UNIVERSITÀ DEGLI STUDI DI TRIESTE

XXXIII CICLO DEL DOTTORATO DI RICERCA IN CHIMICA

DEVELOPMENT OF NEW PRODUCTS FOR HAIR COLORING AND HAIR BLEACHING

Settore scientifico-disciplinare:
CHIM/09 FARMACEUTICO TECNOLOGICO APPLICATIVO

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ABSTRACT

Products claiming to be environm-entally sustainable, naturally derived and free from “chemicals”, “paraben free”, “vegan” have gained much attention in the consumer market. Herbal dyes are biodegradable, non-toxic, soft and without any side effects. Keeping in mind these factors we did a study on plants that color hair. During our research we found different plants with health benefits derived from various parts of the tree such as flowers, bark, seeds, leaves and roots. The extracts powder have been diluted in water and tested by an acidic pH and a basic pH. Furthermore, some plants were tested in synergy with other plants or with various miscellaneous plants.

In the second part of this project we focused on the research of a new raw material that allows a reduction of skin irritation and sensitivity reaction due to synthetic dyes and in color formulation with monoethanolamine. The most common products for hair color formulated with synthetic dyes, allow to obtain good results, but such products can damage the structure of hair. It becomes more porous and in many cases can cause allergic reaction, such as itching, redness, desquamation, etc. PPD (Para-phenylenediamine) is a key ingredient found in the majority of permanent and semi-permanent hair dyes. It is the most common cause of an allergic reaction to hair coloring. Other chemicals in hair dyes, such as ammonia, peroxide, PTDS (para-toluenediamine sulfate), fragrance and pigments can also trigger scalp inflammation and itchiness. During our research we found the innovative formulation of Shield P-17 that allows without affecting the color results a net reduction of sensitization reactions and allergy due to PPD/PTD in the dye event in presence of MEA. It can be added directly into the dye’s preparatory formula after dyes have been added and around 60°C. In this part of the project we formulated four formulas Black, Brown, Red, Blonde following the mechanism of oxidation dyes. The study in vivo and salon test was done to evaluate scalp irritation, skin discomfort and performance during and after the application of a hair color formulated with Shield P-17. In the end we selected four formulas for each color to verify the product stability, meaning how long the product can maintain its original form without any visible changes, its intended physical, chemical and microbiological qualities as well as functionality under appropriate conditions.

The third part of the project focused on the hair bleaching products. The most common products for hair bleaching are formulated with ammonium persulphate and potassium persulphate which allow to obtain good results, but such products can damage the structure of hair. It becomes more porous and in many cases can cause irritating, redness, desquamation, allergic reaction etc. Keeping in mind these factors we did a research of new raw materials that have the capacity to improve the bleaching hair process and the sensoriality. Zeosafe CL-07 (INCI Name Zeolite) is a mineral of volcanic origin, with a regular and microporous crystal structure, characterized by a huge amount of void volumes inside the crystals. Chemically, zeolite is a hydrated aluminosilicates with three dimensionally structures with regular channels and interconnected pores of 4° diameter, contains water and cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}..) inside the structure. It has characteristics as molecular sieve, absorbent and cation exchange capacity with high selectivity. Due to these characteristics it has used to increase the bleaching process. Our study consists of five phases: application of the bleaching powder with a new chelant; the research of new actives to improve the final sensoriality; the application of bleaching powder with the active; the application of bleaching powder containing chelant and active substances; Other test by changing temperature, dilution, time, volume of hydrogen peroxide.

1. INTRODUCTION

Products claiming to be environmentally sustainable, naturally derived and free from “chemicals”, “paraben free”, “vegan” have gained much attention in the consumer market. This trend is becoming more and more popular not only as regards to the skincare products, but lately has also involved the hair color market. [1]

The global market value for natural cosmetics and personal care expected a positive increase from almost 34.5 billion dollars in 2018 to roughly 54.5 billion dollars expected for the year 2027. [2] These data are a proof of the growing importance of the natural and organic beauty market. And like other product consumers, many hair dye users share the opinion that a hair dye employing solely natural or organic ingredients should be kind to hair. Such hair colorants are easy to find, and bear claims for water bases or natural plant and mineral ingredients. Natural hair dyes are usually temporary to semi-permanent in their duration. The degree of colorfastness is inferior to demi-permanent or permanent colors since these products do not contain peroxide or alkaline amines. As such, they are, indeed, usually milder to hair and less likely to cause damage to hair fibers. Furthermore, herbal dyes are biodegradable, non-toxic, soft and without any side effects. Natural dyes, also, are easily available with low cost. They can be used in food, medicine, perfume, leather and textile industries.
Keeping in mind these factors we did a study on plants that color hair. During our research we found different plants with health benefits derived from various parts of the tree such as flowers, bark, seeds, leaves and roots.

Firstly, to evaluate the coverage of plant extracts on hairs and the final colouring we used different types of hair: yak that used as a reference standard for white hair, salt and pepper that used as a standard for dark hair and bleached hair that used as a standard for bleached hair. The extracts powder have been diluted in water in a ratio from 1: 1 to 1: 6 in order to obtain a semi-liquid mixture. All individually tested plants are characterized by an acidic pH of 2.0-3.5. But we decided to add an alkaline agent, monoethanolamine, which allows you to open the scales cuticle and promote the penetration of the active ingredient on the hair, to increase the intensity of the color.

Furthermore, some plants were tested in synergy with other plants or with various miscellaneous plants.

In the second part of this project we focused on the research of a new raw material that allows a reduction of skin irritation and sensitivity reaction due to synthetic dyes and in color formulation with monoethanolamine. The most common products for hair color formulated with synthetic dyes, allow to obtain good results, but such products can damage the structure of hair. It becomes more porous and in many cases can cause allergic reaction, such as itching, redness, desquamation, etc.

PPD (Para-phenylenediamine) is a key ingredient found in the majority of permanent hair dyes. It is the most common cause of an allergic reaction to hair coloring. Other chemicals in hair dyes, such as ammonia, peroxide, PTDS (para-toluenediamine sulfate), fragrance and pigments can also trigger scalp inflammation and itchiness.

For decades, formulators and manufacturers tried to make high performance hair dyes without the use of PPD/PTD that signed the market, in terms of color and brilliance, in a way today is difficult to give up.
During our research we found the innovative formulation of Shield P-17 that allows without affecting the color results a net reduction of sensitization reactions and allergy due to PPD/PTD in the dye event in presence of MEA. It can be added directly into the dye's preparatory formula after dyes have been added and around 60°C.
In this part of the project we formulated four formulas Black, Brown, Red, Blonde following the mechanism of oxidation dyes. So, the primary intermediates bound to the modifiers intermediate forming a chromogen group that reacts with hydrogen peroxide and develops the color.

The study in vivo and salon test was done to evaluate scalp irritation, skin discomfort and performance during and after the application of a hair color formulated with Shield P-17. In the end we selected four formulas for each color to verify the product stability, meaning how long the product can maintain its original form without any visible

changes, its intended physical, chemical and microbiological qualities as well as functionality under appropriate conditions.

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Zeosafe CL-07 (INCI Name Zeolite) is a mineral of volcanic origin, with a regular and microporous crystal structure, characterized by a huge amount of void volumes inside the crystals. Chemically, zeolite is a hydrated aluminosilicates with three dimensionally structures with regular channels and interconnected pores of 4° diameter, contains water and cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}..) inside the structure. It has characteristics as molecular sieve, absorbent and cation exchange capacity with high selectivity. Due to these characteristics it has a lot of use in cosmetic products such as: reduce free radicals in skin tissue, draw out impurities, protect against radiation and UV lights, regenerate cells, counteract inflammation, boost the immune system and bleaching.

Our study consists of five phases: application of the bleaching powder with a new chelant; the research of new actives to improve the final sensoriality; the application of bleaching powder with the active; the application of bleaching powder containing chelant and active substances; Other test by changing temperature, dilution, time, volume of hydrogen peroxide.

The work presented in this thesis was performed at the company Deimos srl (Milan, Italy), during the period Novembre 2017-October 2020, under the supervision of Prof. Guglielmo Zingone and the thesis was co-supervised by Dr. Sabrina Romani.

2. MORPHOLOGICAL, MACROMOLECULAR STRUCTURE AND HAIR GROWTH

Human hair is a keratin-containing appendage that grows from large cavities or sacs called follicles. Hair follicles extend from the surface of the skin through the stratum corneum and the epidermis into the dermis, see Fig. 2.1. Hair provides protective, sensory and sexual attractiveness functions. Hair is characteristic of all mammals and in humans grows over a large percentage of the body surface.

Regardless of the species of origin or body site, human hair grows in three distinct stages and has certain common structural characteristics. These three cyclical stages of hair fibers are called anagen (growing stage), catagen (transition stage) and telogen (resting stage), see Fig.2.2.

Morphologically, a fully formed hair fiber contains three and sometimes four different units or structures. At or near its surface, hair contains a thick protective covering consisting of one or more layers of flat overlapping scale-like structures called cuticles or scales see Fig. 2.3.

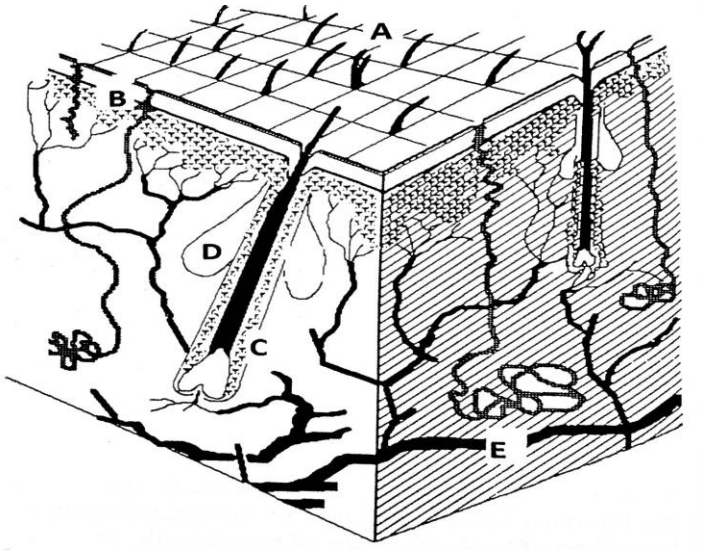


Fig. 2.1 A section of human skin illustrating a hair fiber in its follicle as it emerges through the skin and how it is nourished

A. Stratum Corneum **B. Epidermis**
C. Pilosebaceous Unit **D. Sebaceous gland**
E. Blood Vessel

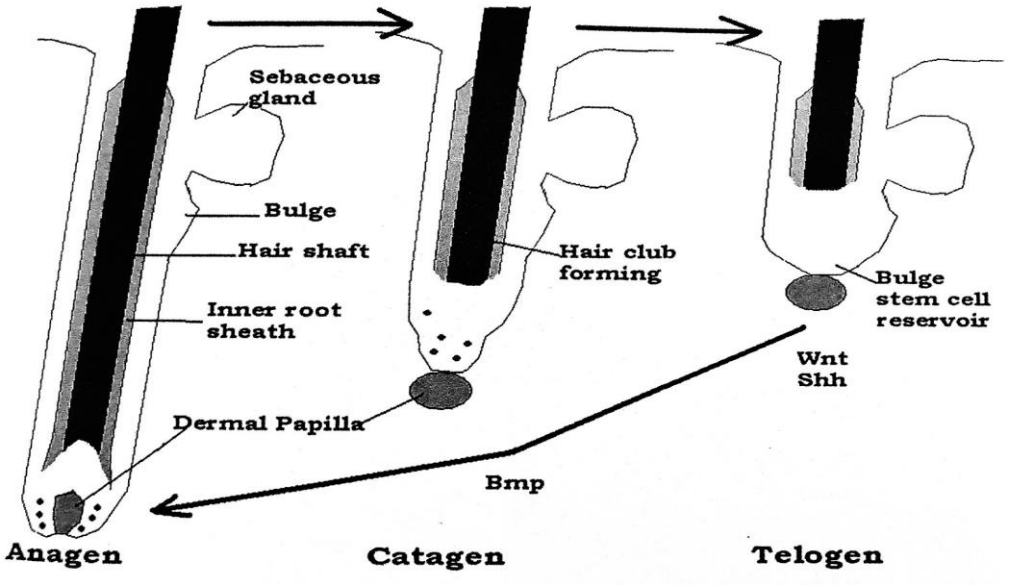
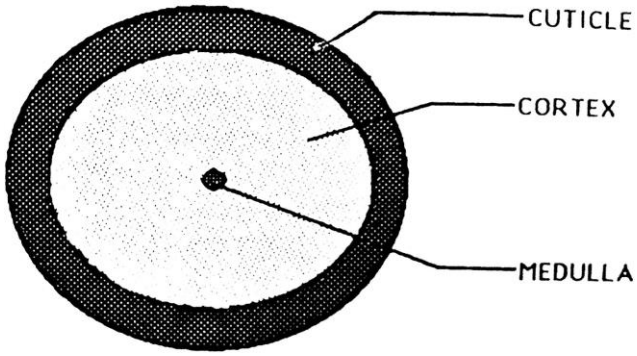


Fig. 2.2 Schematic illustrating the three stages of growth of human hair fibers

Fig. 2.3 Schematic diagram of a cross section of a human hair fibers



The cuticle layers surround the cortex, but the cortex contains the major part of the fiber mass. The cortex consists of spindle-shaped cells that are aligned parallel with the fiber axis. Cortical cells contain many of the fibrous proteins of hair. Coarser hairs often contain one or more loosely packed porous regions called the medulla, located near the center of the fiber. The fourth important unit of structure is the cell membrane complex, the “glue” that binds or holds all of the cells together.

2.1 The General Structure and Hair Growth

The schematic diagram of Fig. 2.1.1 illustrates an active growing human hair fiber inside the follicle, which is the sac that originates in the subcutaneous tissue of the skin and contains the hair fiber with several surrounding structures involved in its growth. The dermal papilla, located near the center of the bulb is involved in important growth functions during anagen (Fig. 2.2). The basal layer that produces hair cells nearly surrounds the bulb. Melanocytes that produce hair pigment also exist within the bulb close to the dermal papilla. Blood vessels (Fig 2.1) carry nourishment to the growing hair fiber deep within the skin at the base of the bulb. [19]

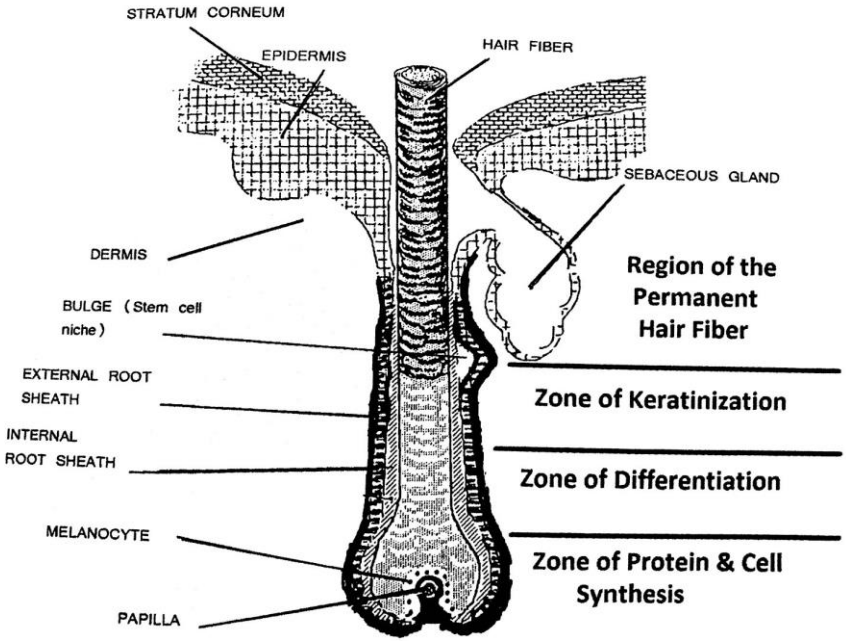


Fig. 2.1.1 Pilosebaceous unit with a hair fiber in its follicle and the zones of protein and cell synthesis, differentiation, keratinization and the region of the permanent hair as the fiber emerges through the scalp.

The human hair fiber beneath the skin can be divided into several distinct zones along its axis (Fig. 2.1.1). The zone of biological synthesis and orientation resides at and around the bulb. This zone is sometimes divided into a lower region called cell proliferation or cell matrix. Moving upward in the growing fiber is the region of cell differentiation which leads into the zone keratinization, where stability is built into the hair structure by the formation of cystine linkages. The next zone that begins below the skin line and eventually emerges through the skin surface is the region of the permanent hair fiber. The permanent hair fiber consists of fully formed dehydrated cornified cuticle, cortical and sometimes medullary cells, but always the cell membrane complex which acts like a natural adhesive, binding the hair cells together.

The diameter of human scalp hair fibers varies from 40 to 120 µm. Others provide a somewhat larger range varying from about 20 to 125 µm. The low values for this latter estimate are undoubtedly due to the inclusion of hair of infants and young children. For adult hair we estimate the variation from means of subjects to be primarily between 45 and 110 µm. The range for individual hairs on individual scalps can exceed these values.

Hair Growth

Generally around the fifth fetal month, the follicles and their growth machinery have been developed, although not entirely mature. Each individual hair after birth is programmed to grow in cycles involving three distinct stages (see Fig. 2.2).

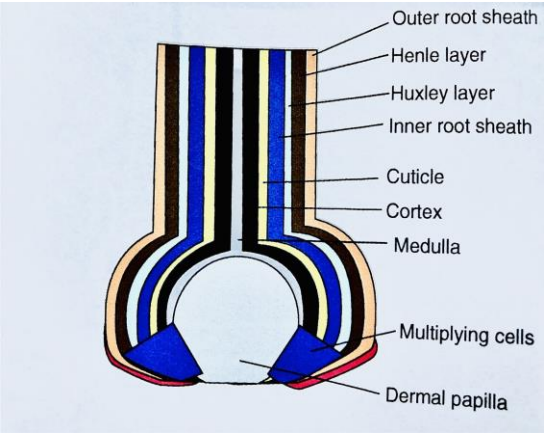


Fig. 2.1.2 Schematic of an active hair bulb with a hair fiber illustrating the important layers with regard to growth

The anagen stage, or the actual growing stage, is characterized by intense metabolic activity in the hair bulb. For scalp hair, this activity generally lasts 2-6 years producing hairs that grow to approximately 100 cm in length; however, human scalp hair longer than 150 cm is frequently observed in long hair contests, indicative of a longer anagen period. *The catagen stage* or the transition stage lasts for only a few weeks. During catagen, metabolic activity slows down, and the base of the bulb migrates upward in the skin toward the epidermal surface. Molecular regulators that promote the transition from anagen to catagen are: Growth factors (FGF5 and EGF1) and neurotrophins (BDNF, p53, TGFß1). Telogen or the resting stage also lasts only a few weeks (generally 4-8). At this stage, growth has stopped completely and the base of the bulb has atrophied to the point at which it approaches the level of the sebaceous canal. The life cycle of a hair fiber is initiated by chemical messengers that act on stem cells in the bulge. Hair growth is partially controlled by androgens and the local tissue most likely through specific receptor sites. Testosterone and DHT are the primary androgenes that determine whether hairs increase or decrease in size with age and some other aspect of hair growth and hair loss. During various stages of growth, signaling molecules and metabolites are transported between the different cell layers of Fig. 2.1.2 to the site where they activate the tissues. In spite of the fact that each follicular unit can function independently, the response by the local tissue tends to be a regional response and it determines whether hairs grow or whether the hair cycle is shortened and ultimately leads to baldness.

2.1.1 The Cuticle

The cuticle consists of flat overlapping cells (scales) that surround the central fiber core. Fig. 2.1.1.1. The cuticle cells are attached at the proximal end (root end), and they point toward the distal end (tip end) of the hair fiber, like shingles on a roof. The shape and orientation of the cuticle cells are responsible for the differential friction effect in hair. Each cuticle cell is approximately 0,5 µm thick, with about a 6-7 exposed axial surface or scale interval, and approximately 45-60 µm long.

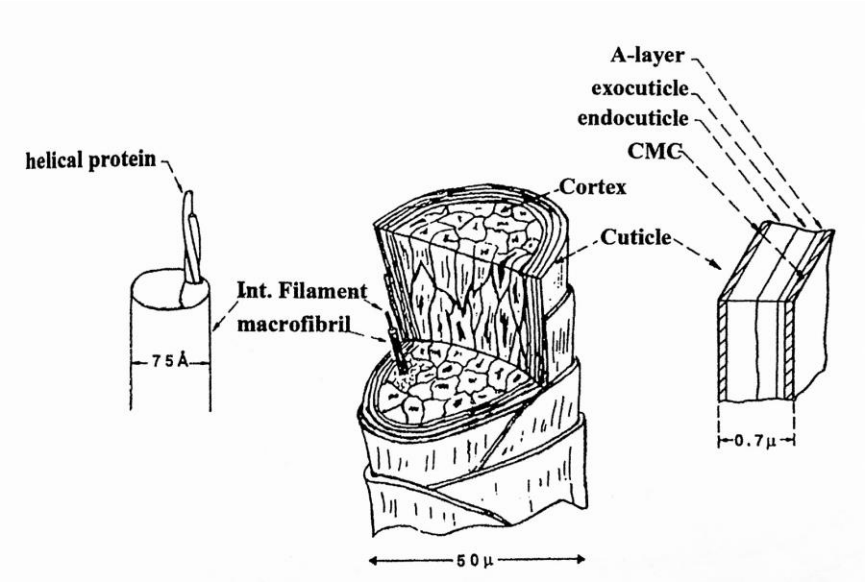


Fig. 2.1.1.1 Stereogram of the hair fiber structure, illustrating substructure of the cuticle and the cortex

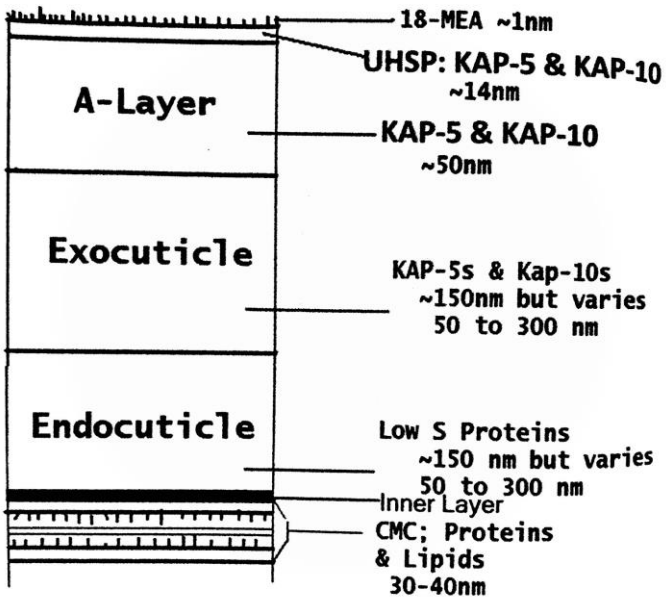


Fig. 2.1.1.2

Schematic diagram of the proposed structure of a cuticle cell in cross section

Beneath the cuticle cell membranes are three major layers; the *A layer*, a highly cross linked resistant layer about 50-100 nm thick (see Fig. 2.1.1.2). The A layer contains a

high cystine content (>30%) and additional cross links called isopeptide bonds. The *exocuticle*, sometimes called the B layer, is beneath the A-layer. It is also rich in cysteine (15-20%) and highly variable in thickness in each cuticle cell averaging about 150 nm. Underneath the exocuticle is the *endocuticle*, low in cysteine content (~3%) and also highly variable in thickness from about 50 to 300 nm within each cuticle scale.

A portion of the under-membrane of Fig 2.1.1.2 is also epicuticle or "epicuticle-like" matter. The outer surface of hair fibers consists of about 75% of a heavily cross-linked protein and about 25% fatty acid that is predominantly 18-methyl eicosanoic acid.

