Chapter 8

HERBAL HAIR FORMULATIONS AND THEIR BIOLOGICAL ACTIVITIES

GENERAL ABOUT HAIRS, MATERIALS AND METHODS, OBSERVATIONS, RESULTS AND REFERENCES
ABOUT HAIRS

Hair are protective appendages on the body. They remain on one or other part of body from the time of birth to death. People who carry luxuriant and lustrous hair are regarded as young and beautiful. Hair is derived from surface ectoderm skin. As the site where a hair follicle is to form, the germinal layer of epidermis proliferates to form a cylindrical mass, that grows down into dermis. The lower and of this down growth becomes expended and is invaginated by a condensation of mesoderm, which forms papilla. The hair itself is formed by proliferation of germinal cells overlying the papilla. As the hair grows the surface, the cell overlying the papilla. As the hair grows the surface, the cell forming the wall of the down growth surrounded it, a typical hair follicle is formed. A hair has a root (bulb or Knob), a shaft and a tip. That portion of hair which lies in the follicle is known as hair root. It is surrounded by loose connective tissues known as root sheath. The root lies in the dermis. The shaft grows from it and projects out side the skin. The distal end of the shaft is known as hair tip.

Types of hair

Three types of hair are known in man: lanugo, vellus, and terminal hair.

Lanugo hair is soft and thin, without medulla, and can be of variable length. It is the hair of the fetus and in postnatal life is replaced by vellus or terminal hair; only premature infants have lanugo hair.

Vellus hair is soft and short, usually not longer than 2 cm, often colourless, it is the general surface hair.

Terminal hair is large and coarse, endowed with medulla and pigment, and can vary in length, at birth it is found as scalp hair, eyebrows, and eyelashes. During life the same follicle can produce vellus hair first and
terminal hair later; this is the normal behaviour of the axilla hair of children of both sexes and of the hair of the beard in the male at puberty, which under the stimulus of the sexual hormones undergo the transformation from vellus to terminal hair.

In male-pattern baldness terminal follicles regress and give origin to vellus hair. In many areas of the body both terminal and vellus hair is present in varying amounts: chest, trunk, and shoulder hair of men is more than 90% terminal hair, while in women it is less than 35% terminal hair.

Plate 8.1   Normal Hair follicle
Hair

The hair consists of three zones: cuticle, cortex, and medulla. The cuticle is the outer zone consisting of scales and forms a certain characteristic pattern. The scales are flattened with serrated edges and surround the shaft completely forming a coronal pattern.

The hair cuticle located peripheral to the hair cortex, consists of overlapping cells arranged like shingles and pointing upward with their peripheral portion. The cells of the hair cuticle are tightly interlocked with the cells of inner root sheath cuticle, resulting in the firm attachment of the hair to its inner root sheath. The hair and inner root sheath thus move upward in unison.

The inner sheath is composed of three concentric layers; from the inside to the outside, these are the inner root sheath cuticle, the Huxley layer, and the Henley layer. None of these three layers contains melanin. All the three layers keratinize, unlike the cells of the hair cortex and of the hair cuticle, by means of trichohyaline granules. Closest to the hair is the single-layered inner root sheath cuticle, consisting of flattened overlapping cells that point downward in the direction of the hair bulb. Since the cells of the hair cuticle point upward, these two types of cells interlock tightly. Trichohyaline granules are few in the inner root sheath cuticle cells. The Huxley layer, which usually consists of two rows of cells, develops numerous trichohyaline granules at the level of the keratogenous zone of the hair. The Henley layer, only one cell layer thick and first layer to undergo keratinization, already shows numerous trichohyaline granules at its emergence from the matrix.

The outer root sheath extends upwards from the matrix cells at the lower and of the hair bulb to the entrance of the sebaceous duct, where it changes into surface epidermis, which lines the upper portion, or infundibulum, of the hair follicle. The outer root sheath is thinnest at the level of hair bulb,
gradually increases in thickness and is thickest in the middle portion of the hair follicle, the isthmus. In its lower portion, below the isthmus, the outer roots sheath is covered by inner root sheath and does not undergo keratinization. The outer root sheath cells have a clear vacuolated cytoplasm because of the presence of considerable amounts of glycogen. In contrast to the surface epidermis lining the infundibulum, which contains active, melanin-producing melanocytes in its basal layer, the basal layer of the outer root sheath contains only inactive amelanotic melanocytes demonstrable with toluidine blue. However, these inactive melanocytes can become melanin-producing cells after skin injuries, such as dermabrasion, when they increase in number and migrate upward into the regenerating upper portion of the outer root sheath and into the regenerating epidermis (Staricoo, 1960). In the middle portion of the hair follicle, the so-called isthmus which extends upwards from the attachment of the erector pili muscle to the entrance of sebaceous duct, the outer root sheath is no longer covered by inner root sheath, which by then has keratinized and disintegrated. The outer root sheath, therefore, undergoes keratinization. This type of keratinization, referred to as trichilemmal keratinization (Pinkus, 1969), produces large, homogenous keratinized cells without the formation of keratohyaline granules. Trichilemmal keratinization is found also in catagen and telogen hair and in trichilemmal cysts and trichilemmal tumors.

