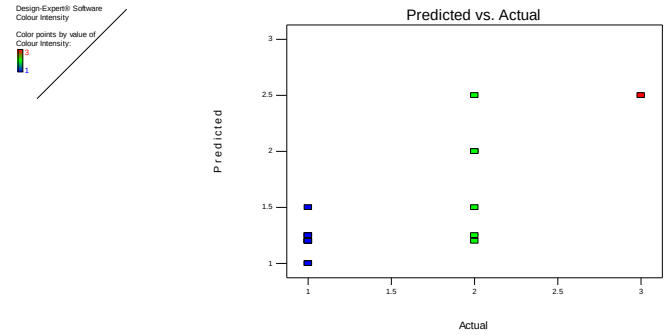



**Figure No. 14: Counter plot for Individuals Response with respect to Predicted vs Actual**

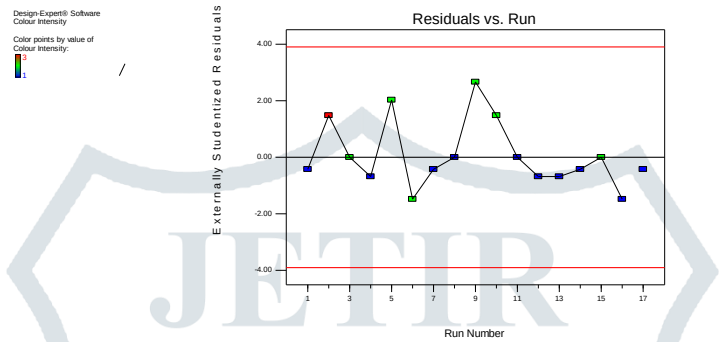



**Fig 15: Residual plot vs. the run i.e. external standard residual with run number**

**Color intensity**

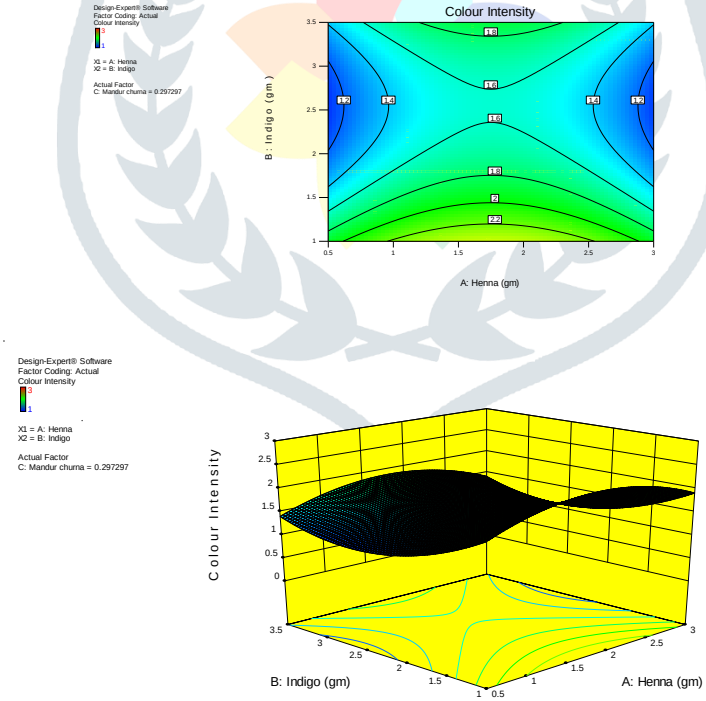



**Figure No16: Counter plot showing combined effect of henna and indigo when Mandur churna at lower level i.e. 0.297gm**

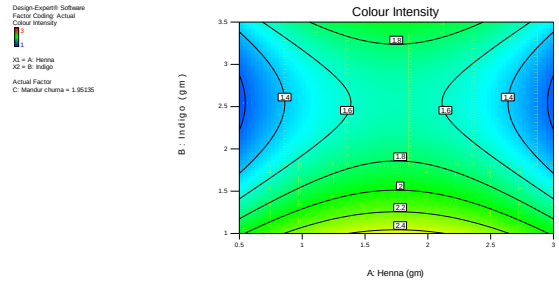



**Figure No.17: 3D Response surface plot showing combined effect of henna and indigo when Mandur churna at lower level i.e. 0.297gm**

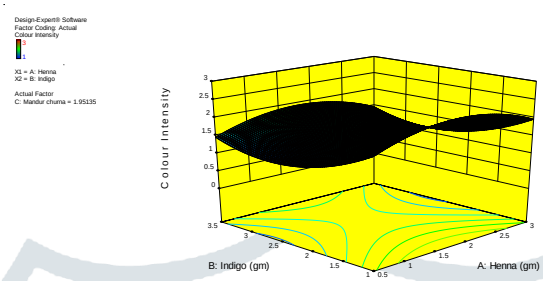



**Figure No18: Counter plot showing combined effect of henna and indigo when Mandur churna at higher level i.e. 1.95 gm**