2.1.2 The Cortex

The cortex constitutes the major part of the fiber mass (70-90%, the lower percentage in fine hair) of human hair and consists of cells and intercellular binding material. Cortical cells of human hair fibers are generally 1-6 µm thick and approximately 50-100 µm long. The cortical cells of human hair are composed of fibrillar components called microfibrils that are connected by inter-microfibrillar material, cytoplasmic remnant and melanin granules. The microfibrils consist primarily of filamentous proteins that form intermediate filaments (IF) that are held together laterally of filamentous and in orientation by amorphous type proteins called keratin associated proteins (KAP's). The filamentous polypeptides of human hair fibers are classified as Type I and Type II and these differ by their amino amino acid sequences resulting in acidic (Type I) and neutral to basic (Type II) proteins. IFs contain precise arrays of the low-sulfur proteins, containing short sections of alpha-helical proteins in coiled coil formation, showing a heptad repeat unit. The coiled coils are interrupted at three positions by non-helical fragments and are terminated by non-helical domains at both the nitrogen (N) and carbon (C) termini of the chain. The individual filament-like protein chains are arranged into coiled coil dimers each containing one strand of type I and a second strand of type II chains (Fig. 2.1.2.1).

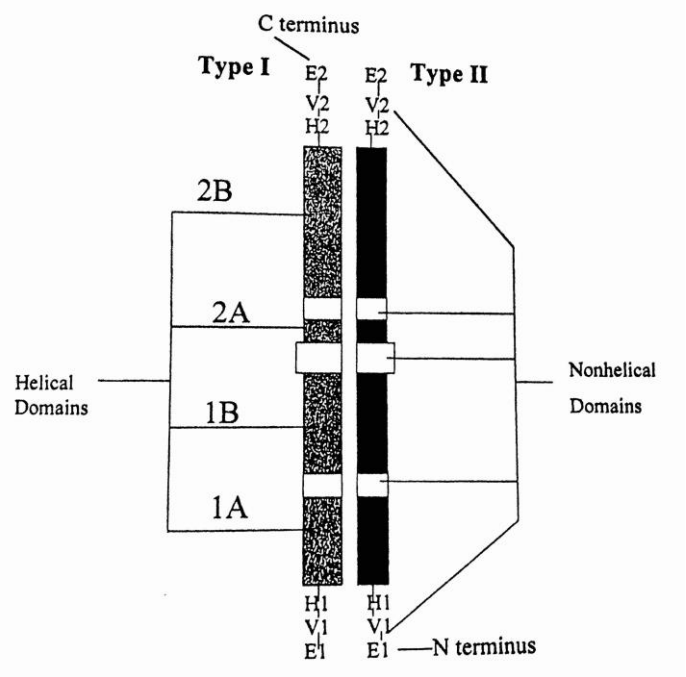


Fig. 2.1.2.1 Schematic illustrating the structure of an intermediate filament protein (type I-type ll dimer). E are the domains (E2 the terminus and the N terminus), V a variable sequence region and H is a sequence region

end

E1

high

2.1.3 The Cell Membrane Complex

The cell membrane complex (CMC) consists of cell membranes and adhesive material that binds or “glues” the cuticle and cortical cells together in keratin fibers. The CMC consists of a central Delta layer approximately 15 nm thick sandwiches by two lipid layers called Beta layers each in the vicinity of 5 nm thick.

Three types of CMC have been described in the literature: cuticle-cuticle CMC representing CMC between cuticle cells, cortex-cortex CMC representing CMC between cortical cells and cuticle-cortex CMC representing CMC at the cuticle cortex boundary.

The CMC of the cuticle contains 18-methyl eicosanoic acid (18-MEA) in its upper beta layer. 18-MEA has never been shown to be in the CMC of the cortex. The CMC of the cuticle has monolayer lipids that are attached by covalent bonds (primarily thioester) with some ester or amide linkages to proteins of the cell membranes on one end and attachment by van der Waals attractive forces to proteins of the delta layer on the hydrophobic end of the fatty acids (Fig.1.2.1.3.1). The evidence shows that the CMC between cortical cells consists of lipid bi-layers that are not attached by covalent bonding to protein layers. The lipid bi-layers of the cortex are bound by salt linkages and polar bonding to the cortical cells membrane proteins on one side and similarly attached to the

Delta layer on the other side of the bi-layer, see Fig.2.1.3.2.

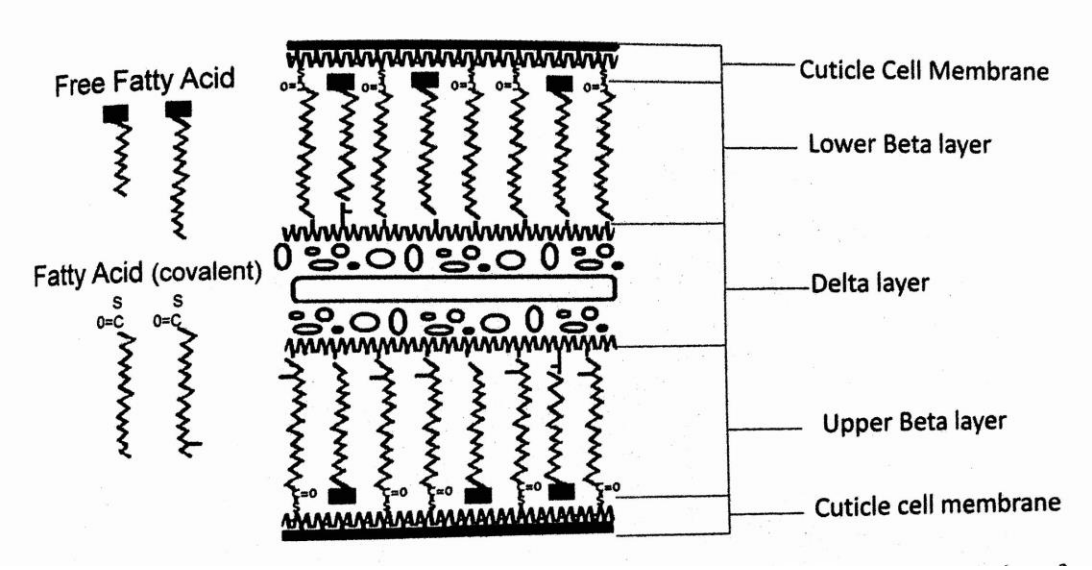


Fig. 2.1.3.1 Schematic proposed for the cuticle-cuticle CMC

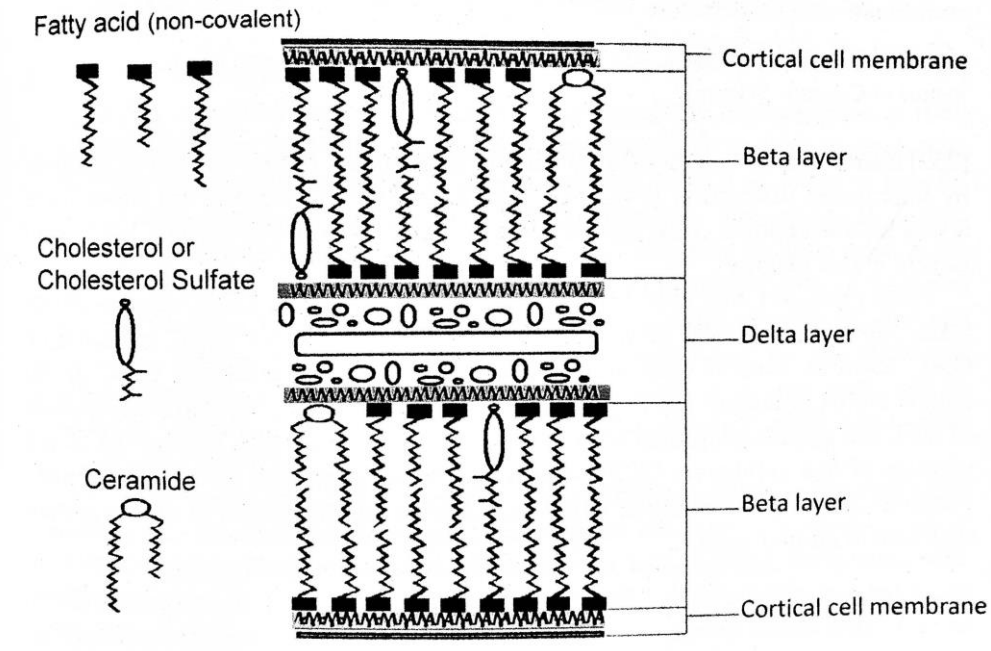


Fig. 2.1.3.2 Schematic representing the cortex-cortex CMC

2.1.4 The Structure of the Medulla

Medullary cells are loosely packed, and during dehydration (formation), they leave a series of vacuoles along the fiber axis. At higher magnification medullary cells appear spherical and hollow inside and are bound together by a cell membrane complex type material (see Fig. 2.1.4.1). Wynkoop classified hairs according to four different medulla types: absent, scanty, broken and continuous. She considered age and fiber diameter vs. medulla type. The amount and type of medulla are not related to age, but the amount of medulla is related to hair fiber diameter and that the finest hair generally does not contain a medulla, medium-sized hairs generally contain a broken medulla and the thickest hairs generally contain a broken medulla and the thickest hairs generally contain a continuous medulla. [19][20]

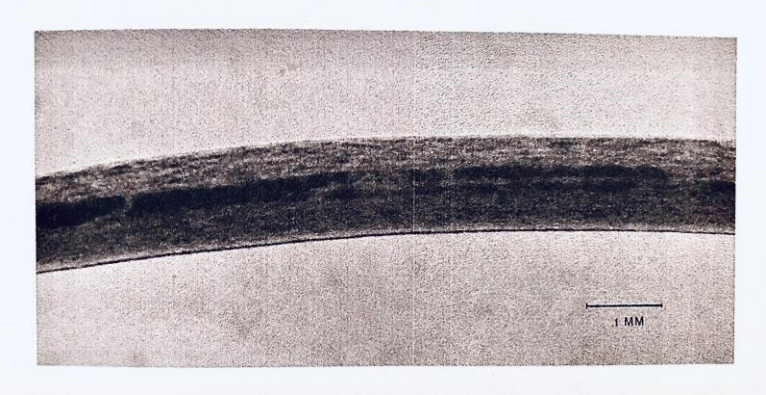
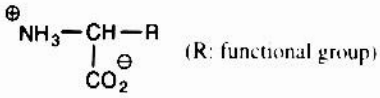
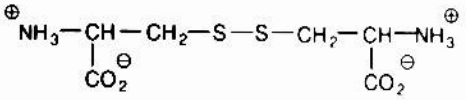
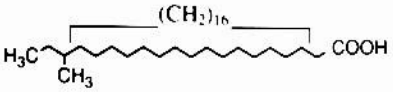


Fig. 2.1.4.1 An optical section of a light micrograph illustrating a hair fiber with a divided or double medulla. Multiple medulas seem more common in facial than scalp hair

2.2 Chemical Composition of Different Hair Types

Depending on its moisture content (up to 32% by weight), human hair consists of approximately 65% to 95% proteins. Proteins are condensation polymers of amino acids. The remaining constituents are water, lipids (structural and free), pigment, and trace elements that are generally not free, but combined chemically with side chains of protein groups or with fatty-acid groups of sorbed or bound lipids Table 2.2.1. The current state of changes in the amino acids, proteins and lipids of hair by morphological region (including KAP and keratin proteins), chemical and sunlight damage, diet, puberty and menopause, and other factors have been and are being made. The species responsible for color in hair is the pigment melanin, which is located in the cortex of the hair in granular form.

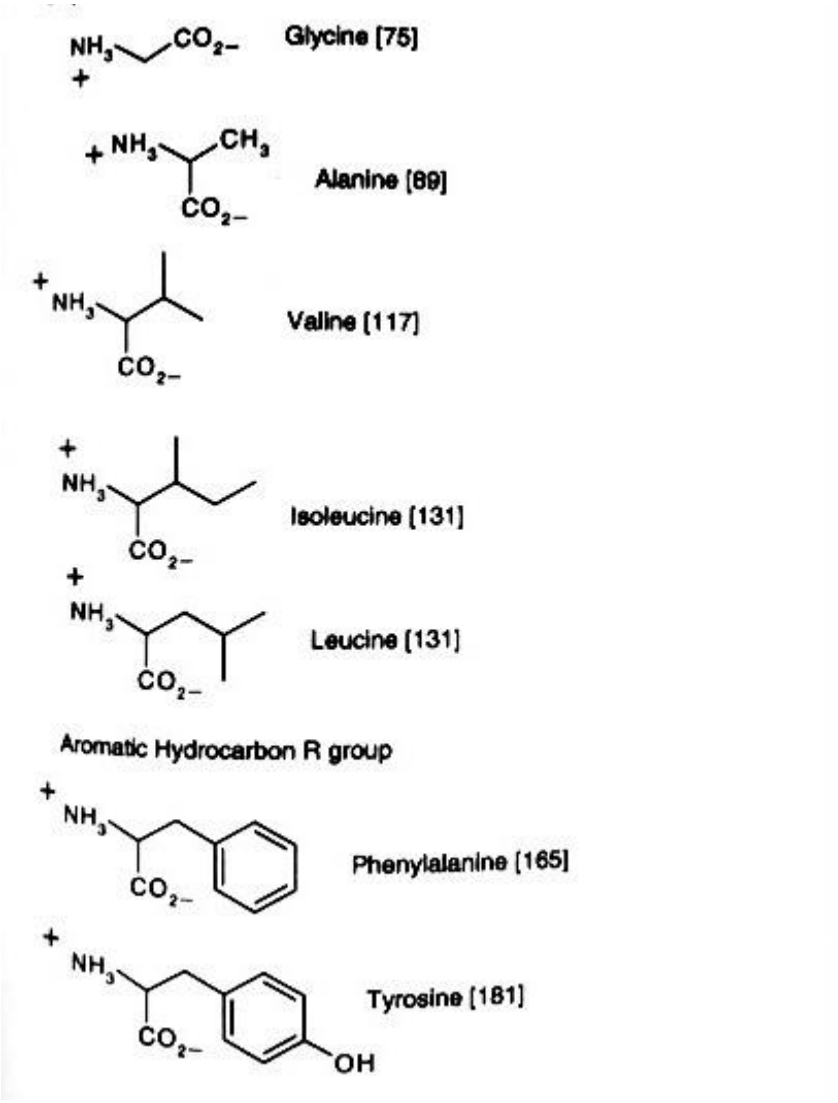
Table 2.2.1 Summary of chemical species present in human hair

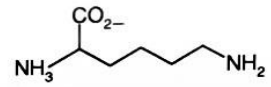
Keratin protein	65–95%
(Amino acids)	 (R: functional group)
Cystine	
Lipids	Structural and free
18-Methyl eicosanoic acid (18-MEA)	
Water	Up to 30%
Pigment and trace elements	Melanin

2.2.1 The Amino Acids and Proteins of Hair

Proteins of hair are made up of long chains of various mixtures of some 20 or 50 amino acids. Each chain takes up a helical or coiled form. The structure of those amino acids that are found in human hair are depicted in Table 2.2.1.1.

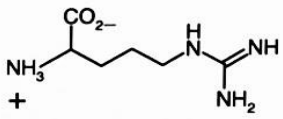
Table 2.2.1.1 Structures of amino acids found in hydrolyzates from human hair (molecular weights of these amino acids are listed in brackets)


Glycine [75]
Alanine [89]
Valine [117]
Isoleucine [131]
Leucine [131]
Aromatic Hydrocarbon R group
Phenylalanine [165]
Tyrosine [181]



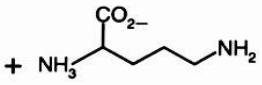
Lysine [146]

+

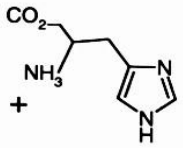


Arginine [174]

+

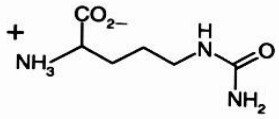


Ornithine [132]



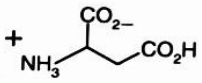
Histidine [155]

+

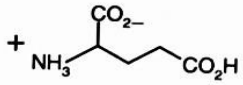


Citrulline [175]

Diacidic Amino Acids

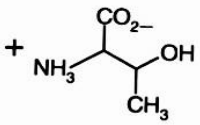


Aspartic Acid [133]

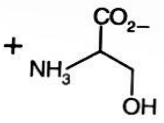


Glutamic Acid [147]

Hydroxyl containing amino acids

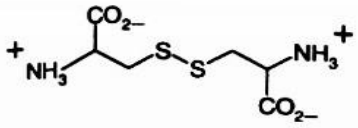


Threonine [119]

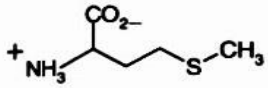


Serine [105]

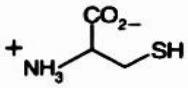
Sulfur containing amino acids



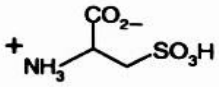
Cystine [240]



Methionine [149]

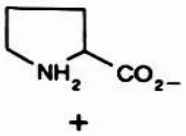


Cysteine [121]

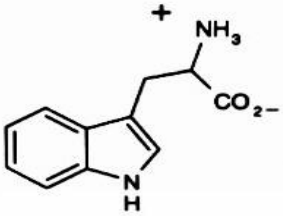


Cysteic Acid [169]

Heterocyclic amino acids in hair



Proline [115]



Tryptophan [204]

Aspartic acid and glutamic acids exist as the primary amides and the free acids in human hair

Among numerous amino acids in human hair, cystine is one of the most important amino acids. Every cystine unit contains two cysteine amino acids in different chains which lie near to each other and are linked together by two sulfur atoms, forming a very strong bond known as a disulfide linkage; see Fig. 2.2.1.1.

In addition to disulfide bonds, hair is also rich in peptide bonds, and the abundant CO- and NH-groups present give rise to hydrogen bonds between groups of neighboring

chain molecules. The distinct cystine content of various cellular structures of human hair results in a significant effect on their physical properties. A high cystine content corresponds to rich disulfide cross-links, leading to high mechanical properties.

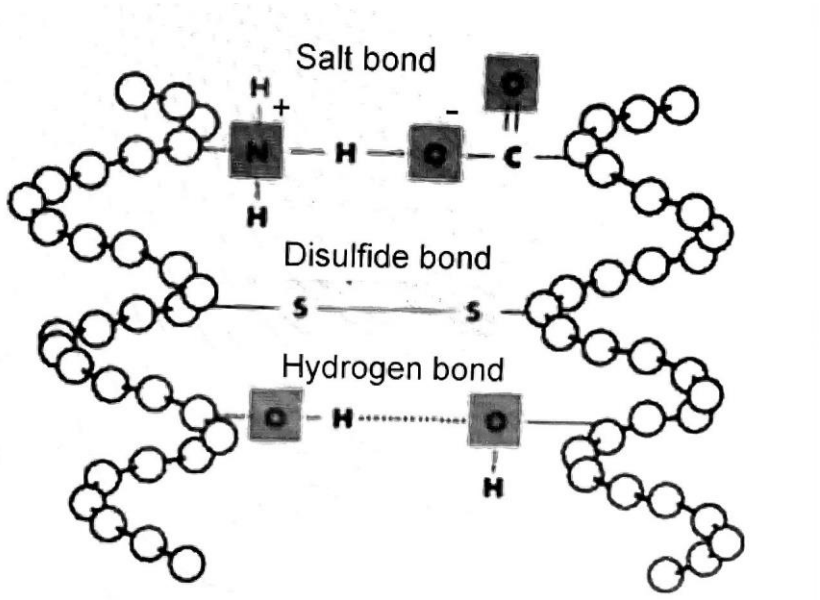


Fig. 2.2.1.1 Various bonds within hair cellular structure

The *cuticle* of human hair contains more cystine, cysteic acid, proline, serine, threonine, isoleucine, methionine, leucine, tyrosine, phenylalanine, and arginine than whole fiber. Average cortex is rich in cysteine (although there is less cystine in the cortex than in the cuticle). The *cortex* is also richer in diacidic amino acids and lysine and histidine than is cuticle. However, the two main components of cortex, the intermediate filaments and the matrix, are very different in chemical composition. The *intermediate filament* proteins are rich in leucine and in glutamic acid and those amino acids that are generally found in alpha-helical proteins. Although small quantities of cystine (~6%), lysine, and tyrosine are also regularly arranged in the intermediate filaments. On the other hand, *the matrix* is rich in cysteine (about 21%, calculated from the sulfur content of gamma keratose of human hair) and proline. *Medulla* has only trace quantities of cystine and appears to have relatively small amounts of hydroxy amino acids and relatively large amounts of basic and acidic amino acids compared to the other two morphological components.

2.2.2 Water: A fundamental Component

The determined moisture content of keratin fibers depends on the conditions selected as the state of dryness as well as on the water. The amount of moisture in hair also plays a critical role in its physical and cosmetic properties. Hair dried with heat can exhibit lower moisture content than hair dried at room temperature. After heat-drying, hair absorbs moisture but does not return to the room temperature dried moisture level until it is either rewet with water or conditioned at a higher relative humidity. Undoubtedly, the several hydrophilic side chains (guanidino, amino, carboxyl, hydroxyl, phenolic, etc.) and peptide bonds of keratin fibers contribute to water sorption, although there is controversy over the primary water-binding groups. Water sorption of unaltered and deaminated fibers, concluded that the binding of water by amino and guanidino groups is responsible for a large percentage of the water sorption capacity of keratin fibers, especially at low humidities. On the other hand, the peptide bonds are preferential sites for hydration. At relative humidity, below 25%, water molecules are principally bonded to hydrophilic sites of the fiber by hydrogen bonds. As the humidity increases, additional water is sorbed, producing a decrease in the energy of binding of water already associated with the protein.

2.2.3 Lipids of Human Hair

The total amount of lipid extractable from hair is generally 1–9% of the weight of the hair. The scientists analyzed human hair and found 41% 18-MEA, 23% palmitic, 25% palmitoleic, 4% stearic, 13% oleic and other fatty acids. These are all non-covalently bound fatty acids with 39% of the total fatty acids being unsaturated (primarily palmitoleic and oleic). Although, it is possible other unsaturated fatty acids were present. 18-MEA is essentially all in the cuticle. It represents more than 40% of the total covalently bound fatty acids in human hair 18-MEA is confined to the upper Beta layer of the cuticle while most (essentially an amount equal to the 18-MEA) of the other covalently bound fatty acids are confined to the lower Beta layer. Therefore, most of the covalently bound fatty acids in hair fiber must be in the cuticle-cuticle CMC with some in the cuticle-cortex CMC and virtually none in the cortex-cortex CMC.

There are at least four different but meaningful classifications of hair lipids. Hair lipids are described as free or bound, as endogenous or exogenous lipids, as internal or surface and by chemical functional group or chemical type. Bound lipids are those that cannot be removed by extracting the hair with lipid solvents because they are covalently bonded to hair proteins. For example, 18-MEA is attached to proteins by thioester linkages, whereas free lipids are extractable from hair using lipid solvents because they are held by weaker bonding forces such as van der Waals attractive forces and sometimes hydrogen bonding or even salt links. Endogenous lipids are those hair lipids that result from biosynthesis in hair matrix cells in the hair follicle, whereas those lipids in the hair that are usually synthesized in sebaceous glands are sometimes called exogenous of an extrinsic source. Internal lipids are those that have either penetrated into the hair or have been incorporated inside the hair fiber as opposed to surface lipids. In this study, hair lipids were extracted and analyzed from both the proximal and distal parts of the hair of 44 Japanese females between the ages of 1 and 81 and the composition determined quantitatively. These scientists separated the lipids into four groups by chemical type: Group A: Squalene (SQ), Wax esters (WE), Triglycerides (TG), and fatty acids (FA); Group B: cholesterol (CH) and ceramides (CER); Group C: hydrocarbons (HC) and Group D: 18-methyl eicosanoic acid (MEA).

These fatty acids are bound either through ester or thioester linkages to the underlying proteins. All other lipids are believed to be free lipids, that is, lipids that are not covalently bonded to hair proteins and they exist on and in the cuticle and the cortex. The scientists found fatty acids (~58%), wax esters (~20%) and hydrocarbons (~10%) comprise the major part of the free lipids in hair, almost 90% of the total lipid (free plus bound) in hair from a population of 44 Japanese females.

The cell membrane complex is laminar in structure and is composed of both protein and lipid layers; however, this structural lipid is not phospholipid like the lipids normally associated with bilayers of cell membranes.