The upper portion of the hair follicle above the entrance of the sebaceous duct, the infundibulum, is lined by surface epidermis, which, like the sebaceous duct, undergoes keratinization with the formation of keratohyaline granules. The glassy or vitreous layer forms a homogeneous, eosinophilic zone peripheral to the outer root sheath. It is thickest around the lower third of hair follicle. Peripheral to the vitreous layer lies fibrous root sheath which is composed of thick collagen bundles.
Cortex - The Cortex is the middle zone of varying thickness and consists of longitudinal keratin fibres and varying amount of pigments. The size, shape, distribution and density of pigment granules along the shaft determine colour of hair.

It consists of cells that, during their upward growth from hair matrix, keratinize gradually by losing their nuclei and becoming filled with keratinized fibrils. The process of keratinization takes place without the formation of kertohyaline granules, as seen in the kertainizing epidermis, or of trichohyline granules, as seen in inner root sheath. Thus, the keratin of the hair cortex represents hard keratin, in contrast to keratin of the inner root sheath, which line that of epidermis, represents soft keratin.

Medulla - The medulla is the inner zone known as medullary canal, the central shaft, the root hair has the appearance similar to that of shaft, except that it is enlarged in the form of bulb or knob. The hair medulla of human hair is often difficult to find by routine light microscopy, since it may be discontinuous or even absent. It is more readily recognizable by the polariscopic examination, since, unlike the cortex, the only partially keratinized medulla contains hardly any doubly refractile structure. If the medulla is seen by the light microscopy in human hair, it appears amorphous because of only partial keratinization.

Keratin is a collagenous fibrous protein that make hair. It is found as coiled coil of a -helices. The amino acids such as glycine, alanine, valine, leucine, proline, crystine, glutamic acid, aspartic acid, argenine and lysine occur to the extents of 1,4,3,13,15,7, 10 and 3% in keratin protein, respectively.

Melanin is the principal pigment responsible for the colour of human hair, and acts as filter that decreases the harmful effects of ultraviolet light providing protection against environmentally induced premature ageing. It is a polymer
formed by the oxidation of tyrosine by tyrosinase to dihydroxyphenylamine (dopa) within melanocytes. The chemical units which predominate these melanins are of the indole type, formed from tyrosine and dopa precursors. In ageing persons, the melanin-producing cells gradually stop working, and as a result hair turn white. The hair seems grey when white hair are seen against the still-pigmented dark hair.

Hair color and texture are racial characteristics and genetically determined. The yellow-brown mongol race has black straight hair. The negroid have black, curly hair and the caucasoids have fair, brown, red or black hair.

**Composition of Animal hair**

Because of the insoluble nature of keratinized hairs, the determination of their composition by solubilization and extraction of the component proteins in undegraded forms has posed many problems. Methods used have involved either the breaking of disulphide bonds or the hydrolysis of peptide bonds. The former has received by far the most attention, and can be achieved by reduction, which produces soluble keratines by oxidation, which produces soluble keratoses or by sulfitolysis (Crewther, 1976; Fletcher and Buchanan, 1977). Reoxidation of the -SH groups in keratines can be blocked by alkylation; for this iodoacetate is commonly used, producing S-carboxymethylkeratines (SCMK). Many procedures have been devised for the extraction of proteins from reduced or oxidized wool and hair.

The soluble proteins extracted from wool and hair have been factionated into three main groups: (a) low-sulphur proteins, e.g., SCMK A and α-keratose, with sulphur contents less than the original wool or hair; (b) high-sulphur proteins, e.g., SCMK B and γ-keratose, with sulphur contents greater than the original wool or hair; and (c) high-glycine -tyrosine proteins (Brunner et al., 1971; Gillespie 1972) rich in glycine and tyrosine. Knowledge of these
proteins has improved with the development of high resolution methods of one-and two-dimensional electrophoresis, listed by Gillespie (1983).

Low -sulphur SCMKA proteins extracted from several species of hair and quill and different breeds of wool have the same general pattern of amino-acid composition (Gillespie, 1983). Part of the variation observed in the proportions of S-carboxymethyl cysteine, proline, glycine and tyrosine is attributable to the presence of small amounts of contaminating high -sulphur and high-glycine -tyrosine proteins, which are difficult to remove completely from low-sulphur proteins (Gillespie, 1983). Components of SCMKA from guinea pig hair are homologous with components from rabbit hair (Shechter et al., 1969).

When the sulphur content of wool is increased by abomasal infusion of sulphur-containing amino acids (Reis and Schinckel, 1963), the high-sulphur proteins are increased in amount the increase being in those components with the highest sulphur content (Gillespie, 1983). These ultra-high-sulphur proteins are not peculiar to sheep, but have also been isolated from hair of 11 Arlrodactyla species (Lindley et al., 1971).

With improvements in techniques, amino acid sequences have been determined on several sub-units of the low-sulphur proteins and on a number of components of the high -sulphur and high -glycine -tyrosine proteins of wool (Lindley, 1977; Parry, 1979; Crewther et al., 1980; Gillespie, 1983).

**Factors affecting hair growth**

1. **Hormonal** effects on hair growth are exhibited in various ways including changes in the onset and duration of anagen, in the rate of growth and thickness of hair during anagen, in the length of telogen and in the release of club hairs. The responses in hair growth obtained by removal of endocrine
glands and/or systemic administration of hormones also vary from species to species.