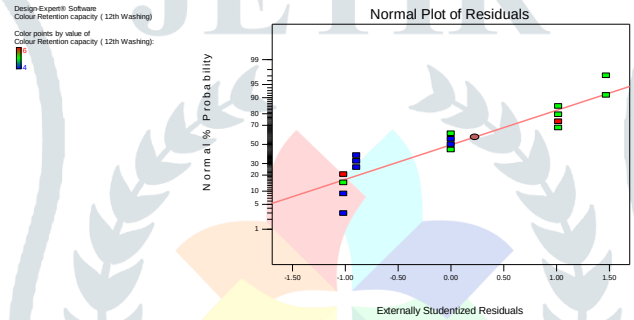



**Figure No19: 3D Response surface plot showing combined effect of henna and indigo when Mandur churna at higher level i.e. 1.95 gm at higher level i.e. 29.45 mg**

## Response surface methodology: (color retention capacity)

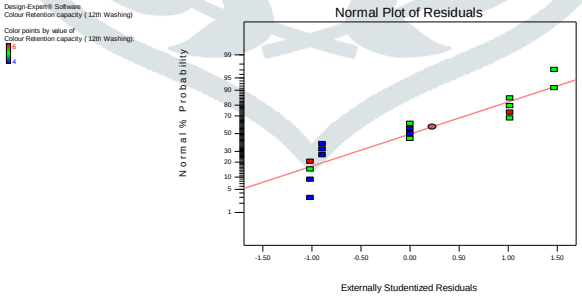



**Fig No 20: Normal Plot of residual graph Normal %Probablity vs Externally Studentized Residuals.**

**Color retention capacity**

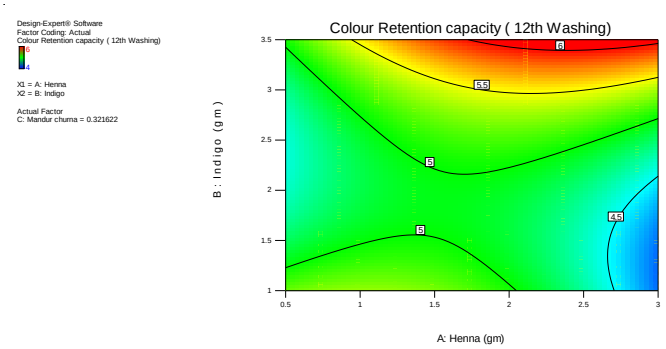



**Figure No 21: Counter plot showing combined effect of henna and indigo when Mandur churna at lower level i.e. 0.32gm**

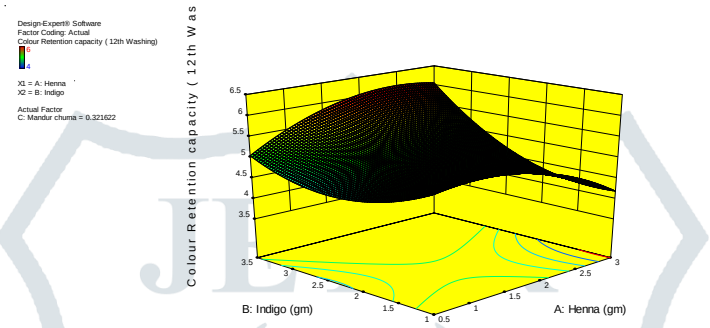



**Figure No.22: 3D Response surface plot showing combined effect of henna and indigo when Mandur churna at lower level i.e 0.32gm**

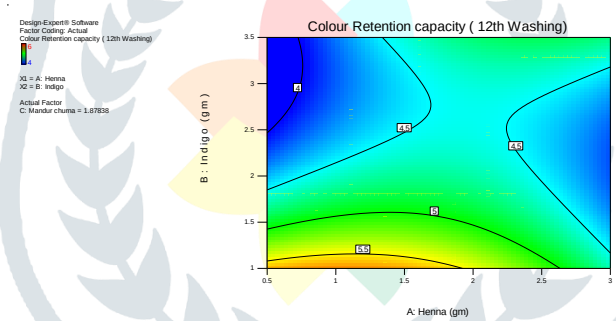



**Figure No 23: Counter plot showing combined effect of henna and indigo when Mandur churna at higher level i.e. 1.87 gm**