2.2.4 Trace Element and Pigments

There are a number of studies describing the quantitative determination of various elements of human hair other than carbon, hydrogen, nitrogen, oxygen, and sulfur. In

particular, the inorganic constituents of human hair appear to be receiving some attention. However, the fact that certain transition metals such as iron and copper can catalyze the formation of free radicals oxidative reactions have picked up interest in cosmetic science too. The mineral content of human hair fibers is generally very low (less than 1%). It is sometimes difficult to determine whether this inorganic matter is derived from an extraneous source (which much of it is) or whether it arises during fiber synthesis. In addition, many metals of human hair exist as an integral part of the fiber structure, such as salt linkages or coordination complexes with the side chains of the proteins or pigments, although the possibility of mineral deposits or compound deposits as in soap deposition also exists. The total ash content of human hair to be as low as 0.26% of the dry weight of the fibers. Among the trace elements reported in human hair are Ca, Mg, Sr, B, Al, Na, K, Zn, Cu, Mn, Fe, Ag, Au, Hg, As, Pb, Sb, Ti, W, V, Mo, I, P, and Se. The actual origin of most of these elements in human hair is due to a variety of sources that are described below. However, from a study involving quantitative analysis of 13 elements in human hair and in hair wash solutions, concluded that a large portion of the trace elements in the hair originate from sweat deposits. In the case of metals, the water supply generally provides calcium and magnesium to hair. Common transition metals such as iron, manganese and copper also deposit in hair from the water supply. Copper from swimming pools has been reported to turn blond hair green at low concentrations. Other sources of metals in hair are sweat deposits, diet, air pollution, and metabolic irregularities. Metal contamination can also arise from hair products that provide zinc or selenium (anti dandruff products), potassium, sodium, or magnesium (soaps or shampoos), and even lead from lead acetate-containing hair dyes.

Hair Pigments

The principal pigments of human hair are the brown-black melanins (eumelanins) and the less prevalent red pigments, the pheomelanin. These latter pigments at one time were called trichosiderin. The brown-black pigments of hair will be referred to as melanins and the yellow-red pigments will be referred to as pheomelanin. The pigments in scalp hair reside within the cortex and medulla as ovoid or spherical granules.

The pigment granules generally range in size from about 0.4-1.0 μm along their major axis; see Fig. 2.2.4.1.

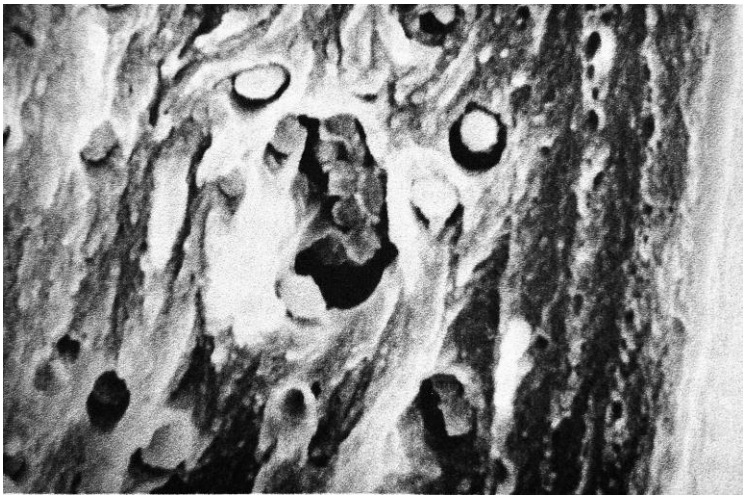


Fig.2.2.4.1 Hair fibers exposed to ultraviolet radiation and then fractured exposing melanin granules

Hair pigments are produced by the melanocytes (melanin producing cells) and are packed into the melanosomes which are pigments containing granules. Melanins are synthesized in melanocytes (melanin producing cells) in a structure called melanosomes; eumelanin from the amino acid tyrosine and/or phenylalanine and pheomelanin from tyrosine and cysteine. Melanin is deposited in melanosomes on a protein matrix inside the melanosomes. Melanin containing melanosomes ultimately become melanin granules after being transferred into keratinocytes. cells that form the shaft of hair fibers.

The intensity or depth of color hair is related to both the size of the melanin granules and the total melanin content or the melanin granule density while the proportion of eumelanin to pheomelanin is believed to be involved in determining the shade of hair color. Graying occurs when the melanocytes become less active during anagen.

We know that highly pigmented hair is both geographically/racially related (georacially) suggesting genetic involvement. For examples those of African and Asian origin tend to have larger amounts of eumelanin in their hair while those of Caucasian extraction

especially originating from Northern Europe tend to have less pigment such as eumelanin and more pheomelanin. [20][21]

2.3 Physical and Mechanical Properties of the Hair Fibers

The main physical properties of the hair depend mostly on its geometry; the physical and mechanical properties of hair involve characteristics to improve: elasticity, smoothness, volume, shine, and softness due to both the significant adherence of the cuticle scales and the movement control, as well as the easiness of combing, since they reduce the bers static electricity.

The evaluation of these effects on hair may be carried out by several methods, as: optical and electron microscopy, mechanical resistance measuring, shine evaluation and optical coherence tomography (OCT).

2.3.1 Hair Physical Properties

Physical properties of hair depend mostly on its geometry. Caucasian hair is oval; Asian hair is circular; Afro hair is elliptic. Several mechanical properties are directly related to bers diameter. The physical properties of hair involve: resistance to stretching, elasticity and hydrophilic power.

2.3.1.1 Resistance to Stretching

In general, the weight needed to produce a natural hair thread rupture is 50-100g. An average head has about 120,000 threads of hair and would support about 12 tons. The resistance to breakage is a function of the diameter of the thread, of the cortex condition, and it is negatively affected by chemical treatments. When a certain load is applied on a hair and its elongation is measured we obtain the graphic representation of its several characteristic regions:

- Hookean’s region or pre-recovering: during the stretching between 0 and 2% the elongation is proportional to the load applied.
- Recovering region: between 25-30% of stretching, the elongation considerably increases without a relationship with the load applied.

- After-recovering region: from 30% stretching load and ber extension are proportional again.

We may consider that in the first and third zones hair acts as a crystal solid; in the second, as an amorphous solid or a fluid since the fiber presents a plastic-type response. The changes undergone by hair during the stretching may be explained by the protein conversion and the possible conversion of α-keratin with an organized and compact helicoid disposition to β-keratin with loose peptide chains. The starting stage is known as Hookean's region. The hair structures consist of stable α-keratin chains with hydrogen bonds and the hair thread seems a crystal solid. The slope of the curb over the elastic region depends on the α-keratin cohesion, and all the factors affecting cohesion will decrease this value. Polypeptides are products of the amino acids chain and they are 18 in the human hair. In steady state, not stretched, polypeptide chains present a helicoid structure which generates several bonds. The cystine-S-S bond is the strongest one and it occurs each 4 cycles of the spiral.

Hydrogen bonds with C=O and H-N groups occur in almost every cycle. Therefore, in the Hookean's region (the elastic region) interference occurs coming from the α-keratin form as stretching resistance, by hydrogen bonds which stabilize the helicoid structure. In the non-Hookean region the transition of α-keratin to β-keratin occurs, when a peptide chain displacement appears without a high degree of resistance. The Third stage, the post non-Hookean region is related with the resistance of the β-keratin configuration to stretching,until reaching the rupture point.

The analysis of the load-elongation curve of threads helps to recognize hair behavior, when several procedures are applied for styling hair. The application of products with reducing character, a process of straightening or waving of reducing antioxidant type or a process of hair discoloration, change the shape of the load-elongation curve. The first and second zones become more extensive; on the other hand, the value of the needed load for starting the transition in first and second regions of the curb is decreased.

2.3.1.2 Hair Elasticity

Hair ber has an elastic characteristic, and it may undergo moderate stretching either wet or dry. Stretching is a hair attribute under the action of a distal force (length) and the thread returns to the original status, when this force stops acting. When dry, the hair thread may stretch 20-30% of its length; and, in contact with water, this may reach up to 50%. In contact with ammonia it becomes more elastic. Chemical and physical treatments, sun exposition and use of electric dryers and heated plates affect this propriety.

2.3.1.3 Hydrophilic Power

Hair absorbs water under both liquid and steam form. Keratin may absorb up to 40% of its own weight in water. Hydration is favored by temperature increase, by changing pH and by all the polar solvents which break hydrogen bonds. Hydration changes the fiber elasticity.

Keratin has a special affinity for water. This absorption depends on the air relative humidity rate and greatly interferes on all the properties of the hair, such as: stretching ability, diameter and internal viscosity of the fibers. Hair tends also to be pervious to water in its liquid form. This absorption is followed by a swelling in the hair, with 15-10% increase in its thread diameter and 0.5-1.0% in its length. Both absorption and swelling essentially depend on the mean pH. Generally, swelling is favored by alkaline pH. Other polar solvents as urea solutions, acetamide and lithium bromide have a similar effect on hair threads.

Normally, the hair resistance to swelling is due to the existence of bonds maintaining the reticular integrity, which avoids the molecules penetration in a volume which is superior to the existing one between protein chains.

2.3.2. Hair Mechanical Properties

Cortex keratin is responsible for this propriety and its long chains are compressed to form a regular structure which, besides being strong, is flexible.

2.3.2.1 Friction

The cuticle surface has high friction coefficient due to its scale shape and it depends on the cuticle geometry and on the physical-chemical status of the hair. The continuous attrition of a thread over another one damages the cuticle. From the roots to the extremities the friction coefficient differs in the dry and wet hair thread, and it is enough combing to damage the hair. Several factors influence the friction, such as:

- relative humidity: friction is higher in wet than in dry hair.
- discoloration of the hair: discoloration increases the friction among threads.
- permanent waving and straightening: due to the chemical composition and high pH of ingredients the friction is increased.
- shampooing: the more is the detergent powder the higher is the friction. Conditioning cream, rinse cream, bath of cream and related products reduce the friction among hair threads.

2.3.2.2 Static Load

When a comb slides over the hair, surface electric load is generated by both friction and high electrical resistance of the hair, which makes handling difficult. The static load dispersion is a function of fibers conductivity or electrical resistance. Quaternary ammonium salts of long chains increase conductivity on the thread surface and reduce the friction. The load potential depends on some factors:

- status of the hair surface, because the presence of an oily layer coming from the sebum or from a cosmetic product influences the static electricity effect, which is reduced or disappears.
- grade of humidity of the hair – thread electric loads tend to bow easier on wet than on dry hair, due to the lower electric resistance. As a consequence, the hair tends to become more ‘electric’ in a dry environment than in a humid one.

2.3.2.3. Combability

Compatibility may be defined as the subjective perception of the east or difficult way for combing the hair. It is directly related to the forces which are opposite to the action of

combing the hair. This is an important attribute in the evaluation of the hair conditioning. For the consumer, a better compatibility reflects a better hair conditioning.
Other factors related with compatibility involve malleability and mechanical damages which may occur when we normally comb the hair, and they are worsened when we detangle it.

2.3.3 Method to Evaluate Hair Products

The desire for products that improve the look and feel of hair has created a huge industry for hair care. The evaluation of hair products efficacy may involve the use of devices which generally have high sensitivity. These tests are specific and only provide information on one attribute for assay. Normally, equipment is used to obtain the image, which is assessed in a subjective way. Analyses performed on predefined regions of hair locks in order to standardize the method and to have more reliable results.

The advantages of these techniques, when compared with merely subjective evaluations are: there is not the need of a volunteers panel; some evaluations may be rapidly performed; use of specific hair locks; condition of standardized assay; they may be used for complex studies. Some of the most used methods for evaluating hair products are listed below.

2.3.3.1 Scanning Electron Microscopy (SEM)

The scanning electron microscopy (SEM) is very used for analyzing hair threads. This technique allows observations of thick and not transparent samples under an electron beam. It also allows determining the shape of a material, the size of its component particles, and its layout. SEM is very used for big magnification of a given sample, generally to evaluate hair surface morphological conditions.

2.3.3.2 Atomic Force Microscopy (AFM)

This equipment allows observing sample images through the microscope, in environment conditions or even when we have a solution. The image is captured by a probe which has physical contact with the sample, and follows a parallel plan to the

surface while acquiring each point of the topographic component. The probe deflection is then measured by a computer program (software) which generates the image.

2.3.3.3 Mechanical Assays

The hair, when considered as a physical body, is a very resistant fiber. The rupture load of a healthy hair thread ranges from 50 to 100g. The relative value is directly proportional to the thread length. To perform this assay dynamometer is used – this is equipment frequently used for evaluating: hair rupture tension, elasticity, compatibility and detangling. The device exerts a tension on the hairthread and measures the needed force versus elongation. The hair lock is tied with a support, and then two combs pass through it as well as the needed force for this action.

2.3.3.4 Piezoelectric Sensors

This analysis is very close to the sensorial perception. The piezoelectric principle is based on the deformation of a crystal by a mechanical action. When this occurs, a load displacement is induced, so creating a voltage signal. In hair, it is possible to apply this technique to the tactile perceptions of hair properties, as: conditioning, cleanliness and surface roughness. During the evaluation, the sensor is placed on a mechanical arm which touches the hair lock and afterwards it is released. This is repeated several times. Results are expressed as voltage arbitrary values.

2.3.3.5 Glossmeters

Glossmeter is a piece of equipment designed to measure the hair shine. The regularity of the hair surface helps to determine the light reflection. When the light follows a uniform surface, as in a mirror, the incidence angle exactly equals the reflection angle. However the hair is not totally uniform and at some points the light beam is reflected forming different angles (0 to 75°) and this kind of reflectance is known as diffuse reflectance.

2.3.3.6 Subjective Tests

This kind of test aims to have a response through a trained panel or a specialized technician, or a group of trained volunteers' subjective assessment, after their evaluation of a test-product, in a way to mimic the final consumers' opinion. These assays allow non-parametric results and the protocols used in them search after standardizing some procedures in order to extrapolate a small group opinion to the target public. The main tests are: salon test and test under normal use conditions.

2.4 Hair Damage

While hair fibers are remarkably strong and can resist substantial external assault, they are not invulnerable and over time, their natural physical properties of elasticity, resistance, water content, porosity, etc., are negatively impacted. One immediate sign that hair is damaged is the lack of shine. In addition, hair fibers feel rough to the touch and lack elasticity and suppleness. This is due to capillary fibers becoming porous and the loss of constitutive elements such as proteins. The hair can become difficult to style, unruly, entangled and brittle. Most of these processes occur on some type of periodic schedule, whether it be daily (while combing the hair) or monthly (haircut and coloring at a salon). In general, hair fiber damage occurs most readily by mechanical or chemical means or by a combination of both (chemo-mechanical). [39] The damage results from large physical forces or temperatures which degrade and wear the outer cuticle layers. External attacks may come from various sources, including:

- combing (generally with plastic objects, and often multiple times over the same area lead to scratching and wearing of the cuticle layers
- scratching (usually with fingernails around the scalp)
- cutting (affects the areas surrounding the fiber tips)
- blow drying (high temperatures thermally degrade the surface of the hair fibers)

2.4.1 Permanent Wave Treatment

Permanent wave treatments saw many advances in the beginning of the twentieth century, but have not changed much with the invention of the Cold Wave around the turn of that century. Generally speaking, the Cold Wave uses mercaptans (typically thioglycolic acid) to break down disulfide bridges and style the hair without much user interaction (at least in the period soon after the perm application).

The Cold Wave process does not need increased temperatures (so no thermal damage to the hair), but generally consists of a reduction period (whereby molecular reorientation to the cuticle and cortex occurs via a disulfide–mercaptan interchange pathway) followed by rinsing, setting of the hair to the desired style, and finally neutralization to decrease the mercaptan levels and stabilize the style. The chemical damage brought on by the permanent wave can increase dramatically when not performed with care.

2.4.2. Chemical Relaxation

Commonly used as a means of straightening hair (especially in highly curved, tightly curled African hair), this procedure uses an alkaline agent, an oil phase, and a water phase of a high-viscosity emulsion to relax and reform bonds in extremely curly hair. A large part of the ability to sculpt the hair to a desired straightness comes from the breakage of disulfide bonds of the fibers.

2.4.3. Dyeing and Bleaching

Hair coloring and dyeing have become extremely successful hair care procedures, due in part to “over-the-counter” style kits which allow home hair care without professional assistance. The most common dyes are para dyes, which contain para-phenylenediamine (PPD) solutions accompanied by conditioners and antioxidants. Hydrogen peroxide (H_2O_2) is combined with the para dyes to effectively create tinted, insoluble molecules which are contained within the cortex and are not small enough to

pass through the cuticle layers, leaving a desired color to the hair. Due to the levels of hydrogen peroxide, severe chemical damage can ensue in the cuticle and cortex.

Bleaching: Like dyeing, bleaching consists of using hydrogen peroxide to tint the hair. However, bleaching can only lighten the shade of hair color, as the H_2O_2 releases oxygen to bind hair pigments. Bleaching may also be applied to limited areas of the hair (such as in highlights) to create a desired look. The chemical damage brought on by bleaching leads to high porosity and severe wear of the cuticle layer.

3. PRODUCT FOR NATURAL HAIR COLOR

Natural dyes are one of the oldest types of hair dye and were the only type of hair dye available until the mid 1800s. These dyes are, as the name implies, from natural sources. This fact has not been lost on the consumer's growing desire for more natural products. The extracts of plants are generally the source of the dye, and the plant is extracted with boiling water. The most common natural dyes used in these products include henna, coffee, black and chamomile tea, carrot and beet juice, walnut shell, etc. Among these, products containing henna dyes are particularly popular for their simultaneous coloring and conditioning effects. Henna hair colors include blonde, brown and black; however, henna plants only contain Lawson as the major coloring ingredient, which is responsible for imparting orange-red color on hair. Chemicals, metallic salts or other vegetable dyes are sometimes added to henna powder to produce colors other than red, and these mixes are referred to as compound hennas. Interestingly, it is also not uncommon to find PPD on a henna hair dye product ingredient list.

3.1 Natural Hair Dyes - Henna

Henna, lawsonia *inermis*, is a plant, a large or small tree, that grows in hot, dry climates. Henna leaves are harvested, dried, and powdered Fig.3.1.1 Henna's leaves have a red-orange dye molecule, *lawsone, 2-hydroxy-1,4 naphthoquinone* Fig.3.1.2. This dye is found in the leaves of the plant. Henna leaves have 1% to 4% lawsone content, depending on climate and soil conditions. There is evidence from Egypt that henna was regularly used to dye hair five thousand years ago.

Henna produces yellow to reddish shades in protein and is normally applied in acidic media. The nonionized (acid) form of henna is yellow while the ionized form is reddish-orange.

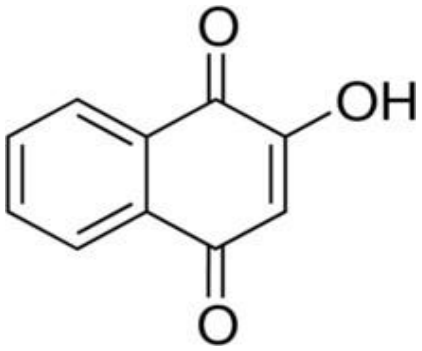
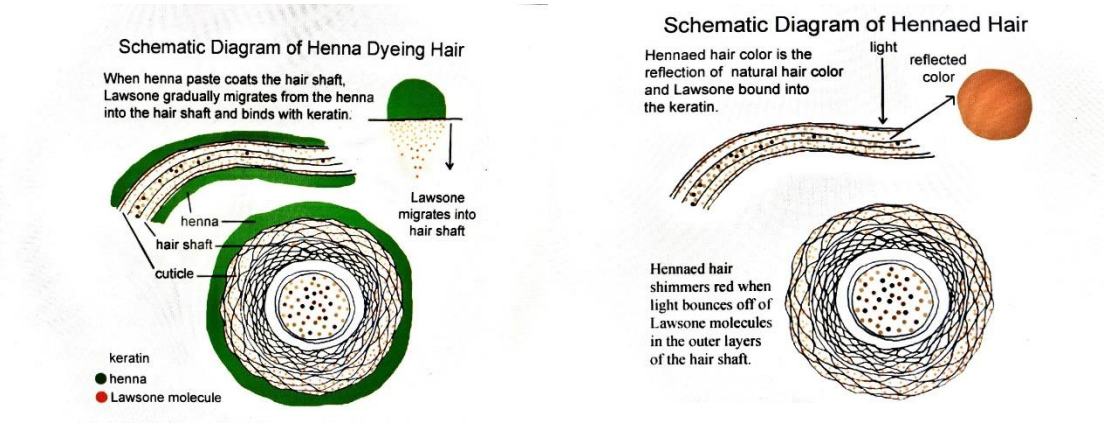


Fig. 3.1.2 *2-hydroxy-1,4 naphthoquinone*

Therefore, acidic pH is optimal to create a higher concentration of protonated sites on the hair to attach to the ionized lawsone in solution. Boxes of commercially produced “henna hair dye” are formulated in a range of colors, “brunette henna,” “strawberry blonde henna,” “black henna,” etc. The range of colors is produced by adding synthetic dyes, metallic salts, and other plant dyes such as Indigo.

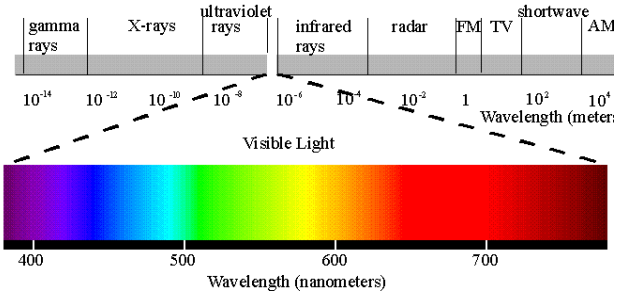
Indigo is a color named after the blue dye derived from the plant *Indigofera tinctoria* and related species. The color is between blue and violet on the electromagnetic spectrum, between 420 and 450 nm in wavelength. If you combine henna and indigo, you will get brunette colors. If there is more henna than indigo, the color will be warm reddish brown. If there is more indigo than henna, the color will be dark brown.



Schema 3.1.1 Diagrams of how henna dyes hair and the appearance of hennaed hair

3.1.1 Fundamentals of Color Theory

Color is the part of perception that is carried to the eye from our surroundings by differences in the wavelengths of light. First, this involves the nature and spectral power distribution in the light from the illuminating light sources. Next, there are several often interrelated processes derived from the interaction of the illumination with matter including absorption, reflection, refraction, diffraction, scattering, and fluorescence. Finally, there is the perception system, involving the eye and the transmission system from eye to brain, leading to the final interpretation reached in the brain.



Lunghezza d'onda, nm	Colore	Colore complementare
400-430		
430-480		
480-490		
490-510		
510-530		
530-570		
570-580		
580-600		
600-680		

Fig. 3.1.1 The electromagnetic spectrum

Fig 3.1.2 The relationship between color and wavelength of absorbed light

Visible light is that part of the electromagnetic spectrum, shown in Fig. 3.1.1, Fig.3.1.2, with wavelengths between the red limit at about 700 nm and the violet limit of 400 nm. Depending on the observer, light intensity, etc, typical values for the spectral colors are red, 650 nm; orange, 600; yellow, 580; green, 550 and 500; and blue, 450.

Hair coloring consists in the transformation of natural color into different shades or intensity of color. The coloring is due to the coloring molecules (also called dyestuff). Dyes are colored substances which are soluble or go into solution during the application process and impart color by selective absorption of light. Dyes are conventionally understood to refer to organic molecules dissolved, as molecular chromophores, in the application medium for examples azo dye, etc. The color imparted by dyestuff to the resulting solution depends on the electronic properties of the chromophore molecule. Dyes are soluble and/or go through an application process which, at least temporarily,

destroys any crystal structure by absorption, solution, and mechanical retention, or by ionic or covalent chemical bonds

Dyestuff colorants tend to have excellent brilliance and color strength, and are typically easy to process, but also have poor durability, poor heat and solvent stability, high migration and have affinity to the substrates to which they are being applied. The coloring molecules have the capacity to: absorb the electromagnetic radiations of specific wavelengths of visible light (400-700nm) falling into the visible field. The color that is observed is the radiation not absorbed, but reflected; the molecules have one chromophore group, carrier of the color (-NO2, -N = N-, -C = C-, -C = O), have a conjugate system, with alternating double and single bonds, exhibit resonance of electrons, which is a stabilizing forces in organic compounds.