The follicle changes involved in seasonal moulting, which has been shown by various workers to be photoperiodic in origin and related to the reproductive cycle, are considered to be mediated via the neuroendocrine system, particularly in wild or primitive species (Johnson, 1976). Photoperiodism is also involved in the seasonal shedding of hair by domestic species, e.g., non-equational breeds of cattle, and in the annual rhythm of wool growth in improved breeds of sheep (Hutchinson, 1976).

2. A low plane of nutrition has been found to delay the seasonal shedding in cattle and retards the sub-adult and adult moults in voles (Pinter, 1968). In non-shedding sheep, e.g., Merino, the effects of plane of nutrition are many and varied, as reviewed by (Ryder and Stephenson, 1968; Chapman et al., 1973 and Allden, 1979). Poor nutrition can reduce follicle initiation and development in the fetus, impair postnatal follicle maturation in lambs, and depress fleece weight, fibre length and fibre thickness in adult animals. However, the nutrition has to be extremely poor before catagen and telogen can be induced nutritionally in follicles in adult sheep. The protein, amino acid, carbohydrate, fat, vitamin and mineral contents of the diet can also affect hair and wool growth in a variety of ways, depending on whether there is an excess or a deficiency.

3. Temperature has modifying effects on seasonal moulting. Low temperature delays the spring moult in some wild species, appears to be required for the growth of a white winter coat by the mountain hare, increases the density of the winter pelage of wild species and the depth of the winter coat of cattle and may stimulate the wool growth of shorn sheep (Ling, 1970; Hutchinson, 1976; Johnson, 1976; Bottomley, 1979).
4. The effect of increase in age on the pelage of various wild species has been reviewed by Ling (1970). The establishment of the adult pelage in some species requires several moults, and sometimes one of these moults may be omitted by animals born late in the breeding season. With increasing age the patterns of the adult moults change. Likewise in the mouse the successive hair waves change in pattern and becomes less frequent with age. In non-shedding sheep, fleece weights are heaviest at ca. 3 ½ years of age and subsequently decline together with fibre length, while fibre thickness increases with age. Also deterioration of staple crimp occurs in an increasing proportion of older sheep, due to abnormal cell proliferation and cyst formation in the proximal outer root sheath of the follicles.

5. Among the most noticeable effects on hair growth produced by a variety of exogenous chemicals are alopecia and change in pigmentation (Flesch, 1963; Ebling and Rook, 1968; Ippen, 1970; Chapman, 1980; Chapman et al., 1982). Attempts have been made to utilize chemically induced hair loss for the biological harvesting of wool (Chapman and Rigby, 1980; Moore et al., 1981; Holis et al., 1983) and for epilating Angora rabbits (Rougeot and Thebault, 1970).

Effects of age on hair

About 70% of men above the age of 50 years face balding and greying of hair. In some, these symptoms of age arrive much earlier. The new-born babies usually possess hair that are fine, soft, downy, non-pigmented, non-medulated and with smooth edged flattened scales. These get slowly replaced by comparatively less fine pigmented, non-medulated and with a more complex scale pattern. At about 14 years in the male and 13 years in female, pubic hair begin to appear. The axillary hair appear a year later. In the beginning, the growth of hair is sparse and its colour lighter. In about one or two years, the growth of hair becomes thick and the colour darker. The sex
hormones, collectively called the androgens e.g. dehydro epi-androsterone, androstandione and testosterone secreted in female and testosterone, dihydrotestosterone, progesterone, etc. in male show their relationship with growth of hair in normal individuals.

The growth of hair at eyebrows and eyelashes are not dependent on androgen. While axillary and lower pubic hair are sensitive to the small amounts of androgen secreted by the adrenal glands. Hair in these regions, therefore, grows approximately equally in men and women. Hair begin to appear on the chin and upper lip in males between 16 to 18 years. Baldness of scalp is not of much value nor the graying of hair, except in a general way. Greying starts on the scalp at about forty years first at the temples, followed later by the beard and moustache and still later the chest. Axillary and pubic hair never turn grey before fifty to sixty years. As age advances, scalp hair become less dense in the male and there is loss of axillary hair in the female.

The age at which a person's hair turns grey is largely decided by heredity. But premature loss of pigment in adults may be caused by a variety of other factors, including illness, certain drugs, worry, even shock and it is irreversible. However, if hair turns white in childhood, it can be a result of malfunction in the body and medical advice is called for. Vigorous brushing can cause hair splits, breaks or uprooting. Washing of hair is essential for them to gleam with a healthy sheen.

Some common diseases of hair

Protein energy malnutrition and parasitic diseases bring out structural changes in the hair. Dandruff is caused by infection of Pityrosporum ovale.

Any inflammatory or destructive disease of the skin on scalp may destroy hair follicles in its wake. Thus, burns, heavy X-ray irradiation, or ringworm infections and similar other events may cause a scarring alopecia. Alopecia in
the presence of normal scalp skin may be patchy and localized or extensive when the skin is diseased. Ring worm of the scalp is caused by fungal infection and is most common in children. The disease causes oval areas of baldness covered with short, broken-off lusterless hair stumps. Premature hair loss or baldness in men is genetically determined and requires adequate levels of circulating androgen for expression. Baldness in women occurs only in old age. Hair are also lost due to the infection of lice.