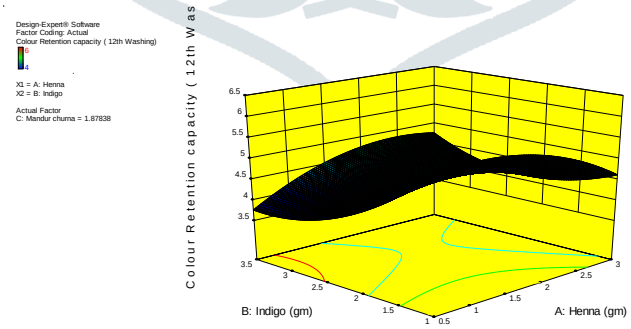



**Figure No 24: 3D Response surface plot showing combined effect of henna and indigo when Mandur churna at higher level i.e. 1.87gm**

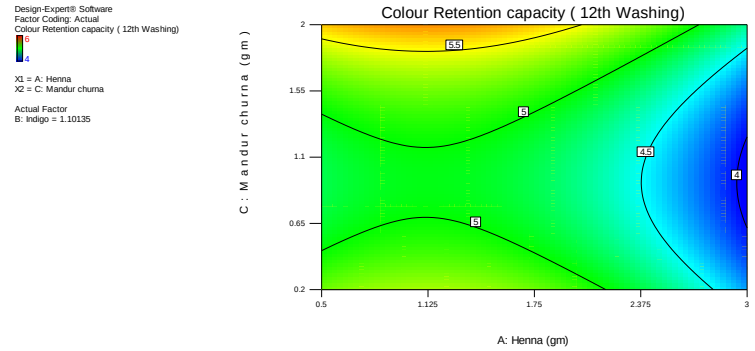



**Fig No 25: Counter plot showing combined effect of henna and Mandur churna when indigo at low level i.e. 1.10gm**

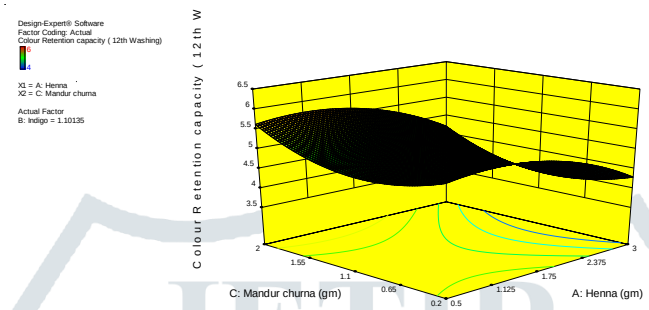



**Figure No26: 3D Response surface plot showing combined effect of henna and Mandur churna when indigo at lower level i.e. 1.10gm**

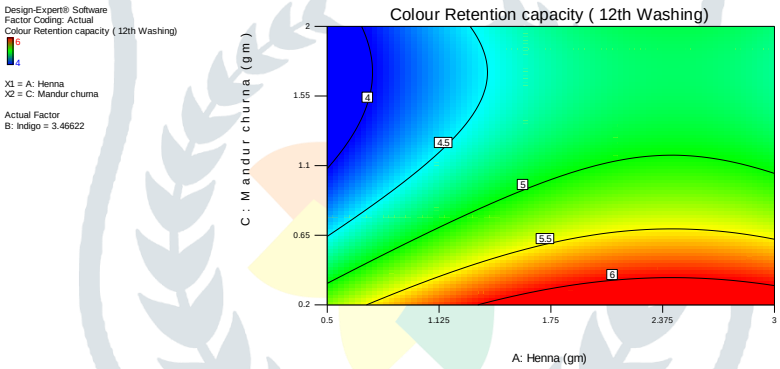



**Fig No 27: Counter plot showing combined effect of henna and Mandur churna when indigo at higher level i.e. 3.46 gm**

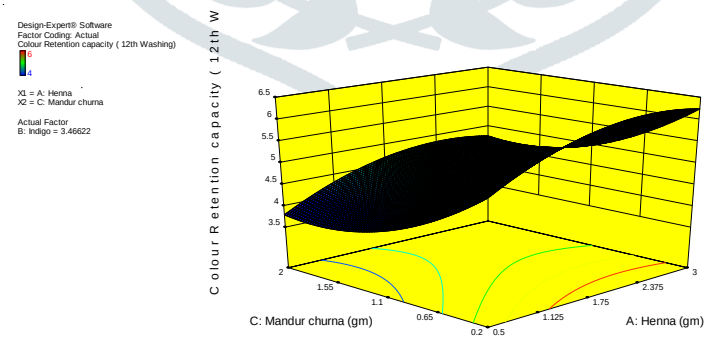



**Figure No.28 : 3D Response surface plot showing combined effect of henna and Mandur churna when indigo at higher level i.e. 3.46gm**