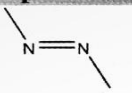
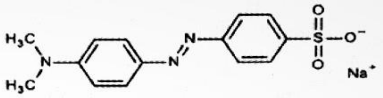
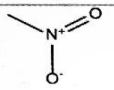
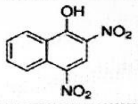
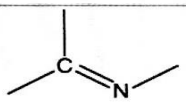
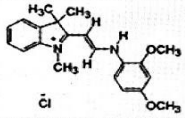
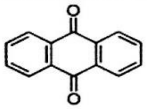
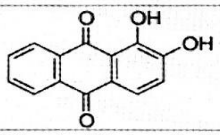
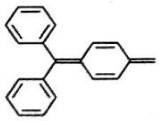
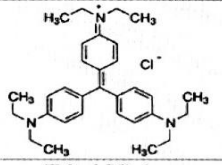
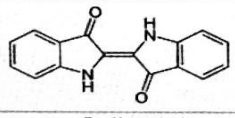
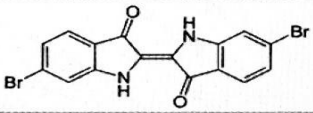
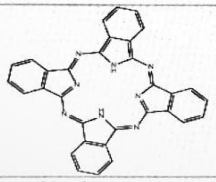
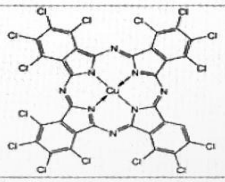
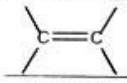
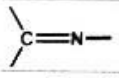
Chromophoric Groups	Sample Dye
	
Azo	Methyl orange
	
Nitro	Martius Yellow
	
Methine	Basic Yellow 11
	
Anthraquinone	Alizarin (Turkey Red)
	
Triarylmethane	Ethyl Violet
	
Indigo	Tyrian purple
	
Phtalocyanine	Pigment green 7

Table 3.1.1 Shows some organic dyes and their chromophoric groups.

The absorption of electromagnetic radiations in the UV and visible regions by a molecule causes the electronic excitation and an electron moves to higher electronic energy level from a lower. A covalently unsaturated group responsible for absorption in the UV or visible region is known as a chromophore. For example, C=C, C≡C, C=O, C≡N, N=N, NO2 etc. If a compound absorbs light in the visible region (400–800 nm), only then it appears colored. Thus, a chromophore may or may not impart color to a compound depending on whether the chromophore absorbs radiation in the visible or UV region.

Chromophores like C=C or C≡C having π electrons undergo π → π* transitions and those having both π and non-bonding electrons, e.g., C=O, C≡N or N=N, undergo π → π*, n → π* and n → σ* transitions. Characteristics of some common unconjugated chromophores are given in Table 3.1.2.

Table 3.1.2 Characteristic of some common unconjugated chromophores

Chromophore	Example	λ_{max}	ε_{max}	Transition	Solvent
	Ethylene	171	15,530	$\pi \rightarrow \pi^*$	Vapor
—C≡C—	Acetylene	150 173	~10,000 6000	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$	Hexane Vapor
	Acetaldehyde	160 180 290	20,000 10,000 17	$n \rightarrow \sigma^*$ $\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	Vapor Vapor Hexane
	Acetone	166 188 279	16,000 900 15	$n \rightarrow \sigma^*$ $\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	Vapor Hexane Hexane
—COOH	Acetic acid	204	60	$n \rightarrow \pi^*$	Water
—$CONH_2$	Acetamide	178 220	9500 63	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	Hexane Water
—COOR	Ethyl acetate	211	57	$n \rightarrow \pi^*$	Ethanol
—NO_2	Nitromethane	201 274	5000 17	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	Methanol Methanol
	Acetoxime	190	5000	$n \rightarrow \pi^*$	Water
—C≡N	Acetonitrile	167	Weak	$\pi \rightarrow \pi^*$	Vapor
—N=N—	Azomethane	338	4	$n \rightarrow \pi^*$	Ethanol

A covalently saturated group which, when attached to a chromophore, changes both the wavelength and the intensity of the absorption maximum is known as auxochrome, e.g., NH2, OH, SH, halogens etc. Auxochromes generally increase the value of λmax as well as εmax by extending the conjugation through resonance. These are also called colour enhancing groups. An auxochrome itself does not show absorption above 200 nm. Actually, the combination of chromophore and auxochrome behaves as a new chromophore having different values of λmax and εmax. For example, benzene shows λmax 256 nm, εmax 200, whereas phenol shows λmax 270 nm, εmax 1450 (both increased). Hence, OH group is an auxochrome which extends the conjugation involving the lone pair of electrons on the oxygen atom resulting in the increased values of λmax and as εmax. When two or more chromophoric groups are conjugated, the absorption maximum is shifted to a longer wavelength (lower energy) and usually to a greater intensity compared to the simple unconjugated chromophore.

In general, the longer the conjugated system, the higher are the values of λmax and εmax. Thus, a compound with sufficient conjugation absorbs in the visible region (400–800 nm) and becomes colored. For example β-carotene, an orange pigment present in carrots, has eleven carbon-carbon double bonds and absorbs in the visible region (λmax 450 nm, εmax 14 × 104) and is colored.

According to this theory, a dye consists of three components: one or more the fused benzene rings attached to the unsaturated groups called as chromophores (e.g., –N=N–, –NO2, –C=O) and basic groups called as auxochromes (e.g., NH2, OH groups). Both of them are responsible for the color.

They realized that a chromophore is usually an electron-withdrawing group, while an auxochrome is commonly an electron-donating group and the two are linked through a conjugated system. This particular approach can be considered as the starting point of the donor–acceptor chromogen concept. In simple terms, it can be considered that the organic dye molecules contain three main components such as chromogen, chromophore and auxochrome.

- The chromogen is a chemical compound that is either colored or could be made colored by the attachment of suitable substituent. The chromophore and the auxochrome(s) are also part of the chromogen.
- The chromophore is a chemical group that is responsible for the appearance of color in compounds (the chromogen) where it is located. The colorants are sometimes also classified according to their main chromophore (e.g., azo dyes contain the chromophore –N=N–).
- The auxochrome is a substituent group found in a chromogen that influences its color. Whereas, the chromophore or chromophoric group is responsible for chromogen which will be colored. The chromophore itself is not capable of determining a particular color and hue. Also, Gurr differentiates two types of auxochrome namely colligators, which are responsible for dye–substrate interactions and which are either ionic (e.g., acidic: –SO_3^-, –COOH, etc., or basic: –N^+, –NH_2) or non-ionic and non-colligators which modify color.

Unlike most organic compounds, colorants possess color because they:

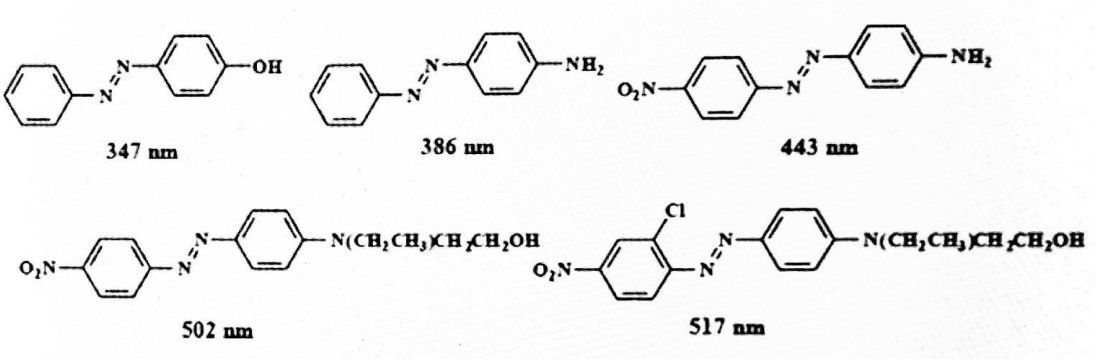
- absorb light in the visible spectrum (400–700 nm),
- have at least one chromophore (color-bearing group),
- have a conjugated system, i.e. a structure with alternating double and single bonds
- exhibit resonance of electrons, which is a stabilizing force in organic compounds.

When any one of these features is lacking from the molecular structure the color is lost. In addition to chromophores, most dyes also contain groups known as auxochromes (color helpers), examples of which are carboxylic acids, sulfonic acid, amino, and hydroxyl groups. While these are not responsible for color, their presence can shift the color of a colorant and they are most often used to influence dye solubility.

Figure 3.1.2 shows the relationships between wavelength of visible and color absorbed/observed. The wavelength spectrum of absorbed light, which determines the

color of the matter, is affected by its chemical structure consisting of components such as the chromophores and auxochromes.

Schema 3.1.1 Effects of substitute groups within an azo-dye system



This is illustrated in Schema 3.1.1 where the following effects of substituents are shown:

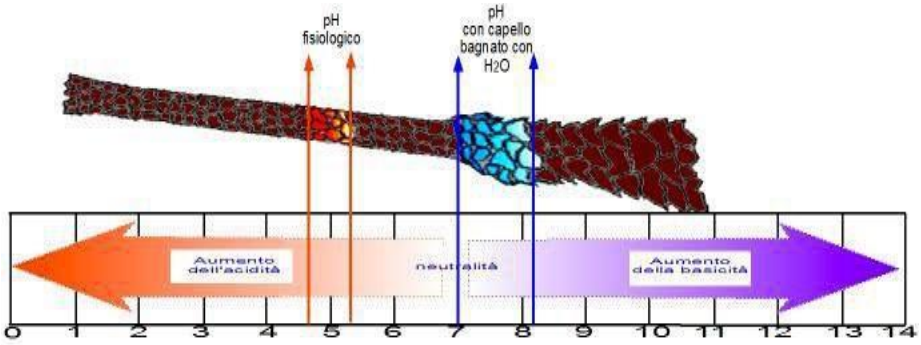
- Adding groups of increasing electron-donating ability to the azobenzene structure has a bathochromic effect (cf. OH vs. NH2).
- Electron-donating (NH2) and electron-accepting (NO2) groups placed in conjugation provide a bathochromic effect. In this regard, nitro groups are especially beneficial, contributing to their prevalence in disperse dye structures.
- Increasing the number of electron-attracting groups conjugated with the electron-donor has a bathochromic effect.
- The electron-donating effects of an amino group are enhanced by adding alkyl groups to the N-atom.

3.1.2 Mechanism of Hair Dyeing

The chromophore groups present in the plants are bound to the functional groups of keratin (carboxyl group, amine group) forming a new conjugated chromophore that settles on the scales of the cuticle and develops the color. New chromophore group works depending on:

- developing time (we have major deposits on the surface of the hair fibers)

- type of hair (color varied depending to the color of the base hair used: for examples yak, bleach, salt and pepper hair)
- mixing plants (major chromophore groups available, major color on the hair)
- pH (changes the conformation of the hair, the acidic pH keeps the cuticle closed, the neutral pH the cuticle with water are swollen, the basic pH keeps the cuticle open) see Schema 3.2.1.1



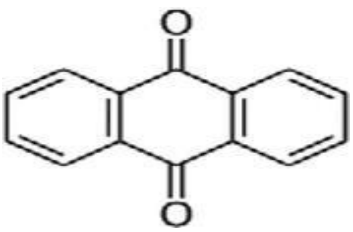
Schema 3.2.1.1 The role of pH value in the hair fiber

3.1.3 Chemistry of Natural Dyes

The pigments contained in the plants belong to various families:

- Carotenoids (beta carotene, vitamin A, lutein)
- Flavonoids (rutin, quercetin, isoquercitrin)
- Anthraquinones (emodin, physcion, chrysophanol)
- Anthocyanins (cyanidin, chrysanthemum, sambucine)
- Tannins (proanthocyanidins, propelargonidins)
- Alkaloids (berberine, palmatine)
- Quinones (arbutin, methyl-arbutin)

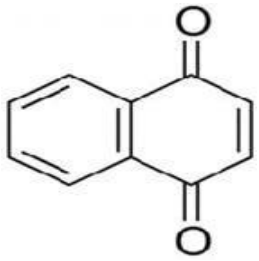
<u>Anthraquinone Class</u>



Dyes that belong to this class are having anthraquinone structure and obtained from plants and insects. The red shade is specific to this class. Madder, lac, kermes and

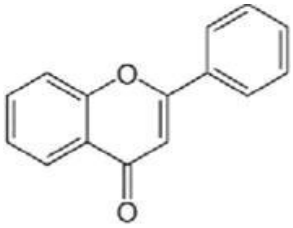
cochineal are some of the examples. The general chemical structure of this class is shown in Figure. Anthraquinone possesses the biggest group of anthraquinone dyes. The extracted dye contains emodin, chrysophanol, aloe emodin and physician. The extracts give the yellow to orange shade.

Alpha Naphthoquinone



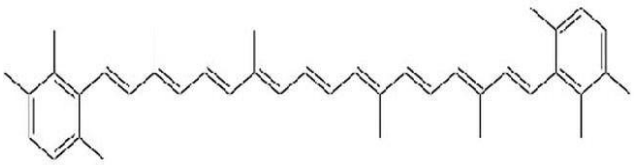
The dyes are having alpha naphthoquinone structures such as 2-hydroxy 1-4-naphthoquinone. Hina, lawsone and juglone are examples of this class. The chemical structure of this class is shown in Figure.

Flavones



The dyes are having yellow shade. The natural dye weld belongs to this category. Most of the dyes are derivatives of hydroxyl and methoxy substituted flavones or isoflavones. The chemical structure of this class of dye is shown in Figure.

Carotenoids

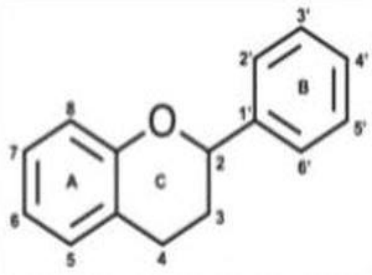


The natural dyes saffron and annatto belong to this class. The dye structure of this class has long-chain conjugated double bonds. The chemical structure of this class is as shown in Figure. Carotenoids are red, yellow and orange pigments present in plants and animals. It has a polyisoprenoid structure with a series of centrally located conjugated bonds. The bright colours of many fruits and vegetables are due to carotenoids. Carotenoids are polyisoprenoid structures which contain conjugated double bonds, which acts as chromophore and responsible for characteristic absorption spectra. Carotenoids are divided into two parts: Hydrocarbon carotenoid and Oxygen containing xanthophylls. Structural changes by hydrogenation, double bond migration,

isomerization and chain lengthening and shortening resulted in many carotenoid structures. Carotenoids possess strong UV light resistance, and β carotene is a typical structure generally found in natural colourants

Pyron Dyes

Pyron dyes contain flavonoids and anthocyanins having structure as shown in Figures. The pyron structure is bound to various sugars by glycosidic bonds. Flavonoids are classified as flavonols, flavones, anthocyanidins, isoflavones, flavon-3,4-diols and coumarins. Yellow flavones and flavonols are used as vegetable dyes. The valuable and very popular flavonoid is yellow quercetin which possesses several bio effects.



Anthocyanins

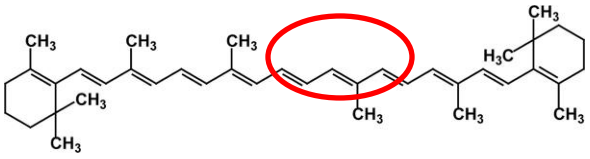
Anthocyanins are found in fruits and vegetables; some are grape wine, sweet and sour cherries, red cabbage, hibiscus and different varieties of oranges. There are more than 500 varieties of anthocyanins that produce red, pink, violet and orange colours. There are some important anthocyanins which are cyaniding, delphinidin, pelargonidin, malvidin, peonidin and petunidin. Many plants besides anthocyanins also contain quercetin and chlorophylls, and the resulting colour is a mixture of all these.

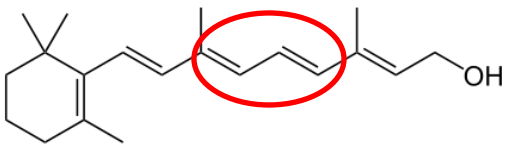
Tannins

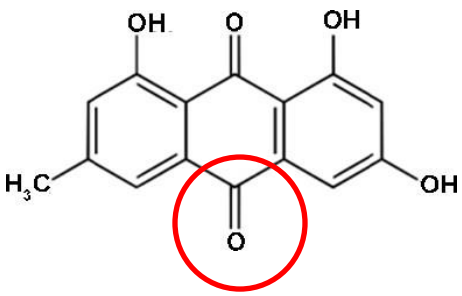
Tannins are polymeric polyphenols with typical aromatic ring structure with hydroxyl constituents and have relatively high molecular weight. In plants two different groups of tannins are found, (a) hydrolysable tannins and (b) proanthocyanidins (condensed tannin). Tannins are present in plant cells and are concentrated in epidermal tissues. Tannins are found in wood, leaves, buds, stems, florals and roots. The hydrolysable tannins are concentrated in the roots of several plants. The plants are the source of

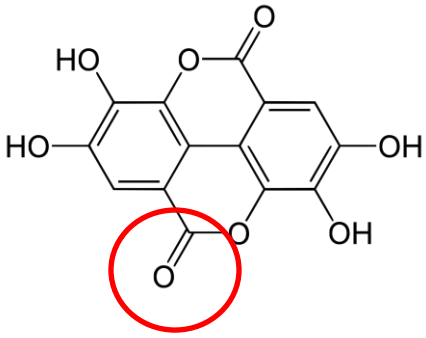
different varieties of tannins. The three major tannins (hydrolysable tannins) are grouped as gallotannins or ellagitannins and which are gallic acid or ellagic acids. The most widespread gallotannins are pentagalloyl glucose. Ellagitannins are esters of hexahydroxy diphenic acids. Gallic acid and hexahydroxydiphenic acid occur together in some hydrolysable tannins. Condensed tannins are polymers of 15-carbon polyhydroxy flavan-3-ol monomer units such as (−) epicatechin or (+) catechin. The complex chemical nature of tannins makes the biosynthesis and polymerisation a difficult task; however, there are some established pathways for biosynthesis. The precursor for biosynthesis of hydrolysable tannins is shikimic acid. The direct aromatization of 3-dehydroshikimic acid produces gallic acid, which upon esterification forms polyol. The biosynthesis of condensed tannins occurs through two different ways (a) by phenylpropanoid and (b) by polyketide. The polyketide pathway takes malonyl moieties for aromatic ring formation in flavonoid biosynthesis. The phenylpropanoid pathway takes aromatic amino acid, L-phenylalanine, which is non-oxidatively deaminated to E-cinnamate by phenylalanine ammonia-lyase.

Following are some formulas of pigments found in plants. The chromophore group responsible for the color that interacts with the functional groups present in keratin has been highlighted in red.






OH
O
OH
H3C
OH
O


O
HO
O
HO
OH
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OH
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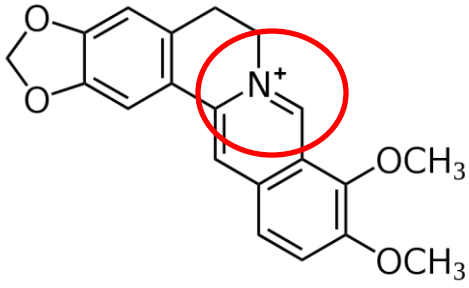
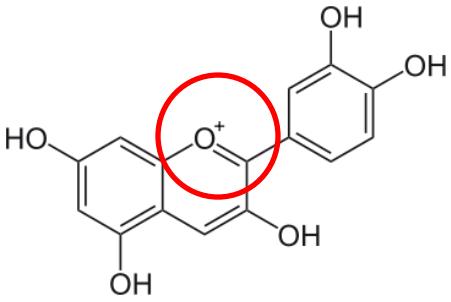
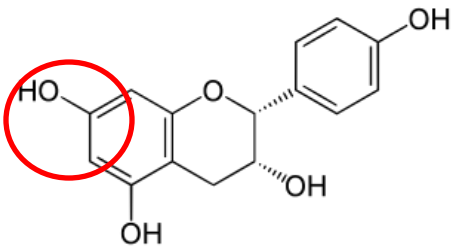
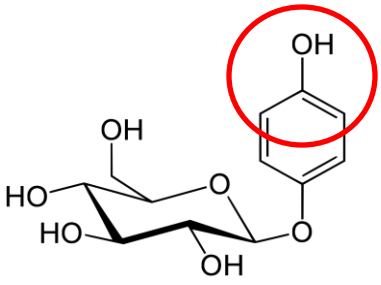

O
O
N+
OCH3
OCH3


OH
OH
HO
O+
OH
OH


OH
HO
O
OH
OH


OH
OH
HO
O
HO
O
OH

3.2 Aim of Project

The most common products for hair color are formulated with synthetic dyes, which allow to obtain good results, but such products can damage the structure of hair. It becomes more porous and in many cases can cause allergic reaction, such as itching, redness, desquamation, etc. Recently, interest in the use of natural dyes has been growing rapidly due to toxic, pollution creating and allergic reactions associated with synthetic dyes. Herbal dyes are biodegradable, non-toxic, soft and without any side effects. Natural dyes, also, are easily available with low cost. They can be used in food, medicine, perfume, leather and textile industries.

Keeping in mind these factors we did a study on plants that color hair. Firstly, to evaluate the coverage of plant extracts on hairs and the final colouring we used different types of hair: yak that used as a reference standard for white hair, salt and pepper that used as a standard for dark hair and bleached hair that used as a standard for bleached hair. The extracts powder have been diluted in water in a different ratio in order to obtain a semi-liquid mixture. All individually tested plants are characterized by an acidic pH of 2.0-3.5. But we decided to add an alkaline agent, monoethanolamine, which allows you to open the scales cuticle and promote the penetration of the active ingredient on the hair, to increase the intensity of the color. Furthermore, some plants were tested in synergy with other plants or with various miscellaneous plants.

At the beginning I would like to give you a short introduction to our research on the plant, then we focus on the different tests performed.

3.3 Material and Method

During our research we found different plants with health benefits derived from various parts of the tree (flower, bark, seeds, leaves and roots). These vegetable sources in powder are not only replaceable but also biodegradable. Here is a brief description of some of the more interesting plants:

3.3.1 **Material**

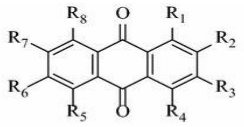
Rhubarb	Sambucus
Hibiscus	Ratanhia
Ginger	Barberry
Red Poppy	Uva Ursi
Juniper Berry	Bilberry
Maca	Hedge Mustard
Cypress	Plantago Afra
Potentilla Erecta	Paprika
Cynodon Dactylon	Spinach
Cacao	Rosa Canina

3.3.1.1 Rhubarb

Rheum palmatum L. and Rheum rhaponticum L. (Polygonaceae)

Rhubarb is one of the most ancient and important herbs with thick roots, hollow and erect stems and small white-green or purple-red flowers clustered on the branches. Rhubarb includes approximately 60 species of plants of the genus *Rheum L*. from the Polygonaceae family. The main chemical compositions of these rhubarb include anthraquinones and their glycosides, [3] anthrones, stilbenes, butyrophenones and chromones, tannins, polysaccharides etc.