Measurement of hair growth

The biological parameters which constitute the trichogram are:

1. **Rate of growth** (= increase in length /10 days): A small area, about 0.05 cm$^2$ of the region to be studied, is shaved with a razor. Ten days later, the increase in length of the shaved hairs is measured *in situ* using a microscope with an ocular micrometer calibrated in 0.05 mm graduations. Measurements are made by applying the instrument directly to the patient's scalp. Since hair stumps stand erect, a glass slide is used to flatten the hairs against the scalp. Three to five measurements are taken and the results are averaged.

2. **Hair density** (number of hairs /cm$^2$). For this measurement a microscope with a x 60 magnification is used with eye piece markings for 1/20 cm$^2$ areas. Lateral illumination is provided by a light source connected to the base of the microscope. Hairs are counted in a given number of fields (three to five) and the results are averaged and expressed as the number of hairs /cm$^2$. When the density is to be determined in regions of lower hair density (axilla, pubis, etc.) the study of this parameter is performed with an eyepiece marked with a 1 cm$^2$ area.

3. **State of hair cycle**: For this determination hairs are plucked off using forceps whose jaws are wrapped with adhesive tape. This facilitates
grasping of hairs of different thickness. The proximal root segments of the hairs are cut, placed over a slide, and covered with a drop of Canadian balsam and a cover glass. The different phases of the hair cycle are identified and counted under the microscope.

Another method for determination of the phases (i.e., "state") of the cycle is bleaching of the hairs. Under the effect of hydrogen peroxide in alkaline medium, the proximal parts of the hair shafts are bleached in their visible extension from the skin. Bleaching involves hairs in different stages of the cycle.

**Hair nourishment and grooming**

Hair nourishment and grooming aids have become increasingly popular throughout the world. Preparations available in the market for men include hair creams, liquid brilliantines, pomades and solid brilliantines, tonics, lotions and conditioners and for women wave sets, hair sprays, special rinses and lotions, brilliantines and conditioners. There are number of herbal plants, utilized in hair care formulations. Examples are given below.

The herbal plants which are used in hair-care formulations can be classified in the following groups:

(i) Plants used as hair cleanser  
(ii) Plants used as hair dyeing agent  
(iii) Plants used as anti dandruff and (iv) Plants used as hair tonic/hair health promotion of growth.

(i) Plants used as hair cleanser  

(a) *Acacia concinna* (Shikakai). Ground pod wall is boiled in water and resulting soapy water, is used as a hair cleanser.
(b) *Cydonia oblonga* (Bihi). The mucilage of cleaned seeds is extracted with hot water and used as a shampoo.

(c) *Sapindus mukorossi* (Ritha). Fruit coat is boiled in water and the soapy water thus obtained is used as a natural shampoo.

(d) *Trigonella foenum-graecum* (Methi). Seeds in coarse powdered form are boiled in water and filtrate is used as an ingredient of natural shampoos.

(l) Plants used as hair dyeing agent

(a) *Acacia arabica* (Kikkar). Fresh or dried leaves are made into aqueous paste and applied as a pack to hair of head as such or after mixing with coffee, babul bark extract (*Cassia nilotica*), Katha (*Acacia catechu*) and walnut fruit shell extract (*Juglans regia*) to impart shades of colours varying from red to dark brown.

(b) *Cydonia oblonga* (Bihi). The seeds mucilage paste with sesame oil is applied on the head to dye the hair.

(c) *Eclipta alba* (Bhringraj). Dried aqueous extract of whole plant, mixed with sesame oil and subbed on hair to dye the hair.

(d) *Haematoxylon campechianum* (Patang). Dried aqueous extract of wood (1 kg) with 100 ml of sesame oil is used for hair drying.

(e) *Hibiscus rosa sinensis* (Gudhal). Its dried alcoholic extract of leaves is mixed with other ingredients, henna catechu, coffee, amla, jatamansi, etc. and applied on hair to impart shades to colour from dark brown to blackish brown.
(f) **Juglans regia** (Akhrot). Its fresh or dried leaves or dried bark or hulls of fruits are made into aqueous paste with other material, e.g., Katha (*Acacia Catechu*), Coffee ( ), etc. and applied as a paste to hair. It produces red to dark brown shades of colour to hair.

(g) **Lawsonia inermis** (Henna). Fresh or dried leaves are made into aqueous paste and applied as a pack to hair after mixing with coffee( ), Catechu (*Acacia catechu*) wall nut fruit shell extract, babul bark extract (*Cassia abratica*), Bhringraj (*Eclipta alba*) etc. to impart shades of colour varying from red to dark brown.

(h) **Pterocarpus indicus** (Nara). The whole plant (1kg) is extracted with hot water, filtered. The filtrate is dried and mixed with 100 ml of sesame oil. It is applied daily on hair to gave a black colour.

(i) **Rubia tinctorum** (Bacho). The whole plant (1 kg) is extracted with hot water, filtered and the filtrate is dried and mixed with 100 ml of sesame oil. It is applied daily on hair to impart black shade of colour.

(j) **Sanssurrea lappa** (Kuth / Kust). The dried alcoholic extract of root is mixed with other herbs and applied on hair, in the form of paste, to give a dark brownish black colour.

(k) **Terminalia belerica** (Behera). Its seed oil is applied on the hair for blackening them.

(l) **Tinospora cordifolia** (Giloe). It is used with gokhru (1:1) in fine powder. The powder (5 gm) is ingested with honey thrice daily for blackening of hair.
(iii) Plants used as anti dandruff agents

(a) *Arctium lappa* (Great burdock). Its roots are kept in 70% alcohol for 5 days, then filtered, the filtrate is diluted with distilled water. This is used for massaging the scalp daily. It is beneficial in removal of dandruff.