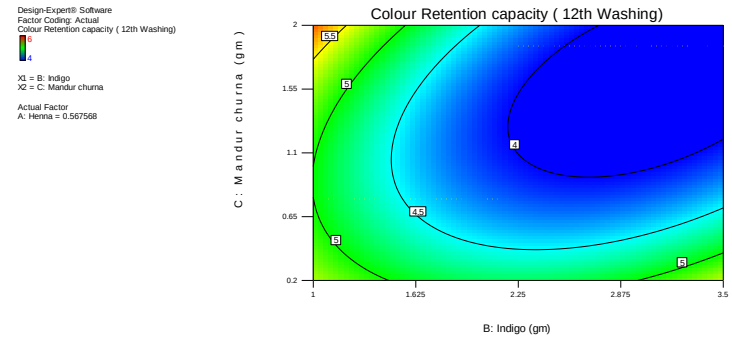



**Figure No 29: Counter plot showing combined effect indigo of and Mandur churna when henna at lower level i.e. 0.56gm**

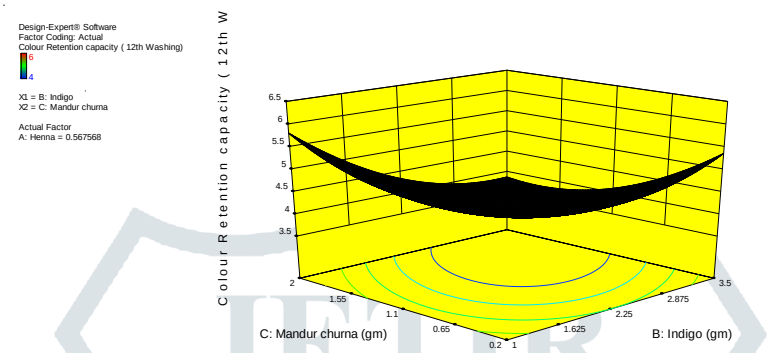



**Figure No.30: 3D Response surface plot showing combined effect indigo of and Mandur churna when henna at lower level i.e. 0.56 gm**

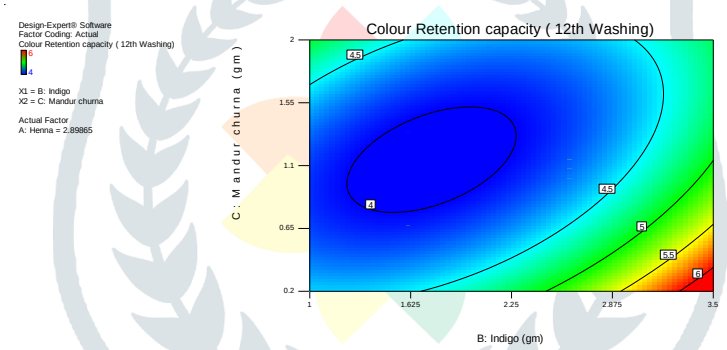



**Figure No 31: Counter plot showing combined effect indigo of and Mandur churna when henna at higher level i.e. 2.89gm**

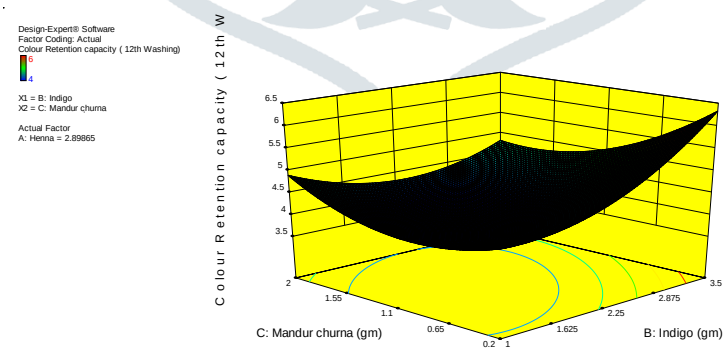



**Figure No.32: 3D Response surface plot showing combined effect indigo of and Mandur churna when henna at higher level i.e. 2.89 gm**