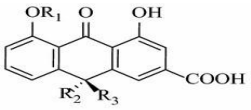
Anthraquinones: The proportion of anthraquinones ranges from 3 to 5% in different species. More than 30 anthraquinones have been isolated and identified from rhubarb. They are divided into free type and combination type. Free anthraquinones mainly contain *rhein, emodin, aloe-emodin, chrysophanol, physcion, iso emodin, chrysaron, laccaic acid D*. Combination anthraquinones are the glycosides combined by free anthraquinones and glycosyl. There are many kinds of anthraquinone glycosides, containing *aloe-emodin-8-glucoside, emodin-8-glucoside, rhein-8-glucoside, physcion diglucoside, emodin-6-glucoside* etc. Main structures of anthraquinones (1–11) are as follows (Fig. 3.3.1.1).[4]



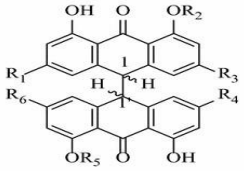
		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1	Rhein	H	COOH	H	OH	OH	H	H	H
2	Emodin	H	CH_3	H	OH	OH	H	OH	H
3	Chrysophanol	H	CH_3	H	OH	OH	H	H	H
4	Aloe emodin	H	CH_2OH	H	OH	OH	H	H	H
5	Physcion	H	CH_3	H	OH	OH	H	OCH_3	H
6	Isoemodin	H	CH_3	OH	H	OH	H	H	OH
7	Laccaic acid D	H	OH	COOH	CH_3	OH	H	OH	OH
8	Aloe-emodin-8-oglucoside	OH	H	H	H	H	CH_2OH	H	OGlu
9	Chrysophanol-8-glucoside	OH	H	CH_3	H	H	H	H	OGlu
10	Physcion-8-glucoside	OH	H	CH_3	H	H	CH_3	H	OGlu
11	Physcion diglucoside	OH	H	CH_3	H	H	CH_3	H	$OGlu^{6}\rightarrow{}^{1}Glu$

Fig. 3.3.1.2 Main structure of rhubarb anthraquinones

Anthrones and dianthrone: also characteristic components of rhubarb, are related to purgative activity. Mainly these include *rheinosides A–D, palmidin A, B, C, rheidin A, B, C, and sennosides A–F*, etc. 26 anthrones have been isolated from the species of this genus. Sennosides have strong cathartic effects though translating to anthraquinones in vivo. The main structures of anthrones and dianthrone (12–25) are as follows (Fig. 3.3.1.2).

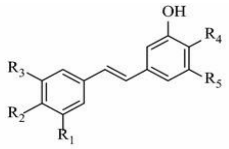


		R_1	R_2	R_3
12	Rheinoside A	Glu	Glu	OH
13	Rheinoside B	Glu	OH	Glu
14	Rheinoside C	Glu	Glu	H
15	Rheinoside D	Glu	H	Glu

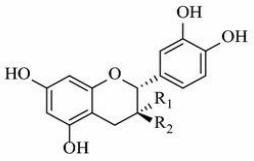


		R_1	R_2	R_3	R_4	R_5	R_6
16	Palmidin A	CH_2OH	H	H	OH	H	CH_3
17	Palmidin B	CH_2OH	H	H	CH_3	H	H
18	Palmidin C	CH_3	H	H	OH	H	CH_3
19	Rheidin A	OH	H	CH_3	COOH	H	H
20	Rheidin B	H	H	CH_3	COOH	H	H
21	Rheidin C	H	H	CH_3	COOH	H	OCH_3
22	Sennosidin A	COOH	H	H	COOH	H	H
23	Sennosidin C	COOH	H	H	CH_2OH	H	H
24	Sennoside A	COOH	Glu 1-1'trans	H	COOH	Glu 1-1'trans	H
25	Sennoside C	COOH	Glu 1-1'meso	H	COOH	Glu 1-1'meso	H

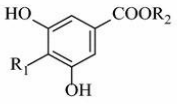
Stilbenes: So far, there are 31 compounds found in rhubarb belonging to stilbenes, such as *rhapontigenin, isorhapontigenin and rhaponticin.* Stilbenes are important components of rhubarb,concerning antihyperlipidemic, antioxidant and hepatoprotective effect. Some representative structures of stilbenes (26–28) are shown at Figure 3.3.1.3.



		R_1	R_2	R_3	R_4	R_5
26	Rhaponticin	OH	H	OH	OCH_3	H
27	Piceatannol 3'-O-glucopyranoside	H	OH	OGlu	H	OH
28	Resveratroloside	H	OGlu	H	H	OH

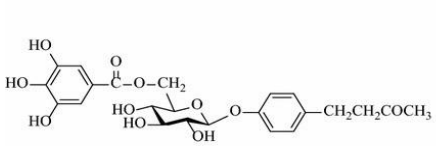


		R_1	R_2
29	(+)-Catechin	H	OH
30	(-)-Epicatechin	OH	H

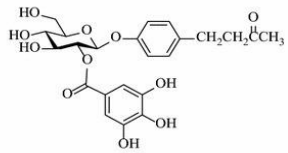


		R_1	R_2
31	Gallic acid	OH	H
32	4-O-methyl gallic acid	OCH_3	H
33	Glucogallin	OH	Glu

Tannins: Tannins in rhubarb generally account for 10–30%. It can be divided into hydrolytic type and condensation type. *Gallic acid* and *d-cate-chin* are the monomers of these tannins. Studies have discovered that tannins are the active elements owing to the stypticity and constipate activity of



Lindleyin
34



Isolindleyin
35

rhubarb. It has been proved that tannins can adjust genotoxicity, oxidative stress, inflammation and apoptosis. The basic structures of tannins (29–33) recorded at Fig. 3.3.1.4.

Butyrophenones and chromones: 6 butyrophenones and 14 chromones have been isolated from rhubarb already. *Lindleyin and Isolindleyin* whose structures are shown at Fig. 3.3.1.4, have been confirmed possessing anti-inflammatory and analgesic activity.

Polysaccharides play multiple roles and have extensive bioactivities in life process, with an immense potential in healthcare, food and cosmetic industries, due to their therapeutic effects and relatively low toxicity. It has been proved that rhubarb polysaccharides have the following pharmacological activity, lowering the blood sugar, protecting liver, promoting the proliferation of intestinal epithelial cells, antineoplastic, anti-senescence and etc. [3][4]

3.3.1.2. Sambucus

Sambucus nigra L. and Sambucus ebulus L. (Caprifoliaceae)

Sambucus is a genus of flowering plants in the family Adoxaceae. The various species are commonly called elder or elderberry. The genus was formerly placed in the honeysuckle family, Caprifoliaceae, but was reclassified as Adoxaceae due to genetic and morphological comparisons to plants in the genus *Adoxa*. The oppositely arranged leaves are pinnate with 5–9 leaflets (or, rarely, 3 or 11). Each leaf is 5–30 cm (2.0–11.8 in) long, and the leaflets have serrated margins. They bear large clusters of small white or cream-colored flowers in late spring; these are followed by clusters of small black, blue-black, or red berries (rarely yellow or white).

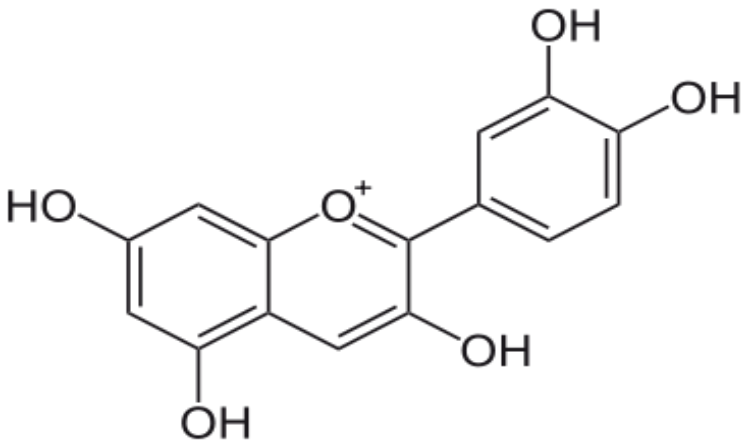
The chemical composition of *Sambucus nigra* is rich and depends on different factors, such as cultivar, location, ripening stage and climatic conditions. **Carbohydrates**: In respect of carbohydrates, elderberry berries contain 7.86–11.50% of total sugar and 2.8–8.55% of reducing sugar. [5] Carbohydrates found in *Sambucus nigra* fruit also include dietary fibre, in particular, *pectin, pectic acid, protopectin, Ca-pectate and cellulose*. **Protein:** Elderberry is a source of whole protein – its content is 2.7–2.9% in berries, 2.5% in flowers and 3.3% in leaves. This protein includes sixteen amino acids, nine of which are essential; the total content of the essential amino acids is approx. 9% in flowers and 11.5% in leaves. *Glutamic acid, aspartic acid and alanine* were reported as the dominant amino acids. **Fats:** Fats are accumulated mostly in elderberry seeds (fat content: 22.4%) and seed flour (fat content: 15.9%). The major fatty acids are polyunsaturated fatty acids, which constitute 75.15% and 21.54% of total fatty acids in seeds and seed flour, respectively, whereas monounsaturated fatty acids (14.21% and 4.21%) and saturated fatty acids (10.64% and 4.81%) make up a significantly smaller share. Polyunsaturated fatty acids that are present in highest concentrations in seeds are *α-linolenic, linoleic and oleic acid*. Organic acids constitute 1.0–1.3% of the berry content. The most abundant of them was citric acid, malic acid, shikimic acid and

fumaric acid. **Minerals**: Minerals are located both in berries and flowers. The mineral matter content represents 0.90–1.55% of the fruit mass, and includes K, Ca, Na, Mg, Fe, Zn, Mn, Cu. **Other**: Elderberry fruit and flowers also include **essential oils** (around 0.01% in fruit), consisting of approx. 53 compounds in berries and 58 compounds in flowers. With regard to **vitamins** present in *S. nigra*, several studies mention ascorbic acid, but the quantitative findings are pretty inconsistent. Furthermore, elderberry seed flour is a source of α-Tocopherol (0.49 µg/g of oil), which has the highest vitamin E bioactivity, as well as γ-Tocopherol (2.63 µg/g), which shows better antioxidant potential.

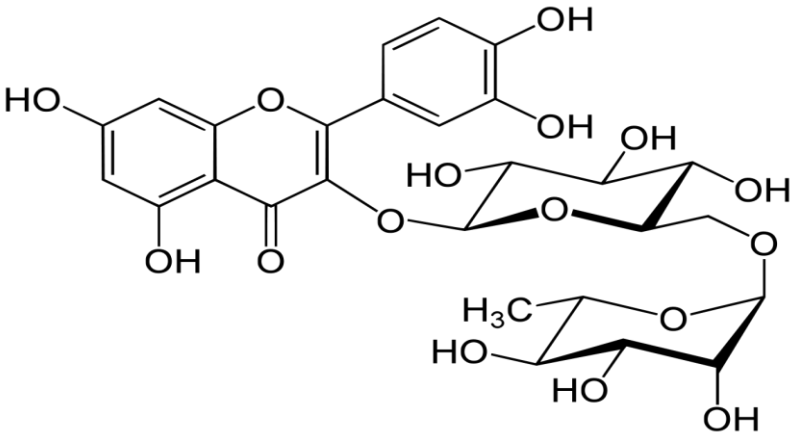
The bioactive compounds found in elderberries are primarily polyphenols and anthocyanins. [6] **Polyphenols**: The fruit of *Sambucus nigra* is an important source of phenolic compounds – their content in the elderberries is relatively high in comparison to other fruits. The main polyphenols in elderberry fruit are *chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, quercetin, quercetin-3-rutinoside (rutin), quercetin-3-glucoside (isoquercitrin), kaempferol-3-rutinoside, kaempferol-3-glucoside (astragalin), isorhamnetin-3-rutinoside and isorhamnetin-3-glucoside.* The primary flavonol in this plant is rutin, while the other flavonols, *isoquercitrin* and *astragalin* occur in elderberries in smaller amounts. The concentration of quercetin measured in the fruit of thirteen elderberry cultivars ranged from 29 to 60 mg/100 g of fruit. Furthermore, *Sambucus nigra* berries contain small amounts of tannins with a low condensation degree. These are procyanidins, such as *epicatechin (*88.4% of total tannins) and *catechin* (11.6% of total tannins) and their thiol derivatives. **Anthocyanins**: They are well-known functional compounds used as food colorants, and they reduce the oxidative stress by scavenging free radicals, which makes them potential chemopreventive agents. The fruit of *Sambucus nigra* contains anthocyanins, especially *cyanidin-3-glucoside* and *cyanidin-3-sambubioside*. Two other (minor) anthocyanins are *cyanidin-3,5-diglucoside*, and *cyanidin-3-sambubioside-5-glucoside*. In addition, trace quantities of cyanidin-3-rutinoside, pelargonidin-3-glucoside and delphinidin-3-rutinoside were identified in the fruit of certain elderberry cultivars.

Thanks to these compounds, elderberry is characterized by high antioxidant activity, which significantly affects its health-promoting properties. It was shown that elderberry

has mainly antibacterial and antiviral properties, can reduce sugar and lipid concentration, and even exhibit antidepressant and antitumor properties. Most studies have focused on the antiviral properties of elderberry fruit, so there is a high need for further research into the other properties of this valuable plant. Considering the growing fashion for natural, organic and health-promoting food, it can be concluded that elderberry as a natural component of food products fits perfectly into this trend and has a good chance to increase its role as a beneficial component of a cosmetic science. [6]



Cyanidin



Rutin

3.3.1.3. Hibiscus

Hibiscus sabdariffa L.

(Malvaceae)

Roselle, also known as jamaica (in Spanish), red sorrel (in English), or karkade (in Arabic), is a perennial plant of the genus Hibiscus (belonging to the Malvaceae family). It is native to India and Malaysia but because it can grow in marginal soils of low fertility and with low moisture retention, its cultivation has expanded to various tropical and subtropical regions including China, Thailand (these two countries, major global suppliers), Indonesia, Saudi Arabia, Vietnam, Sudan, Egypt, Nigeria, and Mexico.

In general, there are two varieties of jamaica, the first Hibiscus var. Altissima Wester, cultivated for having a jute-like fiber; and the second, Hibiscus var. sabdariffa, which presents short and bushy shrubs that have been described in four races: bhagalpuriensi, intermedius, albus and rubber. The most frequently cultivated of them is Hibiscus var. sabdariffa (Hs) ruber. It is characterized by having a herbaceous shrub, with smooth, cylindrical, and typically red stems. Its leaves are green with lengths that vary between 7.5 and 12.0 cm. Its flowers are up to 5 inches (12.5 cm) wide, yellow and may turn pink when they wilt. Its calyx, stems, and leaves are acidic and have a blueberry-like taste (Vaccinium spp.) This characteristic red color is attributed to the content of anthocyanins while its acidic taste is due to the content of organic acids such as citric, malic, tartaric acid, and hibiscus.[16]

The main phytochemicals found in Hs flowers are anthocyanins, flavonoids, organic acids (mostly citric acid, hibiscus acid, and malic acid), glycosides, and fiber.

Phenolic acids and Flavonoids: Roselle calyxes are an abundant and interesting source of bioactive molecules such as polyphenols and flavonoids, which have shown antioxidant, hypocholesterolemic, antihypertensive, antimicrobial, anti-inflammatory, anti-diabetic, and anti-cancer potential. [18] In general, Hs contains flavonol and flavanol

polyphenols in simple or polymerized form. In some studies, the following flavonoids have been detected: hibiscitrin (hibiscetin-3-glucoside), sabdaritrine, gossypetin, gossytrin and other glycosides of gossypetin, quercetin and luteolin; as well as chlorogenic acid, protocatechuic acid, pelargonic acid, eugenol, and sterols (mainly beta-sitosterol and ergosterol).[17][18]

Anthocyanins: The first anthocyanin of the Hs calyx that was isolated was “herbycin” (also called delphinidin-3-sambubioside, cyanidin-3-glucoside and/or delphinidin pentoside-glycoside).

Organic Acids: The studies indicate that Hs contain a high percentage of organic acids, including citric acid, hydroxycitric acid, hibiscus acid, malic acid, and tartaric acid as the main compounds Fig.X. While in a smaller proportion oxalic and ascorbic acids have been found, in general, the percentage of these organic acids is similar in the species of Hibiscus sabdariffa L., although the hibiscus acid is the most representative (13–24%). The rest of the organic acids are: (a) 12-20% citric acid, (b) 2–9% malic acid,
(c) 8% tartaric acid and (d) ascorbic acid between 0.02 and 0.05%, see Fig.3.3.1.5.

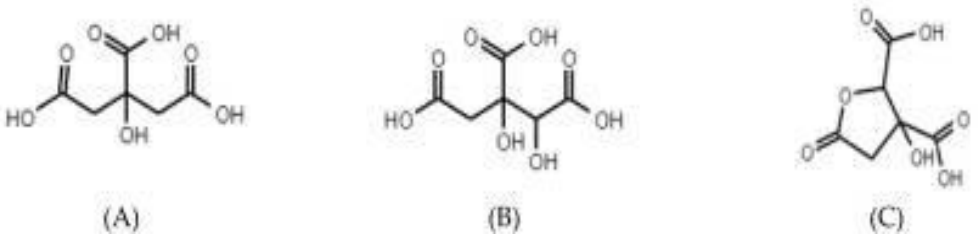


Fig. 3.3.1.5 Chemical structure of the main organic acids of Hs. (**A**) Citric acid, (**B**) Hydroxycitric acid, and (**C**) Hibiscus acid

Citric Acid: Citric acid ((CA), chemically known as 2-hydroxy-1,2,3-propanetricarboxylic acid)) was first isolated by the British Karls Scheels (1874), from lemon juice imported from Italy. Some benefits: Improves the bioavailability of minerals, anti-inflammatory and antioxidant effects, decrease lipid peroxidation and inflammation by reducing cell degranulation and attenuating the release of inflammatory compounds such as myeloperoxidase, elastase, interleukin, and platelet factor 4 etc.

Hydroxycitric acid: (HCA) is a derivative of citric acid found in a variety of tropical plants, including Garcinia cambogia and Hibiscus sabdariffa L. (Hs). Together with hibiscus acid (HA) they are the main organic acids extracted from the calyxes of Hs. However, Garcinia cambogia plants are considered to have the highest amount of HCA. It is important to note that there are four isomers ((+) and (−)-HCA), and ((+) and (−)-hydroxycitric acid), the isomer (−)-HCA being found in plants.

Hibiscus Acid: the empirical formula is C6H6O7•H2O. Through crystallographic analysis and X-ray spectroscopy confirmed that HA is a five-membered lactone ring (similar to HCA), with four carbon atoms and one oxygen atom C3 (sp2) has a double-bonded oxygen atom, C1 an OH group and a COOH group, and C2 a COOH group, respectively (Figure 3.3.1.5).

Tartaric acid: (TA) is a well-known organic acid that is found naturally in many fruits, especially grapes. Depending on the property to rotate the plane of polarized light, there are two enantiomers, L (+)-TA and D (−)-TA, the latter being the one that rarely exists in natural sources. The levogyre form (L (+)-TA) is widely used in the food and chemical industry and for the production of wine (main acidity corrector); while the dextrogyre form (D (−)-TA) is more important in the manufacture of pharmaceutical products.

Malic acid: (MA) is an alpha-hydroxy acid found in various fruits and vegetables. It is frequently used in the cosmetic industry as an exfoliating agent and for the treatment of damaged or dry skin; as well as to control acne.

In general, the uses have been focused on culinary, medicinal issues, as a source of cosmetics and on botanical and/or floral aspects. In the culinary case, the fresh or dried calyces and the flower pods of Hs are used for the preparation of hot and cold drinks, tea, fermented beverages, wines, jams, jellies, ice cream, chocolates, aromatic agents, and cakes. Drinks prepared with Hs have been traditionally consumed by different cultures. For example, in Egypt, calyces are used to make “cacody tea” and fermented beverages, while in Sudan and Nigeria, they are boiled with sugar to produce a beverage known as Karkade or Zoborodo. In West India, the calyces are commonly used as a colorant and flavoring for rum. The flower, used in Mexican cuisine, is used in the drink known as jamaica water or jamaica tea, as well as in different typical dishes.

There is evidence (in Sudan, Malaysia, China and Africa) that its leaves are ingested raw or cooked, like a vegetable, while the seeds are eaten roasted or ground and used to prepare oils or as a substitute for coffee. Regarding its use as a cosmetic agent, Malaysians often use the oil from their seeds to produce scrubs and soaps.

However, the greatest impact of Hs has been in the Traditional Medicine/Complementary and Alternative Medicine (TCAM) where it has shown diuretic, choleretic, analgesic, antitussive, and hypotensive effects. Other effects observed are that it lessens blood viscosity, stimulates intestinal peristalsis and reduces body temperature. Likewise, it has been used to treat nervous diseases, cardiovascular diseases and atherosclerosis, obesity, liver disorders, control arterial hypertension, and genital problems. Certain scientific evidence found in the literature confirms its antioxidant, antidiabetic, antilipidemic, antihypertensive, immunomodulatory, hepatoprotective, diuretic, antimicrobial, antiparasitic, and anti-cancer capacities.

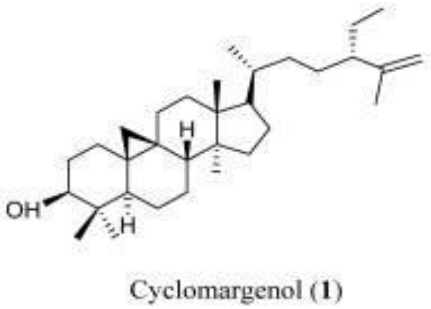
3.3.1.4. Ratanhia

Krameria triandra Ruiz e Pav.

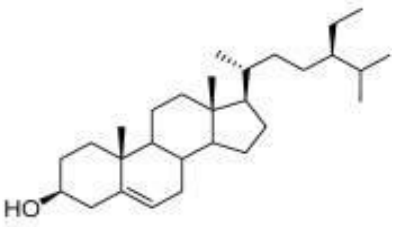
*(*Krameriaceae)

Krameria is the only genus in the Krameriaceae family, of which any of the approximately 18 species are commonly known as rhatany, ratany or rattany. The plant is native to the Bolivian and Peruvian Andes, so called "Ratanhia of Peru". It is a small shrub that grows to knee height, with leafy branches exposed, turned downward, hairy, with leaves covered with silvery hairs and stalked axillary red flowers with 4 sepals and 4 petals. The fruit is globose, ornamented with prickles hooked. [22]

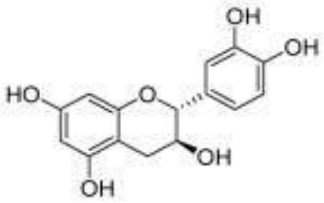
Figure 3.1.1.6 A cycloartane-type triterpene (cyclomargenol, **1**), β-sitosterol (**2**) and several fatty acids were identified from extract. The flavonoids catechin (**3**), epicatechin (**4**), epigallocatechin (**5**) were identified.



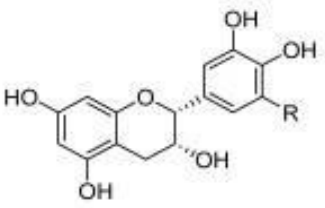
Cyclomargenol (**1**)



β-sitosterol (**2**)

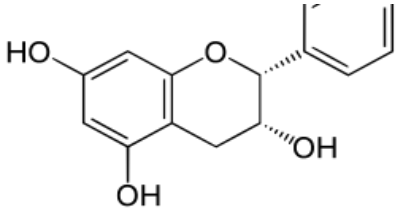


Catechin (**3**)



Epicatechin (**4**) R = H
Epigallocatechin (**5**) R = OH

Has a high tannin content catechists located mainly in the cortex. Oligomeric proanthocyanidins are formed by a variable number (2-14) of propelargonidina and procyanidins.

Propelargonidina 

These macromolecules tend to condense during the storage of the substance, becoming insoluble compounds called pink phlobaphenes which are precisely those responsible for Rathania called red. The tannins are responsible for its disinfectant, tonic and firming effect on the mucous membranes of the mouth. Other benefits: stringent antiviral, antioxidant, anti inflammatory effect.

3.3.1.5. Ginger

Zingiber officinale (Zingiberaceae)

Zingiber officinale, with commonly known name of ginger, named "zangabil" in Persian, belonging to Zingiberaceae family, has been used alone or in compounds as a spice or remedy in ancient recipes of Iranian Traditional Medicine (ITM) manuscripts. This plant is endemic to India and cultivated in South and South-East Asia, Africa, [12][13]
Latin America and Australia. Ginger or ginger root is the rhizome of *Z. officinale*, a perennial plant with annual leafy stems, grass-like and bright green leaves, yellowish green flowers, tuberous and fleshy rhizomes.