(b) *Betula pendula* (Birch). Its fresh or dried leaves are extracted in 70% alcohol for 3 days and filtered. The filtrate is used daily for massaging of scalp, which prevents dandruff.

(c) *Calendula officinalis* (Gule-abbas). Alcoholic (70%) extract of its flowers is mixed with hair oils, shampoos and creams. These preparations are used to cure the dandruff.

(d) *Urtica dioica* (Stinging nettle). The fresh or dried leaves (100g) are dipped in 300 ml of 70% alcohol and distilled water. So that solution will cover the plant material. The solution is kept for 3-5 days and filtered; daily 1-2 spoons of filtrate is applied on the head. It gives relief from dandruff.

(iv) Plants used as hair tonic/hair nourisher:

(a) *Arnica montana* (Arnica) Dried alcoholic (70%) extract of the flower is mixed with vegetable oil and applied on hair, it prevents the falling of hair.

(b) *Centella asiatica* (Mandukarparni). Dried aqueous extract of whole plant is mixed with til oil and applied on hair. It enhances the growth of hair.

(c) *Coccus nucifera* (Nariyal). The oil is rubbed on head. It is also a base for various hair oil formulations.
(d) *Eclipta alba* (Bhringraj). The dried aqueous extract of the whole plant is mixed with sesame oil and rubbed on hair. It is used as a nourisher to hair.

(e) *Morus alba* (Shatoot). The fruits (100g) are extracted with hot water and filtered through a cloth. The filtrate (20ml) is ingested orally twice daily. It prevents the premature and greying of hair.

(f) *Nardostachys jatamansi* (Jatamansi). The rhizome is extracted with water, made the extract alkaline by addition of ammonia and applied on hair. It promotes the growth of hair and also imparts black colour to hair.

(g) *Phyllanthus emblica* (Amla). Its dried aqueous extract is mixed with sesame oil and applied on hair. It promotes the hair growth. When its aqueous extract is kept in iron pen for 3 days, then this can be used with henna, hibiscus, Catechu, etc. as a hair dye which produces black shades in hair.

(h) *Pilocarpus jaborandi* (jaborandi). Its dried alcoholic extract of leaves is mixed with vegetable oil. It helps in thick and healthy growth of hair.

(i) *Thymus serpyllum* (Benajwain). Both alcoholic and aqueous extracts of whole herb are used as a hair tonic, which imparts healthy and then growth of hair.
Hair tonics

About 70% isopropanol applied as a hair tonic after shampooing has an effect on the process of lipid restitution (Gloor et al., 1973). Lipid levels are incased in the distal part of the hair if spirituous hair tonic is applied on the 1st day after shampooing. This may be explained by a transfer of scalp lipids to the distal part of the hair. On the second day, 16% less lipids are traceable after the application of hair tonic. Thus the application of spirituous hair tonic makes the hair seem fattier at first, but it then probably slows the process of lipid restitution. Aqueous hair tonics are also probably effective in the same way (Gloor, et al. 1974).
MATERIALS AND METHODS

EXPERIMENTAL

A. Hair dye formulation

1. Preparation of aqueous herbal extracts from powdered drug

*Hibiscus rosa-sinensis* leaves (250 g), *Nardostachys jatamansi* rhizomes (250 g), *Saussarea lappa* roots (250 g), *Amla* (250 g) and *Kattha* (250 g) were extracted with distilled water for 72 hours. The aqueous extracts were dried on steam bath under vacuum to get dark coloured masses (5-10%).

2. Preparation of formulation

The quantities of dried aqueous extracts and powdered herbs were taken as mentioned in the Table 8.1. All the ingredients were mixed in sufficient quantity of distilled water to prepare uniform viscous pastes (Table 8.1)

3. Application of formulations on sheep wool fibers

The sheep wool (natural) coil, collected from Ludhiana market, Punjab, was cut into small pieces and washed with petroleum ether four times to remove fatty materials. The wool pieces were dipped into each formulation in a beaker for one hour. The wool pieces were dried and then washed with distilled water. The washed wool fibers were divided into three categories to observed the affects of room temperature, sunlight and natural detergent.
Table 8.1. Preparation hair dye formulation from *Hibiscus rosa-sinensis* leaves, *Nardostachys jatamansi* rhizome, *Saussarea lappa* root, henna leaves, coffee, Kattha and Amla

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<th>Formulation</th>
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<th><em>N. Jatamansi</em></th>
<th><em>S. lappa</em></th>
<th>Henna</th>
<th>Coffee</th>
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*a* Dried aqueous extracts were taken (50% weight / volume)

*b* Powdered drugs were taken (in g).
Effect of Room temperature on coloured sheep wool threads
Effect of sunlight on coloured sheep wool threads
Effect of natural detergent on coloured sheep wool threads
a. Effect of room temperature on coloured wool fibers

The coloured wool fibers were pasted on a white paper sheet covered with transparent cellophane sheet and then kept for 30 days at room temperature at the interval of 0, 15 and 30 days photographs were taken (Plates 8.2-8.4).

b. Effect of sunlight on coloured wool fibers

The coloured wool fibers were pasted on a white paper sheet, covered with a transparent cellophane sheet and kept in open sunlight for two hours daily for 30 days. At the interval of 0, 15 and 30 days photographs were taken (Plates 8.5-8.7).

c. Effect of natural detergent on coloured wool fibers

A 30% w/v aqueous solution of Reetha (Sapindus mukorossi) was prepared. The coloured wool fibers were washed with the Reetha aqueous solution for one minute on alternate days and their photographs were taken on 0, 15 and 30th days (Plates 8.8-8.10).