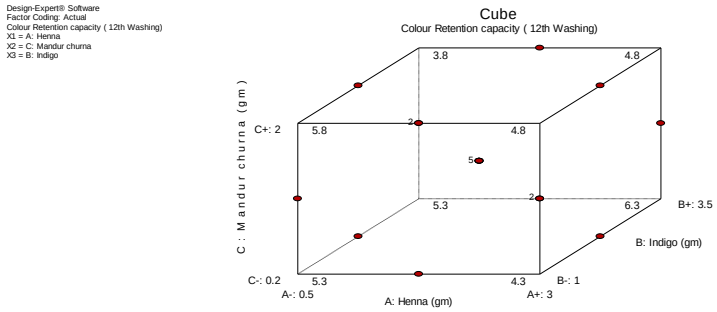



**Fig: 33: Cube of color retention capacity after 12th washing of shampoo**

**Design of experiment for brown color**

**Table No.15: Coded full factorial Box-Behenken design for the 3 independent variables**

| | | Factor 1 | Factor 2 | Factor 3 | Response 1 | Response 2 | Response 3 |
|---|---|---|---|---|---|---|---|
| Std | Run | A:Henna | B:Indigo | C:Mandur churna | Colour Intensity | Colour Retention Capacity (1st Washing) | Colour Retention capacity ( 12th Washing) |
| | | gm | gm | gm | | | |
| 1 | 10 | -1.000 | -1.000 | 0.000 | 2 | 2 | 5 |
| 2 | 16 | 1.000 | -1.000 | 0.000 | 1 | 1 | 3 |
| 3 | 8 | -1.000 | 1.000 | 0.000 | 1 | 1 | 4 |
| 4 | 11 | 1.000 | 1.000 | 0.000 | 1 | 1 | 5 |
| 5 | 12 | -1.000 | 0.000 | -1.000 | 1 | 1 | 5 |
| 6 | 13 | 1.000 | 0.000 | -1.000 | 1 | 1 | 5 |
| 7 | 4 | -1.000 | 0.000 | 1.000 | 1 | 1 | 2 |
| 8 | 9 | 1.000 | 0.000 | 1.000 | 2 | 2 | 4 |
| 9 | 2 | 0.000 | -1.000 | -1.000 | 3 | 3 | 5 |
| 10 | 15 | 0.000 | 1.000 | -1.000 | 2 | 2 | 6 |
| 11 | 6 | 0.000 | -1.000 | 1.000 | 2 | 2 | 6 |
| 12 | 3 | 0.000 | 1.000 | 1.000 | 2 | 2 | 5 |
| 13 | 14 | 0.000 | 0.000 | 0.000 | 1 | 1 | 4 |
| 14 | 7 | 0.000 | 0.000 | 0.000 | 1 | 1 | 4 |
| 15 | 17 | 0.000 | 0.000 | 0.000 | 1 | 1 | 5 |
| 16 | 5 | 0.000 | 0.000 | 0.000 | 2 | 2 | 1 |
| 17 | 1 | 0.000 | 0.000 | 0.000 | 1 | 1 | 5 |

Final Equation in Terms of Coded Factors:

Color Intensity = +1.20+1.979E-016 * A-0.25* B +3.643E-018* C -0.48 * A^2 +0.52* B^2 +0.53* C^2

**Analysis of variance (ANOVA) of SF8 formulation on color intensity**

**Table No16: Analysis of variance (ANOVA) of SF8 formulation on washing**

| **Analysis of variance table [Partial sum of squares - Type III]** | | | | | | |
|---|---|---|---|---|---|---|
| **Source** | **Sum of Squares** | **df** | **Mean Square** | **F Value** | **p-value Prob > F** | |
| **Model** | 3.69 | 6 | 0.61 | 2.41 | 0.1052 | significant |
| ***A-Henna*** | *4.441E-016* | *1* | *4.441E-016* | *1.742E-015* | *1.0000* | |
| ***B-Indigo*** | *0.50* | *1* | *0.50* | *1.96* | *0.1917* | |
| ***C-Mandur churna*** | *4.441E-016* | *1* | *4.441E-016* | *1.742E-015* | *1.0000* | |
| ***A^2*** | *0.95* | *1* | *0.95* | *3.73* | *0.0824* | |
| ***B^2*** | *1.16* | *1* | *1.16* | *4.55* | *0.0587* | |
| ***C^2*** | *1.16* | *1* | *1.16* | *4.55* | *0.0587* | |
| **Residual** | 2.55 | 10 | 0.25 | | | |
| ***Lack of Fit*** | *1.75* | *6* | *0.29* | *1.46* | *0.3726* | *significant* |
| ***Pure Error*** | *0.80* | *4* | *0.20* | | | |
| **Cor Total** | 6.24 | 16 | | | | |

**Model coefficients of thermally F9 formulations estimated by multiple regressions on color retention capacity (significance of regression coefficients)**

| | | | |
|---|---|---|---|
| **Std. Dev.** | **0.50** | **R-Squared** | **0.5910** |
| **Mean** | 1.47 | Adj R-Squared | 0.3457 |
| **C.V. %** | 34.34 | Pred R-Squared | 0.3231 |
| **PRESS** | 8.25 | Adeq Precision | 4.629 |

| | Coefficient | | Standard | 95% CI | 95% CI | |
|---|---|---|---|---|---|---|
| Factor | **Estimate** | **df** | **Error** | **Low** | **High** | **VIF** |
| **Intercept** | 1.20 | 1 | 0.23 | 0.70 | 1.70 | |
| **A-Henna** | 1.979E-016 | 1 | 0.18 | -0.40 | 0.40 | 1.00 |
| **B-Indigo** | -0.25 | 1 | 0.18 | -0.65 | 0.15 | 1.00 |
| **C-Mandur churna** | 3.643E-018 | 1 | 0.18 | -0.40 | 0.40 | 1.00 |
| **A^2** | -0.48 | 1 | 0.25 | -1.02 | 0.073 | 1.01 |
| **B^2** | 0.52 | 1 | 0.25 | -0.023 | 1.07 | 1.01 |
| **C^2** | 0.53 | 1 | 0.25 | -0.023 | 1.07 | 1.01 |