Ginger is abundant in active constituents, such as phenolic and terpene compounds. [12] **The phenolic compounds** in ginger are mainly gingerols, shogaols, and parasols. In fresh ginger, gingerols are the major polyphenols, such as 6-gingerol, 8-gingerol, and 10-gingerol. With heat treatment or long-time storage, gingerols can be transformed into corresponding shogaols. After hydrogenation, shogaols can be transformed into parasols. There are also many other phenolic compounds in ginger, such as quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione. Moreover, there are several **terpene components** in ginger, such as β-bisabolene, α-curcumene, zingiberene, α-farnesene, and β-sesquiphellandrene, which are considered to be the main constituents of ginger essential oils. Besides these, polysaccharides, protein, lipids, organic acids, vitamins (eg, *nicotinic acid* and *vitamin A*), minerals and raw fibers are among the other constituents present in ginger. [13]

Different studies have documented their biological properties such as antimicrobial, antioxidant, cytotoxic, insecticidal, and anti-inflammatory effects as well as food preservative characteristics.[13][14]

These properties have been attributed to the chemical components of *Z. officinale* EO, mainly consisting in monoterpene and sesquiterpene hydrocarbons Fig.3.1.1.7.

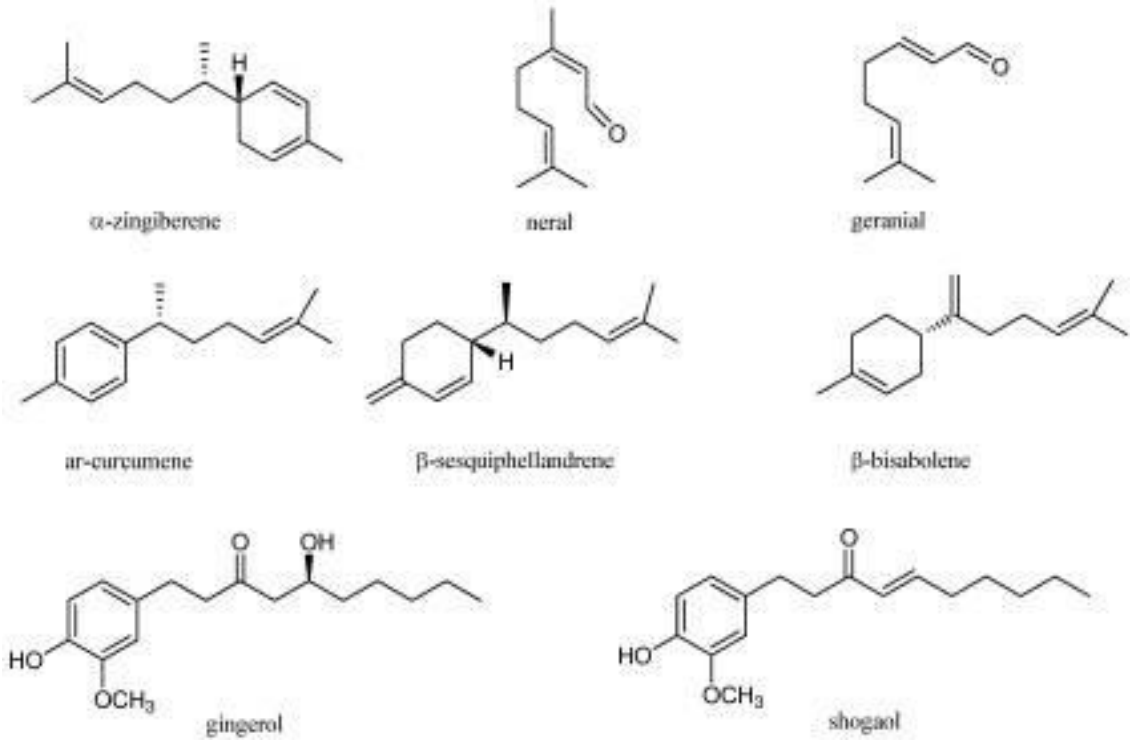


Fig.3.1.1.7 Chemical structures of major components of Z. officinale

The most abundant compounds are α-zingiberene, responsible for the distinctive flavor and aroma, geranial, ar-curcumene, β-bisabolene, β-sesquiphellandrene and neral. Other pungent constituents found in lower amounts are gingerol and shogaol.[15]

3.3.1.6. Barberry

Berberis vulgaris L.

(Berberidaceae)

Berberis spp. are shrubs in the family *Berberidaceae*, native to central and southern Europe, western Asia, as well as northwest Africa. About 500 species of these plants are found in most areas of central and southern Europe, the north-eastern region of the United States, and Asia (including the northern area of Pakistan and Iran). The genus *Berberis* consists of spiny deciduous evergreen shrubs which are characterized by yellow wood and flowers, dimorphic long and short shoots (1–2 mm). Some *Berberis* fruits are small oblong berries 7–10 mm long and 3–5 mm broad and turn blue or red upon ripening during the late summer or autumn. The branches of these plants are cylindrical, angular, or striate and sometimes covered by wax. The leaves of long branches are transformed to single, binary, or triple thistles, sometimes clawed or leaf-like and located in short branches or short small branches with batch oriented, lint-less, without petioles or with short petioles, the lower surface is sometimes covered with a waxed layer. The flowers are yellow and sometimes with a differently colored strip, red-stripped, and with scenery sepals or petals. Petals are often smaller than sepals. The stamens are scenery and often smaller than petals. The fruits of the plants from the Berberidaceae family are bright red or blackish red and taste sour. After falling leaves in autumn and passing through winter, the shrubs of barberries begin to flourish in March and flower in June. The fruits are rape and can be harvested in October. [7]

The main compounds, found in various species of Berberis, are berberine and berbamine. Phytochemical analysis of various species of this genus revealed the presence of alkaloids, tannins and phenolic compounds. **The triterpenes**: *lupeol*, separated from its fruits, and *oleanolic acid*, isolated from ethanolic extract; **the sterol**: *stigmasterol*, obtained from hexane extract, and *stigmasterol glucoside*, from ethyl

acetate extract; **the alkaloids**: *berbamine* (an isoquinoline alkaloid) and *berberine* are the major bioactive constituents. Protoberberine and bisbenzyl-isoquinoline alkaloids, such as berbamine, tetrandrine and chondocurine, which have been known for their anti-inflammatory and immunosuppressive properties, have been detected by phytochemical analysis of the root and stem back extracts of *B. vulgaris*. Other important alkaloids: *oxyberberine*, *columbamine*, *isocorydine*, *lambertine*, *magnoflorine*, *oxycontin* have been reported from this plant. Cytoprotective compounds including *N-(p-trans-coumaroyl) tyramine*, *cannabis in G*, and *(±)-lyoniresinol* have been isolated from ethyl acetate extract of *B. vulgaris*. [8][9]

The fruits contain a high amount of alkaloids, tannins, phenolic compounds and oleanolic acid, gum, pectin, oleoresins, organic acids, anthocyanins and carotenoids. In addition, palmitine, stigmasterol and its glycoside have all been detected in various species of the *Berberis* plant.

The gas chromatography coupled to mass spectrometry (GC-MS) analysis of various parts of *B. vulgaris* revealed that benzaldehyde, benzyl alcohol, 1-hexanol and I-2-hexenal were major compounds of the EOs from fruit, while *p*-cymene, limonene and ocimene were identified as major compounds of the EOs from leaves and flowers

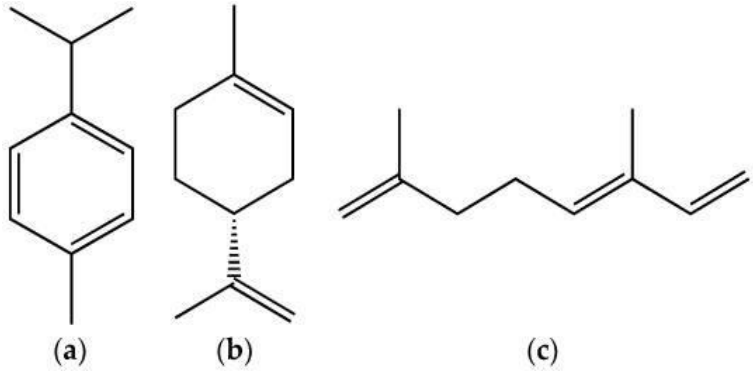
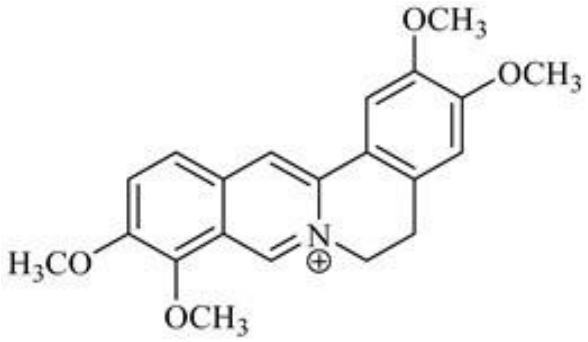
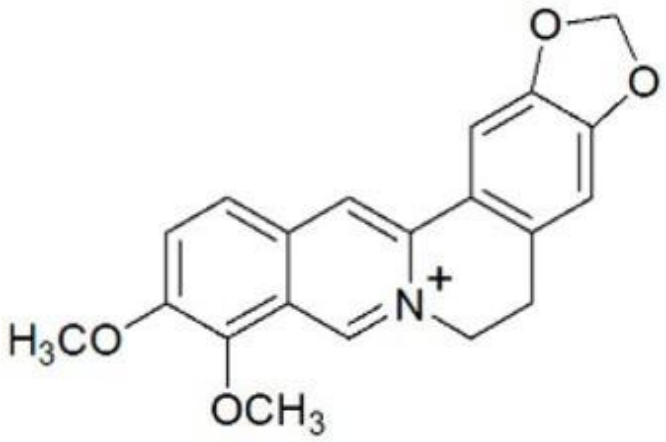


Fig. 3.1.1.8 Major compounds of the essential oils (EOs) of Berberis vulgaris leaves and flowers. (a) p-cymene; (b) limonene; (c) ocimene

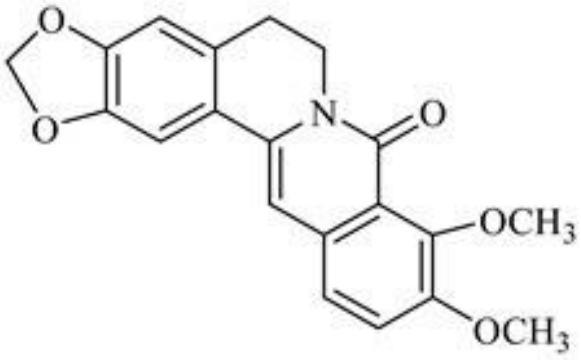
The plant is valued for its antipsoriatic effects and its antibacterial, antifungal, anti-inflammatory, analgesic and antioxidant activity. It also has been used for treating acne, eczema, and candida infection.[8][9]



palmatine



Berberine



oxyberberine

Fig. 3.1.1.9 Alkaloids from Berberis species

3.3.1.7. Red Poppy

Papaver somniferum L.

(Papaveraceae)

Poppies are herbaceous annual, biennial or short-lived perennial plants belonging to the subfamily Papaveroideae of the family Papaveraceae. Some species are monocarpic, dying after flowering. Poppies can be over a meter tall with flowers up to 15 centimetres across. The flowers are red and showy. Flowers of species (not cultivars) have 4 to 6 petals, many stamens forming a conspicuous whorl in the center of the flower and an ovary of from 2 to many fused carpels. The capsules are truncated at the top, smooth, short, obovate in shape and contain many, very small. The petals are showy, may be of almost any color and some have markings. The petals are crumpled in the bud and as blooming finishes, the petals often lie flat before falling away. In the temperate zones, poppies bloom from spring into early summer. The pollen of the oriental poppy, *Papaver orientale*, is dark blue, that of the field or corn poppy (*Papaver rhoeas*) is grey to dark green. The opium poppy, *Papaver somniferum*, grows wild in eastern and southern Asia, and South Eastern Europe. It is believed that it originated in the Mediterranean region.

The opium poppy contains up to 80 different alkaloids, including the *phenanthrenes*: principally morphine, codeine, thebaine and oripavine, and the *benzylisoquinolines*: principally papaverine and noscapine which have been used by man for the treatment of severe pain for generations but are also subject to misuse. Based on evidence from the currently available data set and background information, the CONTAM Panel divided the poppy seed samples with known countries of origin into two groups. The '*high-morphine*' group, which is assumed to represent primarily varieties grown for the pharmaceutical sector and the '*low-morphine*' group, which is assumed to represent primarily varieties grown for the food sector. In poppy seed samples, highest mean middle bound (MB) concentrations were reported for morphine (147 mg/kg in the

'*high-morphine*' group and 16.4 mg/kg in the '*low-morphine*' group) and thebaine (92.5 and 3.92 mg/kg, respectively) compared to codeine (22.7 and 2.88 mg/kg, respectively) and oripavine (20.0 and 2.14 mg/kg, respectively). For noscapine and papaverine, the mean MB concentrations were smaller and not exceeding 2 mg/kg. In the '*high-morphine*' group, thebaine was present at a concentration higher than morphine in more than 25% of the samples and codeine was

higher than morphine in about 5% of the samples. In the '*low-morphine*' group, morphine was the opium alkaloid present at the highest concentration in almost all samples.

Figure 3.1.1.10 shows the chemical structures of the predominant alkaloids reported to occur in poppy seed samples. The opium alkaloids with a phenanthrene structure are also called morphinans, which are typified by an aromatic A ring and (partly) saturated B and C ring and with an additional nitrogen-containing D ring, spanning carbons 9 and 13 of the phenanthrene structure. In the biosynthetic pathway, thebaine is the precursor of oripavine and of codeine, which are both precursors of morphine. The benzylisoquinolines noscapine and papaverine are not closely related in the biosynthetic pathway.

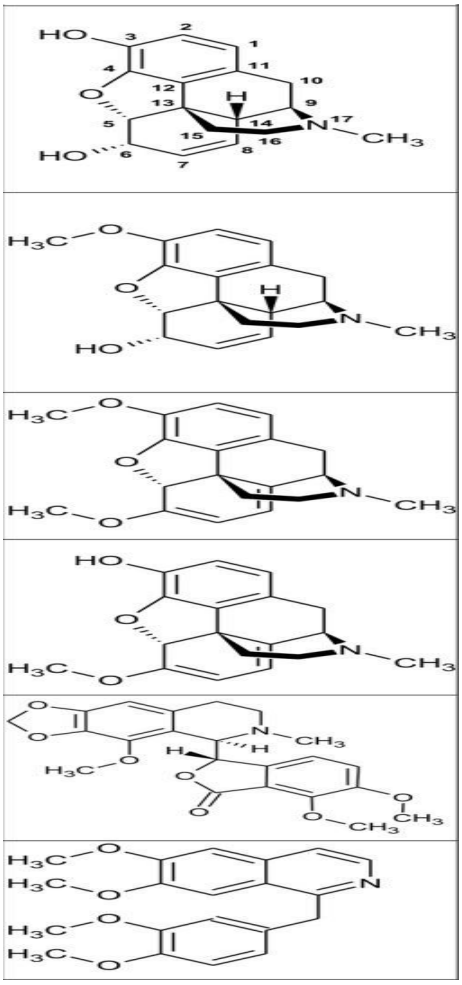


Fig. 3.1.1.10
Structures of opium alkaloids: (a) morphine; (b) codeine; (c) thebaine; (d) oripavine; (e)noscapine (f) papaverine

3.3.1.8. Uva Ursi

Arctostaphylos uva ursi L.

(Ericaceae)

Arctostaphylos uva-ursi is a small procumbent woody groundcover shrub 5–30 cm (2–12 in) high. The leaves are evergreen, remaining green for 1–3 years before falling. The fruit is a red berry. The leaves are shiny, small, and feel thick and stiff. They are alternately arranged on the stems. Undersides of the leaves are lighter green than on the tops. New stems can be red if the plant is in full sun, but are green in shadier areas. Older stems are brown. In spring, they have white or pink flowers. The genus name of *Arctostaphylos uva-ursi* comes from the Greek words *arctos* (meaning bear) and *staphyle* (meaning "bunch of grapes") in reference to the fruits which form grape-like clusters. The specific epithet, *uva-ursi*, comes from the Latin words *uva* (meaning grape) and *ursus* (bear), reflected by the *bearberry nickname*. The common name, *kinnikinnick*, is an Algonquin word meaning "smoking mixture". Native Americans and early pioneers smoked the dried *uva-ursi* leaves and bark alone or mixed with other herbs, tobacco or dried dogwood bark in pipes. Numerous common names exist, depending on region, such as mealberry, sandberry, mountain-box, fox-plum, hog-crawberry, and barren myrtle.

The plant contains diverse phytochemicals, including ursolic acid, tannic acid, gallic acid, some essential oils and resin, hydroquinones (mainly arbutin, up to 17%), tannins (up to 15%), phenolic glycosides and flavonoids*. Arctostaphylos uva-ursi* leaves contain arbutin, which metabolizes to form hydroquinone, a potential liver toxin.

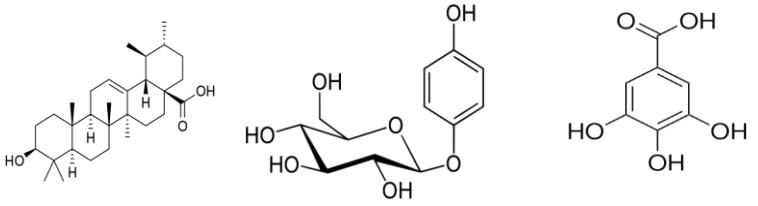


Fig. 3.1.1.11

(a) Ursolic acid

(b)Arbutin

(c)Gallic acid

3.3.1.9. Juniper Berry

Juniperus communis L.
(Cupressaceae)

The genus *Juniperus* (Family Cupressaceae) is evergreen aromatic shrub or tree mostly distributed throughout the cold and temperate regions of the Northern Hemisphere with some species extending as far South as Tropical Africa. The genus consists of approximately 75 species depending on taxonomic features. The widely known and perhaps most useful species is *Juniperus communis* L. commonly known as juniper, has the largest range of distribution than any woody plant extending from the Arctic regions of Asia, Europe and North America. In Asia, the plant grows naturally in the Himalayas and is found at an altitude of 3000–4000 m from Afghanistan to South-west China. Other common important *Juniperus* species of the Himalayan range include *J. indica, J. recurva* and *J. squamata*.

J. communis is a small coniferous evergreen tree or shrub variable in form ranging from 10 m tall to a low, often prostate spreading shrub in exposed locations. It has green needle-like leaves in whorls of three with a single white stomatal band on the inner surface. The fruits are berry-like cones initially green which ripen in 18 months to purple-black with a blue wax coating. These berries are spherical 4–12 mm in diameters and usually have three (occasionally six) fleshy fused scales, each scale with a single seed. The astringent blue-black seed commonly is too bitter to eat raw and is dried for its use as a culinary component in different regions of the world. The dried berries are crushed or grounded to release their flavor before these are added to a dish. These are used to flavor meat, soups, sauces, stews, stuffing and pickled foods. The berries are also used to flavor certain alcoholic beverages like beer and gin.

J. communis plant is not only a rich source of nutrition but also is rich in aromatic oils and their concentration varies in different parts of the plant (berries, leaves, aerial parts, and root). The fruit berries contain essential oil (0.5% in fresh and 2.5% in dry fruit)

invert sugars (15–30%), resin (10%), catechin (3–5%), organic acid, terpenic acids, leucoanthocyanidin besides bitter compound (Juniperina), flavonoids, tannins, gums, lignins, wax, etc. Various flavonoids like biflavonoids (amento-flavone), flavones (apigenin), flavonols (quercetin, isoquercetin) and vitamins (vitamin C) have also been found to be present in juniper berries. [12]

Phytochemical profiling of *J. communis* berry essential oils has mainly focused on the terpenoid content. The main terpenoids of essential oils are hydrocarbons of monoterpenes, sesquiterpenes and diterpenes whereas their oxygenated derivatives are only minor constituents. The mono-terpenoids of berry essential oil amounted to 83% of which 69.4 % was found to be monoterpene hydrocarbons. The main monoterpene hydrocarbons were α-pinene, ß-pinene, ß-myrcene sabinene, limonene whereas oxygenated monoterpene hydrocarbons include terpinen-4-ol, myrtenol, ß-citronellol, linalool, camphene hydrate, borneol, etc. Despite the domination of monoterpene compounds in the oils, there are differences in their quantitative composition due to a number of factors like geographical location, degree of ripeness, the age of berry fruit, production method, etc.

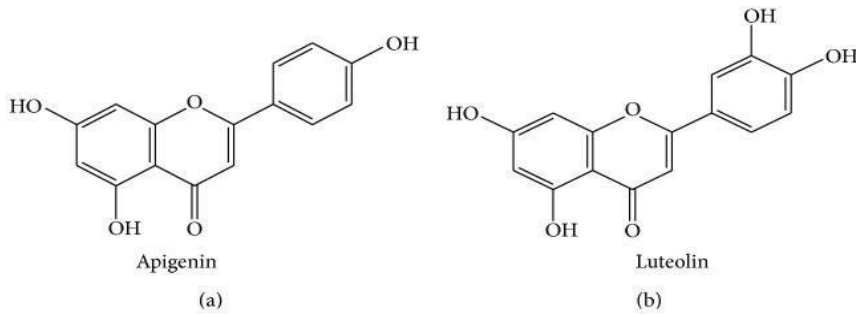
Similarly, sesquiterpenes accounted for about 13.4% of the total berry oil and these are found to be both sesquiterpenes hydrocarbons and oxygen-containing sesquiterpenes. The major sesquiterpenes hydrocarbons present in the berry essential oil are germacrene B and D, α- and β-selinene, α-humulene, epi-α-bisabolol, α-muurolene, β- and δ-elemene whereas oxygenated sesquiterpenes included α-cadinol, spathulenol, eudesmol, viridiflorol, germacrene D-4-ol, caryophyllene oxide, etc.

Bicyclic diterpenes in berries essential oil included imbricatolic acid, junicedral, trans-communic acid, iso-cupressic acid, aryl tetralin and lignin.

The presence of high amounts of other important components such as sabinene, germacrene D, myrcene, ß-pinene and limonene in juniper oil have also been reported. Similarly, essential oils from needle and wood were found to have high proportion of sesquiterpenes especially those bearing a tricyclic skelton (cedrone and longifolene) whereas monoterpenes were present at very low amounts.

Bellowing is a summary of the various chemical compounds of the Junipper communis:

Flavonoids: Berries contain apigenin, rutin, luteolin, quercetin-3-O-arabinosyl-glucoside, quercetin-3-o-rhamnoside quercitrin, scutellarein, nepetin, amentoflavone, and bilobetin



Leaves contain the cupressuflavone, hinokiflavone, biflavones, isocryptomerin amentoflavone, and sciadopitys. The seeds contain haemagglutinin. Plant also contains several labdane diterpenes and diterpenoids (methanolic extract).

Volatile Olio: The juniper berry oil is largely comprised of monoterpene hydrocarbons such as *β*-pinene (5.0%), *α*-pinene (51.4%), sabinene (5.8%), myrcene (8.3%), and limonene (5.1%) Fig.3.1.1.12. The seeds and fruits of the plant contain d-*α*-pinene, camphene, pectins, glycolic acid, malic acid, formic acid, acetic acid, cyclohexanol, terpene, proteins, fermentable sugars, wax, gum, ascorbic acid, dihydroquinine, *β*-pinene, hydrocarbon-junene, cadinene, juniper, and camphor.

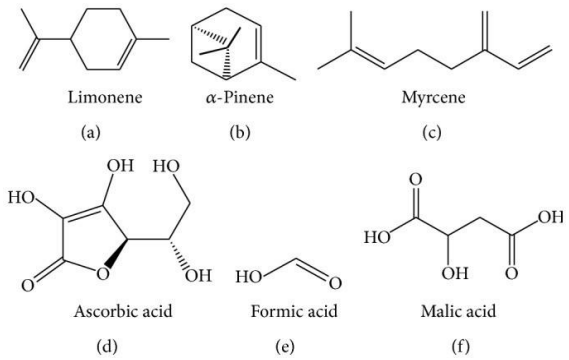
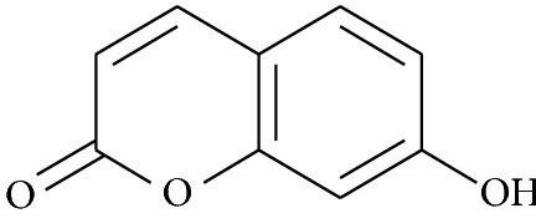


Fig. 3.1.1.12 Some volatile oils (a) limonene, (b) *α*-pinene, (c) myrcene, (d) ascorbic acid, (e) formic acid, (f) malic acid

Coumarins: They contain umbelliferone; see Figure.