4. Clinical trial of hair dye formulations

Six formulations HD-1, HD-3, HD-4, HD-13 and HD-14 were selected. One each formulation was applied to a group of 25 volunteers. The formulations were applied on hair of volunteers and air dried for 2 hours and washed off with water. All these formulations were also tested for patch test (allergic reactions).

Patch test

A small quantity of paste is applied on the back of ear. After 15 minutes this paste is removed and the area is watched carefully. If there was irritation/allergic reaction, the application of that formulation was avoided.
Plate 8.11

TS of rabbit skin showing hair follicle, matrix cell root sheath, vascuclar nuclei with formulation HN-1.

Plate 8.12

TS of rabbit skin showing hair follicle, matrix cell root sheath, vascuclar nuclei with formulation HN-2.

Plate 8.13

TS of rabbit skin showing hair follicle, matrix cell root sheath, vascuclar nuclei with formulation HN-3.
Plate 8.14

TS of rabbit skin showing hair follicle, matrix cell root sheath, vasicular nuclei with formulation HN-4.

Plate 8.15

TS of rabbit skin showing hair follicle, matrix cell root sheath, vasicular nuclei with formulation HN-5.

Plate 8.16

TS of rabbit skin showing hair follicle, matrix cell root sheath, vasicular nuclei with formulation HN-5.
Plate 8.17

TS of rabbit skin showing hair follicle, matrix cell root sheath, vascular nuclei with formulation HN-6.

Plate 8.18

TS of rabbit skin showing hair follicle, matrix cell root sheath, vascular nuclei with control (coconut oil).
B. Hair Nourisher formulations

1. Preparation of hair nourishing formulation

One or more dried powders of *N. jatamansi*, *S. lappa* and *H. rosa sinensis* were mixed with coconut oil (Table 8.2). Coconut oil was also used as a blank formulation for comparative study.

The air dried coarsely powdered drug was/were mixed in coconut oil, warmed at 6-70° C on a water bath for 5-6 hours and then kept at room temperature for 72 hours. The material was filtered through an absorbent cotton pad. The filtrate was kept in an air tight container (sufficient quantity of anti-oxidant was added). Coconut oil was used as a blank application.

2. Application of formulations on animals

Rabbits were selected for application. Three rabbits were used for each formulation. Rabbits' hair were shaved with razor at three places. One spot was used for formulation, second spot for the use of blank (coconut oil) and third spot for observing normal growth of hair. All the seven formulations were applied on the hair removed portion of each group of rabbits daily for 30 days. After the interval of 7, 14, 21, 28 days length of hair of each rabbit was measured and a small portion of skin was dissected from each portion of application and control which contained hair root, after giving local anaesthetic to the rabbits (one from each group). The histological studies of these dissected skin pieces of rabbits were prepared. These slides were examined under trinocular research microscope and their photographs were taken with the help of camera (Plates 8.11 - 8.18).
Table 8.2. Preparation of Herbal hair nourishing formulations from herbal powders

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Nardostachys jatamansi (g)</th>
<th>Sausurea lappa (g)</th>
<th>Hibiscus rosa sinensis (g)</th>
<th>Coconut oil (ml)</th>
<th>Others (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN-1</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>HN-2</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>HN-3</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>0.5 (a, b)</td>
</tr>
<tr>
<td>HN-4</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>HN-5</td>
<td>50</td>
<td>50</td>
<td>-</td>
<td>100</td>
<td>0.5 (d)</td>
</tr>
<tr>
<td>HN-6</td>
<td>50</td>
<td>-</td>
<td>50</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>HN-7</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>150</td>
<td>-</td>
</tr>
</tbody>
</table>

a - volatile oil of jatamansi  
b - volatile oil of lemon peel  
c - volatile oil of orange peel  
d - volatile oil of cymbopogon grass
Photographs showing the human hair growth
3. Application of formulations on human beings

Three formulations, HN-3, HN-4 and HN-5, were selected out of these seven formulations and were applied on a group of 3 persons. Skull hair of one person from each group were shaved with razor from three different places. Each formulation was applied daily for 30 days (Plates 8.19-8.21), along with the controlled preparation. The third place was kept without formulation and coconut oil. After an interval of 7, 14, 21, 28 days the length of hair of each group was measured. Before applying formulations to these volunteers, all were tested for patch test/ allergic reaction test. It was found that all the formulations were free from any allergic reaction in their application to human skin.
OBSERVATIONS

Six hair dye formulations, viz., HD-1, HD-3, HD-4, HD-11, HD-13 and HD-14, out of 14 formulations blackened better colourless sheep wool threads than the individual herbal extracts. The black colouring capacity of the formulation HD-3 was maximum. The black colour remained for the longest duration of period when the fibers kept at room temperature, in sun light and washed with natural detergent, the order of blackening wool fibers by the herbal formulations was in order of HD-3 > HD-4 > HD-1 > HD-13 > HD-14 > HD-11.