**Response surface methodology:**

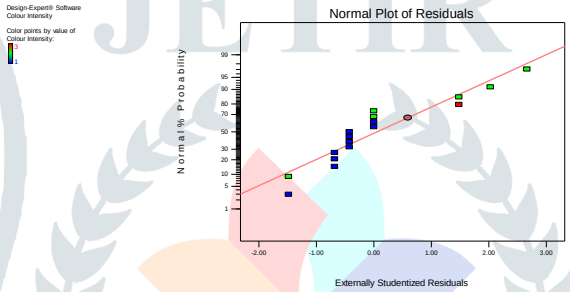



**Figure No.34: Normal Plot of residual graph Normal %Probablity vs Externally Studentized Residuals.**

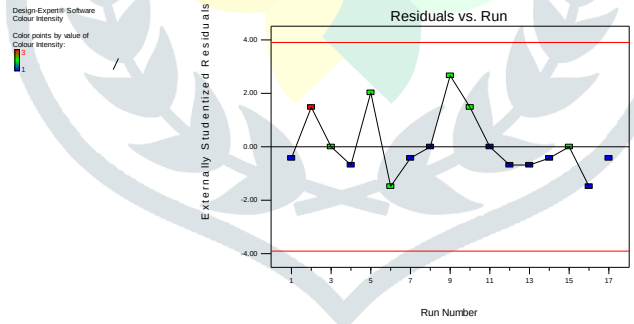



**Figure No. 35 : Counter plot for Individuals Response with respect to Predicted vs. Actual**

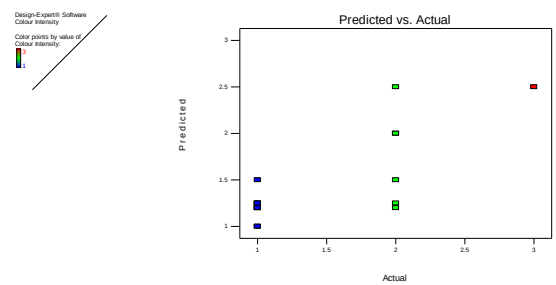



**Figure No. 36 : Counter plot for Individuals Response with respect to Predicted vs Actual**

**Color intensity**

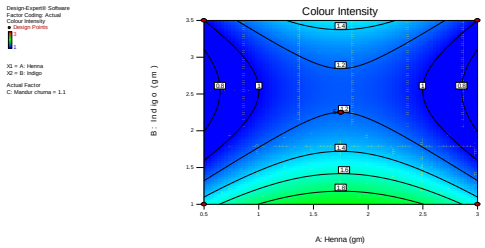



**Figure No37: Counter plot showing combined effect of henna and indigo when Mandur churna at high level i.e. 1.1 gm**

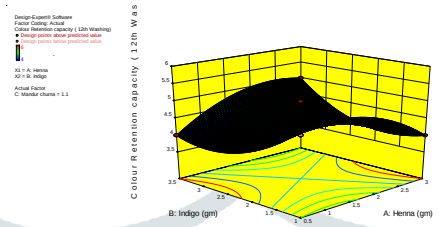



**Figure No.38 : 3D Response surface plot showing combined effect of henna and indigo when Mandur churna at high level i.e. 1.1gm**

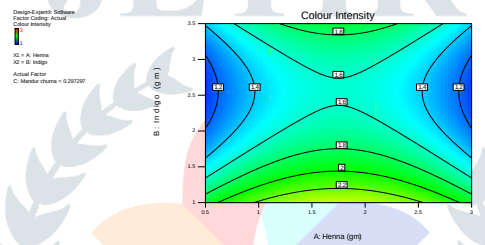



**Figure No39 : Counter plot showing combined effect of henna and indigo when Mandur churna at lower level i.e. 0.2gm**

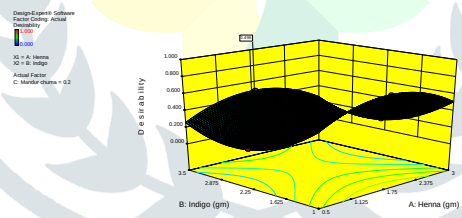



**Figure No.40 : 3D Response surface plot showing combined effect of henna and indigo when Mandur churna at lower level i.e. 0.2gm**