Umbelliferone

Bicyclic Diterpenes: They contain imbricatolic acid, Junicedral, *trans*-Communic acid, diterpenes, isocupressic acid, aryl tetralin, and lignan deoxypodophyllotoxin. Three new diterpene acids have been identified as 15-dien-18-oic acid, 7-oxo-13-epi-pimara-8, 7*α*-hydroxysandaracopimaric acid.[11]

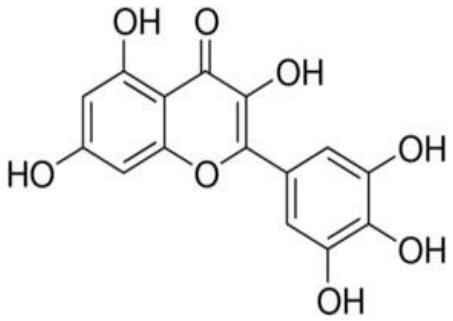
The plant has been reported as diuretic, having anti-inflammatory properties, antifungal activity, analgesic activity, hepatoprotective activity, antidiabetic and antihyperlipidemic activity, antimicrobial activity, antioxidant activity, antihypercholesterolemic activity, antibacterial activity and neuroprotective activity in Parkinson's disease. The analysis of the volatile fraction of *J. communis* berries was done by HS-SPME coupled to GC/MS for gin aromatization and more than 20 constituents have been reported.[10]

3.3.1.10. Bilberry

Vaccinium myrtillus L.
(Ericaceae)

Bilberry (*Vaccinium myrtillu*s L.) is a dark blue fruit that belongs to the genus *Vaccinium*, family *Ericaceae,* which comprises around 450 species of trees, shrubs, sub-shrubs and hemiphytes distributed all over the world. These fruits are usually consumed in the fresh form, however, due to their short shelf life, they are also frozen, dried or processed in the form of jams, juices and wines or liqueurs. Bilberries have been conventionally consumed and used in traditional medicine since ancient times, being harvested from wild bushes, although currently the cultivation of these fruits is commonly performed in northern and eastern Europe, and also in northern Africa.

These fruits are described as being an important source of phenolic compounds and carotenoids, also containing moderate levels of micronutrients and phytochemical compounds with health benefits, such as organic acids, sugars, vitamins, fibres, and phenolic compounds. **Phenolic compounds:** *Vaccinium myrtillus* L. is rich in polyphenols, with anthocyanins flavan-3-ols, flavonols and phenolic acids (Fig.3.1.1.13). Flavonoids, such as flavan-3-ols (catechins and proanthocyanidins) and flavonols (*i.e*.kaempferol, quercetin, **myricetin**), phenolic acids (mainly hydroxycinnamic and hydroxybenzoic acids) and derivatives of stilbenes, are the major non-anthocyanin polyphenols present in fruits.



The most common flavan-3-ols are procyanidins, consisting of (epi)catechin oligomers and can be classified into A-type and B-type, depending on the stereo configuration and linkage between monomers. B-type procyanidins are the most abundant, with procyanidins B1, B2, B3 and B4 occurring most frequently.

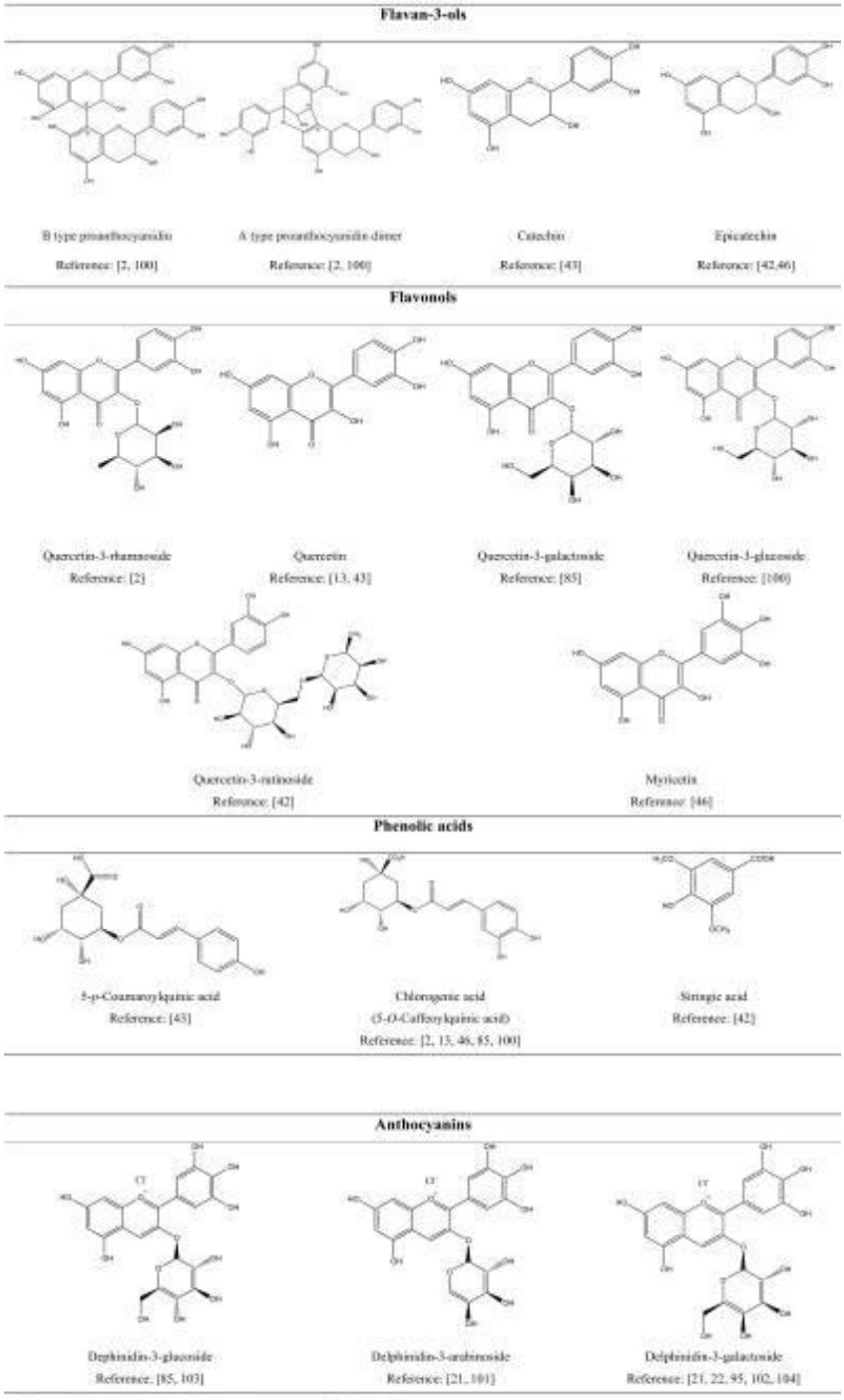
All the reported studies identified kaempferol, quercetin and myricetin glycosides as the main compounds, also reported laricitrin, syringetin and isorhamnetin glycosides.

Phenolic acids are phenols that possess one carboxylic acid function and include two main groups: the hydroxycinnamic and hydroxybenzoic acids. Caffeic, *p*-coumaric, vanillic, ferulic, and protocatechuic acids are widely present in many plants.

Anthocyanins: Anthocyanins are pigments commonly present in plants, where they are responsible for the characteristic blue, red or purple colour. Actually, these are considered the most important water-soluble pigments in plants, being particularly relevant in flowers and berries, namely bilberry, cherry or blackcurrant. The anthocyanin profile in bilberry consists of fifteen main compounds, derived from five aglycones (delphinidin, cyanidin, petunidin, peonidin, and malvidin) linked to different sugar moieties (galactose, glucose and arabinose). Nevertheless, it is due to their high levels that these fruits are recognized for their bioactive properties. Anthocyanins, besides being responsible for the blue color of bilberries, are the major group of flavonoids in these berries and have been associated to many beneficial health effects, such as prevention or treatment of cancers, cardiovascular diseases, obesity, diabetes, aging diseases, urinary tract infections and periodontal diseases. Studies showed that cyanidin-3-glucoside and a quercetin fraction from bilberry have beneficial dermatological effects and inhibit inflammation of injured skin, correcting the Th1/Th2 balance and reducing IL-17. Anthocyanins are classified in the USA as natural food colourings in the fruit (21 CFR 73,250) and vegetable (21 CFR 73,260) category, and in the EU, they are included as additives under code E163.

Mineral: Regarding mineral composition, these fruits have three main macroelements (Ca, P, and Mg) and seven microelements (Fe, Ba, Na, Mn, Cu, Sr, and Zn).

Fig.3.1.1.13 Structural formulas of the main bioactive compounds found in bilberry



3.3.1.11. Maca

Lepidium meyenii Walp

(*Brassicaceae*)

This plant belonging to the Brassicaceae family and Lepidium genus was discovered more than 2000 years ago in the Andes highlands of Peru, where it grows exclusively between 3500 and 4500 m above sea level. The most relevant plants related to *Lepidium meyenii* are rapeseed, mustard, turnip, black mustard, cabbage, garden cress, and watercress. Lepidium constitutes one of the largest genera in the Brassicaceae family. Maca is characterized by an overground and an underground part. The overground part is small and flat in appearance. This seems to be the result of an adaptation process to prevent the impact of strong winds. The underground part is the hypocotyl-root axis. The principal and the edible part of the plant is a radish-like tuber that constitutes the hypocotyl and the root of the plant. This hypocotyl-root axis is 10–14 cm long and 3–5 cm wide and constitutes the storage organ storing a high content of water. After natural drying, the hypocotyls are dramatically reduced in size to about 2–8 cm in diameter. There are many types of maca that can be characterized by the color of their hypocotyls ranging from white to black. Recently, it has been demonstrated that different types of maca (according to its color) have different biological properties.[23]

The plants are rich in elements, such as carbohydrates, proteins, lipids, essential amino acids, and free fatty acids. Furthermore, Maca contains several secondary metabolites, such as macamides, macaridine, alkaloids, and glucosinolates. The most abundant glucosinolates detected in Maca are aromatic glucosinolates, namely benzyl glucosinolate (glucotropaeolin) and *m*-methoxybenzyl glucosinolate (glucolimnanthin). Maca has been mainly classified in three ecotypes according to the color of the hypocotyls: red, yellow, and black. These showed different biological activity depending on the type of cultivation, processing, and extraction, and on the concentration of different bioactive metabolites.

Maca contains 80% water and has high amounts of iron and calcium, 10.2% proteins, 59% carbohydrates, 2.2% lipids, and 8.5% of fibre. Free fatty acids are also present in maca, the most abundant being linoleic, palmitic, and oleic acids. Saturated fatty acids represent 40.1% whereas unsaturated fatty acids are present at 52.7%. Maca contains amino acids (mg/g protein) like leucine (91.0 mg), arginine (99.4 mg), phenylalanine (55.3 mg), lysine (54.3 mg), glycin (68.30 mg), alanine (63.1 mg), valine (79.3 mg), isoleucine (47.4 mg), glutamic acid (156.5 mg), serine (50.4 mg), and aspartic acid (91.7 mg). Other amino acids present but in less proportion are histidine (21.9 mg), threonine (33.1 mg), tyrosine (30.6 mg), methionine (28.0 mg), hydroxyproline (26 mg), proline (0.5 mg), and sarcosine (0.70 mg). Minerals reportedly found in maca were iron (16.6 mg/100 g dry matter), calcium (150 mg/100 g dry matter), copper (5.9 mg/100 g dry matter), zinc (3.8 mg/100 g dry matter), and potassium (2050 mg/100 g dry matter) among others. [24]

Maca contains several secondary metabolites such as macaridine, macaene, macamides, and maca alkaloids are only found in this plant. Macaenes are unsaturated fatty acids. Other compounds include sterols as beta-sitosterol, campesterol, and stigmasterol.

Different glucosinolates as the aromatic glucosinolate glucotropaeolin have been described within maca. Benzyl glucosinolate has been suggested as a chemical marker for maca biological activity. However, this has been discarded since glucosinolates may easily metabolize to isothiocyanates and these in other smaller metabolites. It has been observed that maca batches from different producers significantly vary in the amount of macaene, macamides, sterols, and glucosinolates. In 2005 appeared the first publication indicating that different maca color types have different properties. These compounds individually or acting in synergy may be acting favoring the reported biological properties from maca.

The main chemical components of Maca were considered to be responsible for its aphrodisiac, antioxidants, immunostimulant, and energizing properties.[23[[24]

3.3.1.12. Hedge Mustard

Sisymbrium officinale L.

(Brassicaceae)

Mustards are members of the Brassicaceae family, and are among the earliest cultivated plants. Their seeds are one of the oldest recorded spices with use and cultivation dating back over 5000 years. Different types of Brassicaceae mustards, namely *Alliaria petiolata*, *Brassica alba*, *B. carinata*, *B. juncea*, *B. nigra*, *B. rapa*, *Erysimum repandum*, *Neslia paniculata*, *Sisymbrium officinale*, *S. orientale* and *S. erysipeloides* have been naturalized and adapted for use as food, incorporated into traditional medicine and play an important role in the agriculture.

Sisymbrium officinale L. Scop., synonym *Erysimum officinale*, known as erysimum, English watercress, hedge mustard, St. Barbara's hedge mustard, common hedge, singer's plant, and thalictrum, is an annual or biennial mustard. It is found on roadsides, wastelands and as a weed of arable land in Eurasia, the Mediterranean, north-western Africa, Scandinavia and Asia, and naturalized in Australia and New Zealand. Hedge mustard is sometimes regarded as an environmental weed in the Australian Capital Territory, Victoria and South Australia. It has been listed for official medicinal plant use in Australia. In Australian and New Zealand folk medicine, the seed and plant extracts were made into a syrup, with honey or sugar and flowers, and used to make a strong infusion. Allyl isothiocyanate, 4-hydroxybenzyl isothiocyanate and *p*-hydroxybenzyl isothiocyanate cause the sharp and hot pungency of mustards by stimulating the heat and acidity sensing TRPV ion channel, TRPV1, in the mouth and nasal cavity. Phenethyl isothiocyanate, benzyl isothiocyanate and sulforaphane are relatively less pungent. The sulfoxide group present in sulforaphane (4-methylsulfinyl butyl-ITC, CH3-SO-(CH2)4—N=C=S) is structurally similar to a thiol [(R–S-H) group] which produces onion or garlic-like odors in food. Use of mustards as food crops has some drawbacks as they contain metabolites that are considered anti-nutritional. [25]

3.3.1.13. Paprika

Capsicum annuum

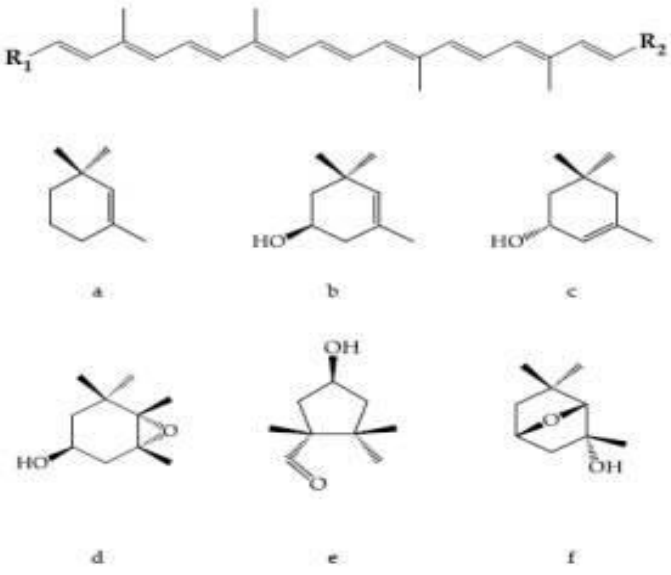
(Solanaceae)

C. annuum, which is a suffrutescent annual shrub, grows up to 0.75–1.8 m in cultivated locations with many angular twigs. The leaves are simple and are of different shapes, and alternate, elliptical to lanceolate, with smooth margins (entire) that are usually wrinkled. The small flowers (around 1.5 or 1 cm in diameter) are white or violet, in groups of two or more. The fruits are many-seeded berries which may be long, cylindrical, ovoid, obtuse, or oblong, but with no sutures; they are red when ripe, with a smooth shiny surface. The fruit is up to 25 cm in length and 7 mm in breadth, with many seeds which are yellow, smooth, round, and discoid, with a protuberant spine-aroma on the edge. *C. annuum* fruits have a characteristic odor and pungent taste.

C. annuum fruits are rich in capsaicinoids, carotenoids, flavonoids, vitamins, and minerals. The leaves contain alkaloids, tannins, and flavonoids, whereas the roots contain steroids, alkaloids, coumarins, glycosides, and triterpenoids. *C. annuum* contains a considerable quantity of L-asparaginase.[26]

The **capsaicin** is the most powerful natural compound, with a pungent, painful, and desensitizing effect, since its structure has an amide bond and a double bond (linked to an ether). **Capsaicinoids**, which are synthesized in the fruit placenta after enzymatic condensation, are alkaloid, which confers to the fruits a strong and pungent taste that is popular as a spice; they are characterized by the presence of a nitrogen atom. **Piperine** is an *N-acyl piperidine* that belongs to the vanilloid family of compounds, including capsaicin, with an antimutagenic activity. Recently, they have been studied by researchers, as they are antioxidants and can protect organisms from free radicals produced during the cell metabolism. Researchers have evaluated the composition of two flavonoids (quercetin and luteolin) after the acid hydrolysis of a phenolic portion of a

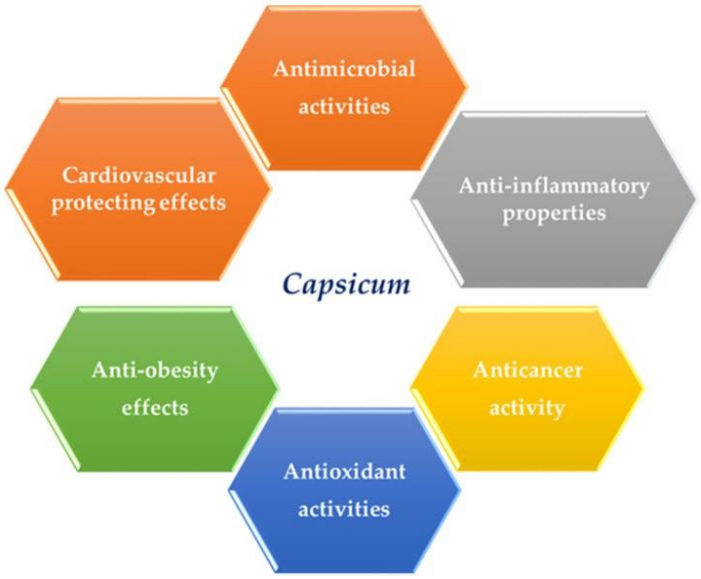
C. annuum extract.[27] **Flavonoids** are the other compound with an antioxidant property. They stop the activity of some enzymes, such as prostaglandin synthase, lipoxygenase, and cyclooxygenase, which are involved in cancer genesis. In fruits and plants, they are glycosides, with a sugar linked to 3-carbon. Quercetin and luteolin are the principal polyphenolic flavonoids found in *C. annuum*. **Carotenoids** are responsible for the product color, and they are at epicarp. The coloring is the result of more than 30 types of carotenoids: capsanthin and capsorubin are accountable for the red color, while the yellow shade is given by xanthophylls and carotenes. All the carotenoids in chili peppers are isoprenoids with 40 carbon atoms and contain nine double bonds in the central chain; the diverse final groups (b, e, k, 3-idrossi-5, 6-episode) contain the modification chromophore properties of all pigments, [28] permitting their classification into two isochromatic families: red (R) and yellow (Y). The red portion is rich in capsanthin, capsanthin-5,6-episode capsorubin (together with other minor carotenoids), while the yellow portion consists of all the other pigments (principally zeaxanthin, violaxanthin, antheraxanthin,β cryptoxanthin, β-carotene, cucurbita xanthinA). Thus, the different colours of pepper fruit can be characterized by the pigment profile Fig 3.1.1.14.



Carotenoid	R1	R2
β-Carotene (1)	a	a
β-Cryptoxanthin (2)	b	a
Lutein (3)	b	c
Zeaxanthin (4)	b	b
Antheraxanthin (5)	d	b
Violaxanthin (6)	d	d
Capsanthin (7)	b	e
Capsanthin-3,6-epoxide (8)	f	e
Capsoroubin (9)	e	e

Fig. 3.1.1.14 Main carotenoids of different fruit colours of Capsicum species

β- Carotene is a naturally occurring retinol (vitamin A) precursor obtained from certain fruits and vegetables with potential antineoplastic and chemopreventive activities. Peppers are also rich in vitamins. In the *Capsicum* genus, the plants are abundant in **vitamins A, C, E, D,** and **B**. **Ascorbic acid** has antioxidant properties in biologic organisms and restricts worsening processes.



C. annuum was used traditionally to cure toothache. The fruits are employed to activate gastric activities and cause an upsurge in blood circulation. It is also a stimulant and a carminative, and is utilized traditionally for neuralgia and rheumatism.

3.3.1.14. Cacao

Theobroma cacao (Malvaceae)

Cacao (*Theobroma cacao*) belongs to the genus *Theobroma* classified under the subfamily Byttnerioideae of the mallow family Malvaceae. Cacao is one of 17 species of *Theobroma*. In 2008, researchers proposed a new classification based upon morphological, geographic, and genomic criteria: 10 groups have been named according to their geographic origin or the traditional cultivar name. These groups are: Amelonado, Criollo, Nacional, Contamana, Curaray, Cacao guiana, Iquitos, Marañon, Nanay, and Purús.

The generic name is derived from the Greek for "food of the gods"; from θεός (*theos*), meaning 'god', and βρῶμα (*broma*), meaning 'food'. The specific name *cacao* is the Hispanization of the name of the plant in indigenous Mesoamerican languages. The cacao was known as *kakaw* in Tzeltal, K'iche' and Classic Maya; *kagaw* in Sayula Popoluca; and *cacahuatl* in Nahuatl as "bean of the cocoa-tree". [29]

Leaves are alternate, entire, unlobed, 10–40 cm (3.9–15.7 in) long and 5–20 cm (2.0–7.9 in) broad. The flowers are produced in clusters directly on the trunk and older branches; this is known as cauliflory. The flowers are small, 1–2 cm (0.39–0.79 in) diameter, with pink calyx. The fruit, called a cacao pod, is ovoid, 15–30 cm (5.9–11.8 in) long and 8–10 cm (3.1–3.9 in) wide, ripening yellow to orange, and weighs about 500 g (1.1 lb) when ripe. The pod contains 20 to 60 seeds, usually called "beans", embedded in a white pulp. The seeds are the main ingredient of chocolate, while the pulp is used in some countries to prepare refreshing juice, smoothies, jelly, and cream. Usually discarded until practices changed in the 21st century, the fermented pulp may be distilled into an alcoholic beverage. Each seed contains a significant amount of fat (40–50%) as cocoa butter. The fruit's active constituent is the stimulant theobromine, a compound similar to caffeine.

Among these, *T. cacao* is the only species that is cultivated extensively. This species has three genetic groups based on morphological and anatomical

characteristics—Criollo (*T. cacao* Spp. Criollo), Forastero (*T. cacao* Spp. Sphaerocarpum) and Trinitario. Of these, the Criollo type is well known for its superior flavour and provides the raw material from which fine flavour chocolates are produced; these represent 5%–10% of world chocolate production. Today, most of the world's chocolate production (approximately 80%) comes from the Forastero type of cacao; this variety is favoured over the Criollo for its disease-resistant and high-yielding nature, and beans from this variety are relatively cheaper than those from the Criollo type. The third genetic group, Trinitario, is a hybrid produced from crosses between Criollo and Forastero varieties. This variety was initially developed in Trinidad, and today, it is cultivated in many parts of South and Central America, Africa, South-East Asia and Oceania for its aroma, productivity and disease-resistant character.