The black colour retaining power of the fibers retained for sixty days at room temperature (Plates 8.2 -8.4). In sun light, the colour stain faded gradually. After 15 days, the stain remained half of the original stain. It indicated that UV rays present in sun light effected the hair stain/hair dye (Plates 8.5 - 8.7).

Washing of the coloured fibers with the natural detergent on alternate days did not affect the stain of the threads. The black colour of the thread dipped in formulation HD-11 completely faded within 30 days. In other cases the colour started fading after 25 days. The colour intensities of thread dipped in formulations HD-13 and HD-14 became half whereas the intensities of the colours of fibers blackened with HD-1 and HD-4 were lesser then half after 35 days (Plates 8.8 - 8.10). These six formulations were prepared in bulk quantities, six group of volunteers of different age groups were constituted and each group had 25 volunteers. Each formulation was applied on the scalp of 25 volunteers for two hours, air dried and then washed with water. The results were observed daily for 30 days. The percentage of acceptance of these formulations by different groups of volunteers is given in Table 8.3. The formulation HD-3 was the most accepted hair dyeing formulation and the percentage acceptance was 96 %. The percentage of acceptance of the
herbal formulations was in the order: HD-3 > HD-4 > HD-1 > HD-13 > HD-14 > HD-11.

Table 8.3. Volunteers percentage for the various formulations of natural hair dye.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Formulation</th>
<th>No. of volunteers accepting the formulation</th>
<th>% of acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HD-1</td>
<td>21</td>
<td>84.00</td>
</tr>
<tr>
<td>2</td>
<td>HD-3</td>
<td>24</td>
<td>96.00</td>
</tr>
<tr>
<td>3</td>
<td>HD-4</td>
<td>22</td>
<td>88.00</td>
</tr>
<tr>
<td>4</td>
<td>HD-11</td>
<td>18</td>
<td>72.00</td>
</tr>
<tr>
<td>5</td>
<td>HD-13</td>
<td>21</td>
<td>84.00</td>
</tr>
<tr>
<td>6</td>
<td>HD-14</td>
<td>20</td>
<td>80.00</td>
</tr>
</tbody>
</table>

Each formulation was applied to a group of 25 volunteers.

The growth of hair length of those rabbits was maximum when the formulations HN-3 was applied to their skin. The order of the growth of the hair with the application of the herbal formulation was in the order:

HN-3 > HN-7 > HN-6 > HN-5 > HN-4 > HN-1 > HN-2 (Table 8.4 - 8.7).
Table 8.4. Measurement of rabbits' hair after one week (length in mm)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Condition s</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HN-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>1</td>
<td>Hair growth (Normal)</td>
<td>A₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av</td>
</tr>
<tr>
<td>2</td>
<td>Hair growth with control sample (coconut oil)</td>
<td>A₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av</td>
</tr>
<tr>
<td>3</td>
<td>Hair growth with test formulation</td>
<td>A₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av</td>
</tr>
</tbody>
</table>

A₁ - A₇, B₁ - B₇ and C₁ - C₇ indicates the animal groups used for formulations.
<table>
<thead>
<tr>
<th>S.N.</th>
<th>Condition</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HN-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>1</td>
<td>Hair growth (Normal)</td>
<td>A₁ 2.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₁ 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁ 2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av 2.05</td>
</tr>
<tr>
<td>2</td>
<td>Hair growth with control sample</td>
<td>A₁ 2.08</td>
</tr>
<tr>
<td></td>
<td>(coconut oil)</td>
<td>B₁ 2.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁ 2.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av 2.06</td>
</tr>
<tr>
<td>3</td>
<td>Hair growth with test formulation</td>
<td>A₁ 2.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₁ 2.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁ 2.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av 2.22</td>
</tr>
</tbody>
</table>

A₁ - A₇, B₁ - B₇ and C₁ - C₇ indicates the animal groups used for formulations.
Table 8.6. Measurement of rabbits' hair after third week (length in mm)

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Condition</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HN-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>1</td>
<td>Hair growth (Normal)</td>
<td>A₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.06</td>
</tr>
<tr>
<td>2</td>
<td>Hair growth with control sample (coconut oil)</td>
<td>A₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.10</td>
</tr>
<tr>
<td>3</td>
<td>Hair growth with test formulation</td>
<td>A₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.28</td>
</tr>
</tbody>
</table>

A₁ - A₇, B₁ - B₇ and C₁ - C₇ indicates the animal groups used for formulations.
### Table 8.7. Measurement of rabbits' hair after fourth week (length in mm)

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Condition</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HN-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>1</td>
<td>Hair growth (Normal)</td>
<td>A₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av</td>
</tr>
<tr>
<td>2</td>
<td>Hair growth with control sample (coconut oil)</td>
<td>A₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av</td>
</tr>
<tr>
<td>3</td>
<td>Hair growth with test formulation</td>
<td>A₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av</td>
</tr>
</tbody>
</table>

A₁ - A₇, B₁ - B₇ and C₁ - C₇ indicates the animal groups used for formulations.
Size of hair root

The size hair root was maximum with the formulation HN-3 and followed by HN-7, HN-6, HN-5, HN-4, HN-1 and HN-2. In all cases size of hair roots was larger than the control one.