**Response surface methodology: (color retention capacity)**

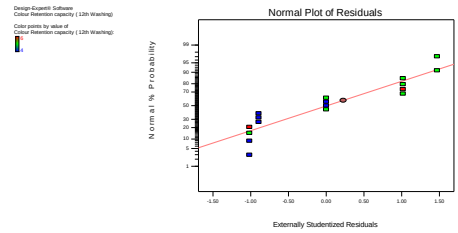



**Figure No.41 Normal plot vs Resedual**

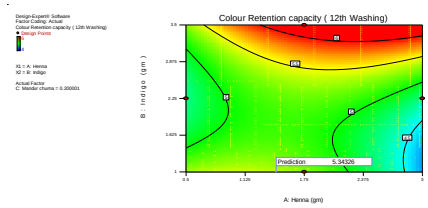



**Figure No 42 : Counter plot showing combined effect of henna and indigo when Mandur churna at lower level i.e. 0.2gm**

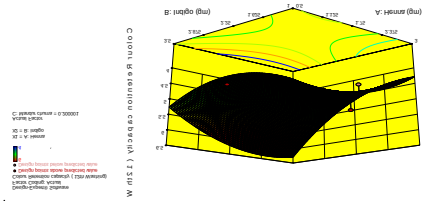
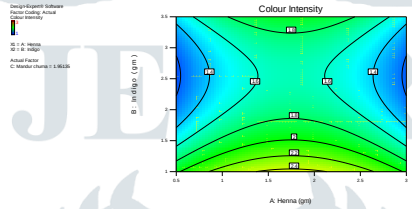


**Figure No.43 : 3D Response surface plot showing combined effect of henna and indigo when Mandur churna at lower level i.e 0.2gm**

**Figure No 44 : Counter plot showing com**




**Combined effect of henna and indigo when Mandur churna at higher level i.e. 1.9 gm**

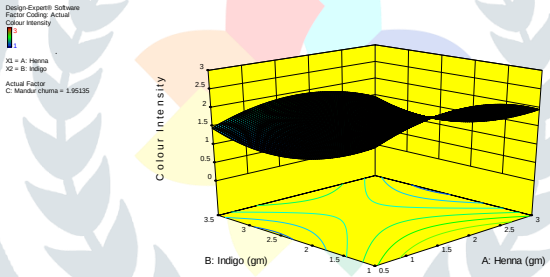



**Figure No 45 : 3D Response surface plot showing combined effect of henna and indigo when Mandur churna at higher level i.e. 1.9gm**

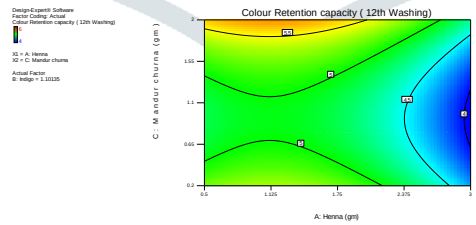



**Fig No 46 : Counter plot showing combined effect of henna and Mandur churna when indigo at low level i.e. 1.10gm**

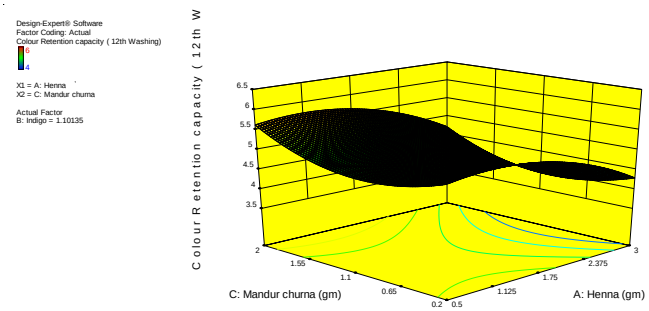



**Figure No 47: 3D Response surface plot showing combined effect of henna and Mandur churna when indigo at lower level i.e. 1.10gm**

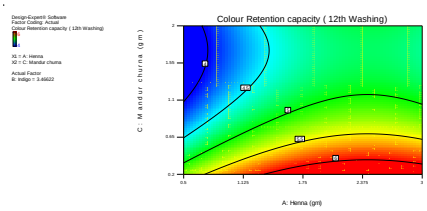



**Fig No 48 : Counter plot showing combined effect of henna and Mandur churna when indigo at higher level i.e. 3.4 gm**

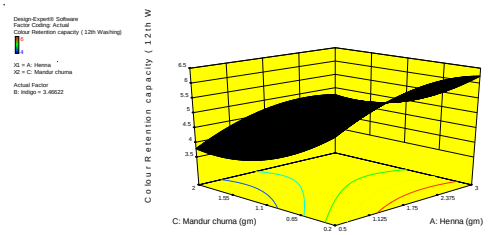



**Figure No 49: 3D Response surface plot showing combined effect of henna and Mandur churna when indigo at higher level i.e. 3.4 gm**

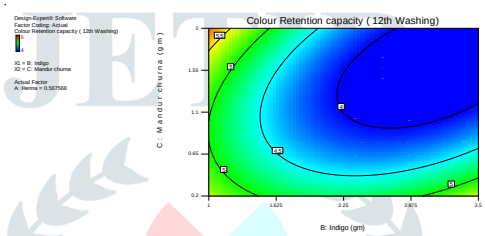



**Figure No 50: Counter plot showing combined effect indigo of and Mandur churna when henna at higher level i.e. 0.5 gm**

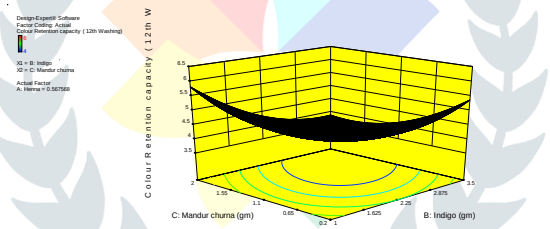



**Figure No.51 : 3D Response surface plot showing combined effect indigo of and Mandur churna when henna at lower level i.e. 0.56 gm**

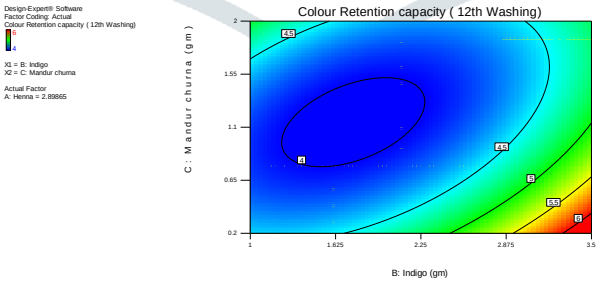



**Figure No 52: Counter plot showing combined effect indigo of and Mandur churna when henna at higher level i.e. 2.8gm**

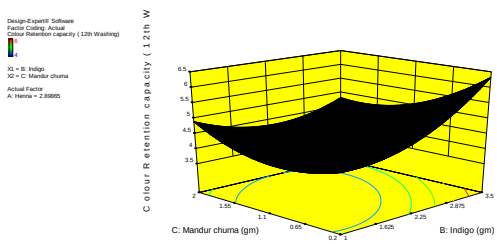



**Figure No.53: 3D Response surface plot showing combined effect indigo of and Mandur churna when henna at higher level i.e. 2.8 gm**

**Summary of Introduction**

In this chapter gives the detailed description of causes of premature of graying also explain the anatomy and physiology of hair structure the types of hair dye the also explain the the chemistry of hair dye the, interaction of hair and hair dye how it interact with hair and which chemical constituents are responsible for the hair dying or hair coloring, also explain The difference between the herbal and synthetic hair dye the route cause analysis of premature of graying the detailed description of dietary factor which essential for hair also explain the holistic approach about premature hair graying and the social survey about the hair coloring product either using the synthetic dye or herbal hair dye

**Summary of Materials and Method**

In that explain the detailed information about the ingredients which are used in formulation and which chemical constituents are responsible for coloring to hair to select the combination of the ingredients to produce or obtain proper color on hair as we want

To check the physicochemical property of herbal formulation and the marketed formulation the color intensity and color retention property is also checked by washing the hair by herbal shampoo and synthetic shampoo explain the microscopy of the hair after applying the herbal formulation to check the primary irritation testing, open patch testing of hair dye

**Summary of Result and Discussion**

In that explain the result of the formulation and shows the result after applying the hair dye on hair the result of the ten times washing after applying the color on hair either on synthetic shampoo or herbal shampoo shows their result result of microscopy of hair after applying the hair dye i.e. marketed as well as herbal means compare the standard formulation and test formulation shows the result of the irritation testing conducted on albino rat.

**Conclusion**

The formulation was found to produce excellent coloration on application on hair. It can be concluded from the investigation that by changing the proportion of henna and indigo a suitable off black and brown color could be obtained for hair. At pH of 6.2 was best for penetration of hair colorant. Repeated application of the henna and indigo product gives an increase in the color intensity. And retention capacity Addition of ferric chloride enhances the color retaining property. It was observed that when indigo leaves are subjected to ageing give more color intensity. No influence of ageing was observed in color intensity with henna powder. Advantage of this natural hair colorant is that it does not cause any irritation. Staining, hypersensitivity reaction, or any kind of allergic reaction on skin or nails or fingers while preparing the hair colorant formulation or paste which is the main problem with marketed products. At the same time the color does not stick to the clothes which come into contact with the product. The Herbal hair formulation which is semi-permanent in nature and exhibit better dying efficiency than marketed formulation.

The developed herbal formulation is prepared by purely natural substances free from any chemical and adulterate substances so, it is considered to be nontoxic and devoid of any side effects such as hair fall, dandruff production etc. Further studies should be performed to evaluate dying efficiency and toxicity on human volunteers and it does not see any kind of allergic reaction and hypersensitivity reaction. Also, the study is done by DoE method i.e. the design of Experiment which also called the QbD approach Give the Detailed Information of Combination of three ingredients which shows the responses like color intensity and color retention capacity

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