Matrix cell

In the hair bulbs, there was maximum increase of the cells of hair matrix in case of HN-3 formulation. These cells were having large vesicular nuclei and the basophilic cytoplasm. The number of the cells were in the following decreasing order HN-7, HN-6, HN-5, HN-4, HN-1 and HN-2. With HN-4, HN-1 and HN-2 the number of hair matrix cells were similar.

Follicle papila

The follicle papila was prominent and large in all the test formulation in comparison to control one. However, it was maximum in HN-3.

Root sheath

The outer root sheath constituted by clear cells and these were more prominent in HN-3 and HN-6. The Henle's layer was equally prominent and thick in all test formulations than control.

Concentration of hair

The number of hair follicle appeared to be maximum per unit area in HN-3, followed by HN-7, HN-6, HN-5, HN-4, HN-1 and HN-2. In case of all test formulations numbers of hair were significantly higher than the control. However, enlargement of the Hair follicle with increase in proliferation of different cellular components were identical in all the test formulations (Plates 8.11 - 8.18).
The formulation HN-3, HN-4 and HN-5 were tested on three groups of human beings having three members/ volunteers in each group. The length of hair measured from all three formulations (Plates 8.19-8.21). Regular growth was reported from these three formulations were the maximum growth (length) of hair was from formulation HN-3 (Table 8.8 - 8.11).

Table 8.8. Measurement of Human Hair after one week (length in mm)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Condition</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HN-3</td>
</tr>
<tr>
<td>1</td>
<td>Hair growth (Normal)</td>
<td>A₁,B₁, C₁ (Average)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.71</td>
</tr>
<tr>
<td>2</td>
<td>Hair growth with control sample</td>
<td>2.77</td>
</tr>
<tr>
<td>3</td>
<td>Hair growth with formulation</td>
<td>2.98</td>
</tr>
</tbody>
</table>

A₁ - A₃, B₁ - B₃, C₁ - C₃ stands for human beings.

Table 8.9. Measurement of Human Hair after second week (length in mm)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Condition</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HN-3</td>
</tr>
<tr>
<td>1</td>
<td>Hair growth (Normal)</td>
<td>A₁,B₁, C₁ (Average)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.32</td>
</tr>
<tr>
<td>2</td>
<td>Hair growth with control sample</td>
<td>5.52</td>
</tr>
<tr>
<td>3</td>
<td>Hair growth with formulation</td>
<td>6.01</td>
</tr>
</tbody>
</table>

A₁ - A₃, B₁ - B₃, C₁ - C₃ stands for human beings.
Table 8.10. Measurement of Human Hair after third week (length in mm)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Condition</th>
<th>Formulations</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HN-3</td>
<td>HN-4</td>
<td>HN-5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Hair growth (Normal)</td>
<td>$A_1, B_1, C_1$ (Average)</td>
<td>$A_2, B_2, C_2$ (Average)</td>
<td>$A_3, B_3, C_3$ (Average)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.16</td>
<td>8.09</td>
<td>8.18</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Hair growth with control sample</td>
<td>8.32</td>
<td>8.18</td>
<td>8.36</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Hair growth with formulation</td>
<td>8.98</td>
<td>8.69</td>
<td>8.48</td>
<td></td>
</tr>
</tbody>
</table>

$A_1 - A_3, B_1 - B_3, C_1 - C_3$ stands for human beings.

Table 8.11. Measurement of Human Hair after fourth week (length in mm)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Condition</th>
<th>Formulations</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HN-3</td>
<td>HN-4</td>
<td>HN-5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Hair growth (Normal)</td>
<td>$A_1, B_1, C_1$ (Average)</td>
<td>$A_2, B_2, C_2$ (Average)</td>
<td>$A_3, B_3, C_3$ (Average)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.76</td>
<td>10.73</td>
<td>10.82</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Hair growth with control sample</td>
<td>10.98</td>
<td>10.92</td>
<td>11.14</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Hair growth with formulation</td>
<td>11.98</td>
<td>11.54</td>
<td>11.32</td>
<td></td>
</tr>
</tbody>
</table>

$A_1 - A_3, B_1 - B_3, C_1 - C_3$ stands for human beings.
RESULTS AND DISCUSSIONS

In case of hair dye formulations, the best formulation was HD-3 out of 14 formulations. This formulations gave good results on sheep wool threads as well as on the human hair. *Nardostachys jatamansi* has been already reported for blackening of hair. The black colour of hair (from dye) was increased with the addition of other herbal ingredients, *i.e.*, *Hibiscus rosa sinensis*, *Saussurea lappa* and *Lawsonia inermis* to the formulations. These herbs acted synergically whereas in case of formulations for hair growth the formulation HN-3, which is having *Nardostachys jatamansi* with volatile oils of jatamansi and lemon peel, gave good response and the maximum hair growth was observed from this formulation followed by formulation HN-5 and HN-4, respectively. In formulation HN-5, there were two herbs, namely *Nardostachys jatamansi* and *Saussurea lappa*, but this formulation was not able to increase the length of hair. The length of hair was somewhat equal to HN-3 formulation. In formulation HN-4, formulated by adding *Hibiscus rosa sinensis* and *Saussurea lappa*, was not able to give the length of hair equal to that of formulation HN-3. The formulation HN-3 and HN-4 were also very effective against dandruff hair might be due to the present of lemon and orange volatile oil.

In rabbits, the formulation HN-3 caused the enlargement of hair follicle with hyperplasia of cellular constituents which was proportional to hair length.
REFERENCES


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