The potential health implications of biologically active substances present in cocoa seeds are well documented. They are rich in natural antioxidants, such as polyphenols and tocopherols. Thanks to this antioxidant property, many of these compounds, especially **flavonoids**, exhibit also a wide range of pharmacologic effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory actions. The high levels of flavanols are responsible for the bitterness of cocoa that represent a fundamental aspect of the organoleptic and palatability characteristics of chocolate, and contribute to cocoa health benefits. **Epicatechins** (that are classified as flavan-3-ols, based on their structure) are the most abundant cocoa phenolic components; they mainly include monomeric (−) epicatechin and (+) catechin (as well as oligomeric and polymeric proanthocyanidin flavanols), gallocatechin and epigallocatechin. Epicatechin represents approximately 35% of polyphenol content of unfermented Forastero cocoa beans. The antioxidant properties are also related to the presence of **tocopherols** and **tocotrienols**, which reduce oxidative stress and delay the progress of a variety of degenerative disorders, such as cardiovascular diseases and cancer. In addition, they have been shown to regulate cellular signalling, cell proliferation and gene expression. The total tocopherol content in cocoa beans is reported to be in a range of 100–300 mg/Kg fat, values that are similar to those generally observed for wheat germ oil. The predominant isomer in a cocoa bean is the gamma-tocopherol and a different distribution of the four isomers in seed parts.

Precursors of tocopherols and polyphenols are produced from the plant primary biosynthetic shikimate and acetate pathways. The main products of these pathways are the **aromatic amino acids** phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp) and acetyl-coenzyme A (CoA), respectively. They represent the starting molecules for the biosynthesis of a wide range of secondary metabolites. Other interesting classes of cocoa bioactive constituents are represented by **phytosterols** (collective terms comprising saturated sterols, also known as stanols) and fatty acids. Phytosterols are typical plant lipids which have a structural similarity with cholesterol and inhibit its intestinal absorption, contributing to a lower total plasma cholesterol and low-density lipoproteins levels. In cocoa seeds, the content of plant sterols is 2–3 mg/g fat, with an abundance of β-sitosterol and stigmasterol. **Fatty acids** are organized as triacylglycerol (TAG), the majority of these TAG's being 2-oleyl glycerides (O) of palmitic (P) and stearic (S) acids (POP, POS, SOS). This TAG structure directly affects the way chocolate behaves in the manufacturing process and the characteristics of the final product (texture, viscosity, melting behaviour, flavour and taste). The fatty acids content depends on the variety and region of cultivation of cocoa beans. The biosynthesis of fatty acids requires as precursor the acetyl-CoA, that also represents the starting point of the mevalonate pathway from which arise the secondary metabolites, sterols/phytosterols. The presence of above-mentioned compounds (as polyphenols, phytosterol and fatty acids) is not the only trait influencing the nutritional characteristics and aroma of cocoa beans, their morphological and anatomical traits, as the permeability of the seed coat, are also very important aspects. The **acetic acid** produced during the fermentation moves through the seed coat to contribute to formation of flavour precursors in the cotyledons. The plant content of **mineral** macro and micronutrients such as Ca, Mg, Fe, Zn, Cu and Mn.[30][31]

3.3.1.15. Cypress

Cupressus sempervirens L.

(Cupressaceae)

Cupressus sempervirens L., known as Mediterranean cypress, is an ornamental tree and a member of the Cupressaceae family. Among the habitats of the species, northern America, Africa, southeastern Europe, and western Asia can be cited. Cypress species, represented by 25 different taxa in Mediterranean Region, North America, and Asia, are primarily divided into three main groups: Mediterranean cypress, North American cypress, and Asian cypress. Among them, Mediterranean cypress consists of *Cupressus sempervirens* L., *Cupressus atlantica*, and *Cupressus dupreziana*. Although there are many different subspecies and varieties of this species, the widely accepted varieties by ramification type are branched cypress (*Cupressus sempervirens* L. var. *horizontalis*) and pyramidal cypress (*Cupressus sempervirens*. var. *pyramidalis* (fastigiata = stricta).

Various classes of phytochemical compounds have been reported in different parts of *C. sempervirens*, including **flavonoid** derivatives (rutin, quercetin, quercetin rhamnoside, quercitrin, myricitrin and kaempferol 3-0-rhamnoside, cupressuflavone, amentoflavone, and other biflavonoids), **diterpenes** (isocupressic acid, isocupressic acid, sugiol, communic acid, sandra pimaric acid, imbricatolic acid, acetyl imbricatolic acid, ferruginol, abita-8,11,13-triene-20-ol), **sesquiterpenes** (junepediol), catechins and flavonols oligomers, proanthocyanidins, essential oils, phenolic acids (caffeic acid and *p*-coumaric acid), and fatty acids. The leaf extract of *C. sempervirens*, which was determined to be rich in **polyphenols** expressed as quercetin glycosides (174 µg/mL) and biflavonoids (1460 µg/mL).

Depending on the phytochemicals found in *C. sempervirens*, it has been reported to possess a number of biological activities. The leaves and cones of *C. sempervirens*

have been used as folk remedies for antiseptic, antipyretic, anthelmintic, astringent, antirheumatic, antihemorrhoidal, antidiarrhoeal, antioxidant and vasoconstrictive purposes. Early studies revealed that *C. sempervirens* had strong antimicrobial and antiviral activity thanks to the proanthocyanidin-rich fraction of *C. sempervirens* and the essential oil of the plant. Nevertheless, the essential oil of the plant displayed insignificant antifungal activity against *Casuarina timber*, *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., and *Mucor* sp.[33]

3.3.1.16. Plantago Afra

Plantaginaceae

Plantago afra is a herbaceous plant in the family Plantaginaceae, commonly called plantains or fleaworts. It has two seeds, reddish-brown, narrow-elliptic, shining, 2–3 mm long. The leaves are sessile, but have a narrow part near the stem which is a pseudo-petiole. They have three or five parallel veins that diverge in the wider part of the leaf. The inflorescences are borne on stalks typically 5–40 cm (2.0–15.7 in) tall, and can be a short cone or a long spike, with numerous tiny wind-pollinated flowers. It is native to Malta and Mediterranean regions, Afghanistan to Pakistan but they are found all over the world, including the Americas, Asia, Australia, New Zealand, Africa and Europe.[34]

Plantago contains several active compounds such as flavonoids, polysaccharides, terpenoids, lipids, iridoid glycosides and caffeic acid derivatives, etc. [35]

Carbohydrates: The seeds contain the monosaccharides glucose, fructose, xylose and rhamnose as well as the disaccharide sucrose and the trisaccharide planteose (*O*-*α*-d-Gal*p*-(1→6)-*O*-*β*-d-Fru*f*-(2→1)-*α*-d-Glc*p*). Planteose acts as a reserve carbohydrate in the seeds.

Lipids: Fatty acids, both free and after hydrolysis of triglycerides, have been isolated from the seeds. According to studies 64.8% of the fatty acids are unsaturated 18:3*ω*3 and 18:2*ω*6 and the saturated fatty acid palmitic acid was most abundant in the leaves. The major components of the leaf wax are the free triterpene acids, oleanolic and ursolic acid, and the linear alkanes C27H56-C33H58.

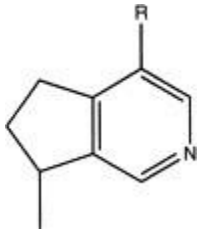


Fig. 3.1.1.15 Alkaloids in Plantago. Indicain: R=CHO; plantagonin: R=COOH

Alkaloids: the plant contains alkaloids such as indicain and plantagonin *Figura. 3.1.1.15.*

Caffeic acid derivatives: the main caffeic acid is plantamajoside, and only small amounts of acteoside (synonym to verbascoside) are present. Plantamajoside is glycosylated with glucose to the central glucose while in acteoside it is glycosylated with rhamnose (Fig. 3.1.1.16).

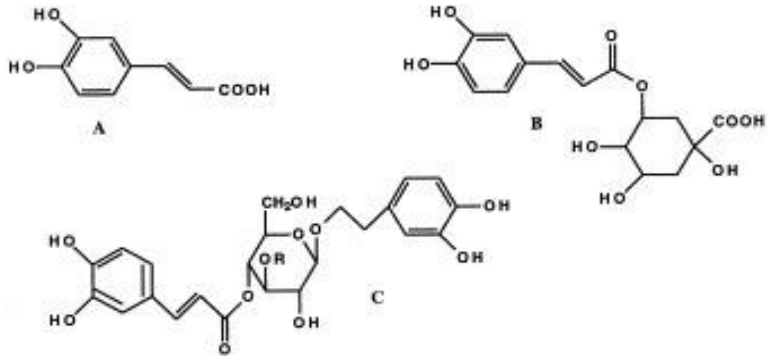


Fig.3.1.1.16 Caffeic acid derivatives in P. major L. (A) Caffeic acid, (B) chlorogenic acid, (C) Plantamajoside R=Glc, acteoside R=Rha.

Plantamajoside has an anti-inflammatory activity and antioxidant activity. It is also known to have some antibacterial activity. Acteoside has superoxide anion and DPPH radical scavenging activities, has antioxidant activity and inhibits lipid peroxidation It has antibacterial, immunosuppressant and analgesic activity.

Flavonoids: Several flavonoids have been isolated such as luteolin 7-glucoside, hispidulin 7-glucuronide, luteolin 7-diglucoside, apigenin 7-glucoside≈nepetin 7-glucoside, luteolin 6-hydroxy 4′-methoxy 7-galactoside. Many flavonoids have antioxidant, anti-inflammatory properties.

Terpenoids: The triterpenoids oleanolic acid, ursolic acid, 18*β*-glycyrrhetinic acid and sitosterol were isolated from the plant. Ursolic acid inhibits cyclooxygenase-2 and cyclooxygenase-1 catalysed prostaglandin biosynthesis in vitro while the structural isomer oleanolic acid is less active. 18*β*-Glycyrrhetinic acid had no significant inhibitory effect. The mechanisms of the anti-inflammatory effects also include inhibition of histamine release from mast cells, inhibition of elastase and inhibition of complement activity. Ursolic acid and oleanolic acid also have hepatoprotective, tumor promotion inhibiting activity and an anti-hyperlipidemic effect.

Vitamins: The vitamin contents have, therefore, been examined. Thus, *Plantago* can be considered as a good source of vitamin C and carotenoids. In addition, the oxalic acid, nitrate and erucic acid were present in low amounts.

Other organic acids: the plant contains fumaric acid, syringic acid, vanillic acid, *p*-hydroxy benzoic acid, ferulic acid, *p*-coumaric acid, gentisic acid, traces of salicylic acid, benzoic acid and cinnamic acid

Plantamajoside and acteoside have antibacterial activities. Some flavonoids and the caffeic acid derivatives plantamajoside and acteoside have antioxidative and free radical scavenging activities. However, the leaves also contain compounds with anti-inflammatory activity, namely plantamajoside, baicalein, hispidulin, aucubin, ursolic acid and oleanolic acid.

3.3.1.17. Tormentil erecta

Potentilla Erecta L. (Rosaceae)

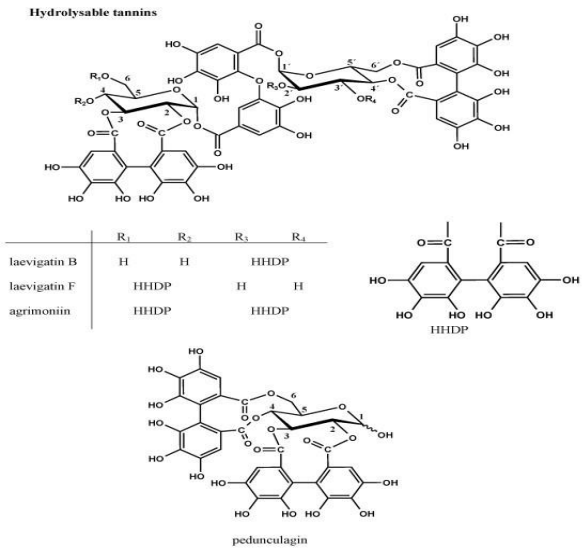
Potentilla erecta (syn. Tormentilla erecta, Potentilla laeta, Potentilla tormentilla, known as the (common) tormentil, septfoil or erect cinquefoil) is a herbaceous perennial plant belonging to the rose family (Rosaceae). Potentilla erecta is a low, clump-forming plant with slender, procumbent to arcuately upright stalks, growing 10–30 centimetres (3.9–11.8 in) tall and with non-rooting runners. It grows wild predominantly in Europe and western Asia, mostly on acid soils and in a wide variety of habitats such as mountains, heaths, meadows, sandy soils and dunes. This plant flowers from May to August/September. There is one yellow, 7–11 millimetres (0.28–0.43 in) wide flower, growing at the tip of a long stalk. There are almost always four notched petals, each between 3 and 6 mm long. Four petals are rather uncommon in the rose family. The petals are somewhat longer than the sepals. There are 20–25 stamens. The radical leaves have a long petiole, whilst the leaves on the flowering stalks are usually sessile or with short petioles. The glossy leaves are alternate, ternate, consisting of three ovate leaflets with serrated margins. The paired stipules are leaflike and palmately lobed. There are 2–8 dry, inedible fruits. Potentilla erecta is found wild throughout Europe, Scandinavia and West Asia.

This genus has been known since ancient times for its curative properties. Extracts of the aerial and/or underground parts have been applied in traditional medicine for the treatment of inflammations, wounds, certain forms of cancer, infections due to bacteria, fungi and viruses, diarrhoea, diabetes mellitus and other ailments.[37]

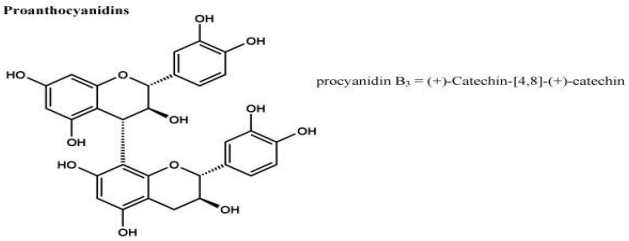
Most of the biological effects of *Potentilla* species can be explained by the high amount of hydrolysable and condensed tannins, flavonoids and triterpenes present in all plant parts. Tannins have been known to be important constituents of *Potentilla Species* and their extracts, respectively, and the cause for the astringent effects. Therefore thorough phytochemical studies on *Potentilla* species starting especially in the 1960s were

primarily focussed on tannins. Several of the polyphenols are identified as ellagic acid and flavonols glycosylated derivatives. Types of flavonoids, including flavones, isoflavonoids, flavanols, and anthocyanins. Content of six phenolic compounds: were identified as (+)-catechin, caffeic acid, ferulic acid, hyperoside, rutin and ellagic acid.

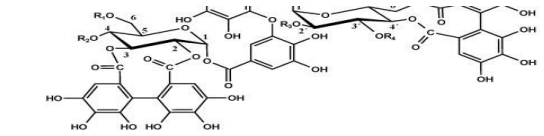
Roots and rhizomes: Due to the high amount of 17–22% of tannins in the rhizomes of *Potentilla erecta* (i.e. 15–20% condensed tannins, ca. 3.5% hydrolysable tannins;), this group of natural compounds has been in the focus of many phytochemical studies.



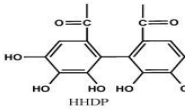
The condensed tannins of *Potentilla erecta* consist of dimeric and trimeric type B proanthocyanidins in which the catechin units are connected via 4,8-, 4,6-, 6,6′- or 6′,8-bonds Figura 3.1.1.17. The [4,8]-2,3-*trans*-3,4-*cis*-bi-(+)-catechin is a rare example of a *cis*-configurated dimeric proanthocyanidin, found in *Potentilla erecta*. Several precursors for condensed tannins were identified for this plant source including (+)-catechin, (−)-epicatechin, (+)-gallocatechin and (−)-epigallocatechin. Several more phytochemical studies have concentrated on the isolation of triterpenoids. A characteristic constituent of triterpenoids is tormentoside (rosamultin), which has originally been isolated from *Potentilla erecta*. A very limited number of flavonoids have only been reported for *Potentilla erecta*. Further ingredients include a series of organic acids and phenol carboxylic acids which were exclusively described for *Potentilla erecta*. Finally, sterols, sugar, amino acids and fatty acids were detected in *Potentilla* species.



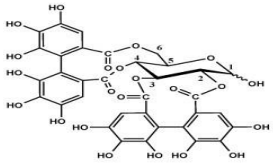
The aerial parts: Predominants in aerial parts of plants are generally flavonoids. A common feature is the mono-, di- and also tri-hydroxy substitution of ring B in the isolated instances. In 15 flavonoid aglycones one or more hydroxyl groups in positions 7, 3′, 4′ and/or 5′ are methylated. Characteristic is also the presence of a large number of flavonoid *O*-glycosides and *O*-glucuronides with a large structural variety of the aglycone. 5–10% of the constituents are tannins, especially hydrolysable tannins. Interestingly, no condensed tannins were elucidated, whereas their precursors, e.g. (+)-catechin, (+)-gallocatechin, (−)-epigallocatechin, were detected in *Potentilla erecta*. Furthermore several other constituents are organic acids and phenolic carboxylic acids, sterols, essential oils and pectin.[36][37]



	R_1	R_2	R_3	R_4
laevigatin B	H	H	HHDP	
laevigatin F	HHDP		H	H
agrimoniin	HHDP		HHDP	

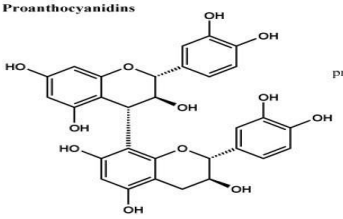


HHDP



pedunculagin

Proanthocyanidins



procyanidin B_3 = (+)-Catechin-[4,8]

3.3.1.18. Cynodon Dactylon

Poaceae

Cynodon dactylon, known as Bermuda grass, *Dhoob*, *dūrvā* grass, *ethana grass*, *dubo*, dog's tooth grass, Bahama grass, devil's grass, couch grass, Indian *doab*, *arugampul*, *grama*, wiregrass and scutch grass, is a grass that is native to most of the eastern hemisphere. Although it is not native to Bermuda, it is an abundant invasive species there. In Bermuda it has been known as crabgrass (also a name for *Digitaria sanguinalis*). The blades are a grey-green colour and are short, usually 2–15 cm (0.79–5.91 in) long with rough edges. The erect stems can grow 1–30 cm (0.39–11.81 in) tall. The stems are slightly flattened, often tinged purple in colour. The seed heads are produced in a cluster of two to six spikes together at the top of the stem, each spike 2–5 cm (0.79–1.97 in) long.

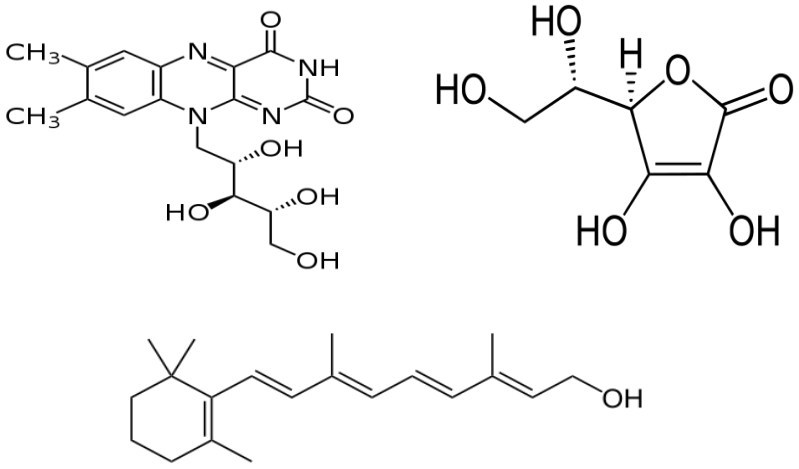
It has a deep root system; in drought situations with penetrable soil, the root system can grow to over 2 metres (6.6 ft) deep, though most of the root mass is less than 60 centimetres (24 in) under the surface. The grass creeps along the ground with its stolons and roots wherever a node touches the ground, forming a dense mat. *C. dactylon* reproduces through seeds, stolons, and rhizomes. Growth begins at temperatures above 15 °C (59 °F) with optimum growth between 24 and 37 °C (75 and 99 °F); in winter, the grass becomes dormant and turns brown. Growth is promoted by full sun and retarded by full shade, e.g., close to tree trunks.

3.3.1.19. Spinach

Spinacia oleracea L. (Amaranthaceae)

The plant *Spinacia oleracea* L., family Amaranthaceae, commonly called spinach or palang (vernacular name), is an annual plant (rarely biennial). It is native to central and southwestern Asia. The leaves are alternate, simple, ovate to triangular-based, variable in size, from about 2 – 30 cm long and 1 – 15 cm broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. The flowers are inconspicuous, yellow-green, 3–4 mm (0.1–0.2 in) in diameter, and mature into a small, hard, dry, lumpy fruit cluster 5–10 mm (0.2–0.4 in) across several containing seeds. It is a favorite food vegetable among Indians in the winter season and is a dietary power house, full of vitamins and minerals. Raw spinach is 91% water, 4% carbohydrates, 3% protein, and contains negligible fat. It is a rich source of vitamin A, vitamin C, vitamin K, folate. Spinach is a good source of the B vitamins riboflavin and vitamin B_6, vitamin E, minerals and dietary fiber. Spinach contains an appreciable amount of minerals such as iron, zinc, manganese, magnesium, calcium, potassium, and a rich source of nitrate. [38] Spinach is well recognized for its antimicrobial, antioxidant activity and it also has been studied in cancer.

Fig. 3.1.1.18 (a) Riboflavin, (b) Vitamin A, (c) Vitamin C



3.3.1.20. Rosehip

Rosa canina L.

The genus Rosa contains over 100 species that are widely distributed mostly in Europe, Asia, the Middle East and North America. Rosa canina L. (dog rose) is an erect shrub of up to 3.5 meters height, sometimes climbing; its branches are often curved or arched. Petals are white to pale pink, rarely deep pink and fruit ripens late. The Rosa canina L. fruits have constituted an important source of food and medicine for many cultures. Common food preparations using rose hips include juice, wine, tea, jelly, jam, as well as mixed with dried salmon eggs.

The dog rose hips (Cynosbati fructus) comprise several biologically active compounds, such as: sugars, organic acids, pectins, flavonoids, tannins, carotenoids, fatty acids, vitamins (particularly vitamin C and also vitamins B1, B2, K, PP, E), macro- and microelements etc. Rose hips are known to have the highest vitamin C content (30–1300 mg/100 g) among fruits and vegetables. In addition, rose hips contain other vitamins and minerals, carotenoids, tocopherols, flavonoids, fruit acids, tannins, pectin, sugars, organic acids, amino acids and essential oils [32] with potential for significant nutritional and therapeutic benefits among natural antioxidants.

Rosa canina L. is well-known for its high phenolic contents. These compounds are known to have antioxidant, antimutagenic and anticarcinogenic effects. Polyphenol compounds are potential antioxidant substances and protective agents against the development of human disease.

Its seeds are rich in oil and mineral substances. The fatty acids from the dog rose oil are mainly: the linoleic, oleic, linolenic, palmitic, stearic and arachidonic acid.

The substances within the dog rose fruit (hips) are endowed with vitaminizant, astringent, cholagogue, choleretic, diuretic, anti diarrhoea, antioxidant properties, etc..

3.3.2 **Method for Natural Dyes**

We followed this method for the realization of our project. Here is a short scheme of our work and more details has been described below.

1) Dilution test
   - water
   - plant
2) Application on hair
   - yak (standard for white hair)
   - bleach (standard for bleach hair)
   - salt and pepper (standard for dark hair)
3) Time of developing
   - 45 min
   - 2 hours
4) Miscellaneous temperature
   - 50°C
5) pH of miscellaneous
   - acidic pH (standard)
   - basic pH (addition of monoethanolamine)
6) Mixing test
   - tea, coffee
   - different plants
7) Rinse off
   - warm water
8) Drying process
   - phon

Fig. 3.1.1.19 Types of hair
(a) yak (b) bleach (c)salt&pepper

3.3.2.1 Dilution Test

The extracts powder have been diluted in water in a ratio from 1: 1 to 1: 6 in order to obtain a semi-liquid mixture, see Fig 3.3.2.1

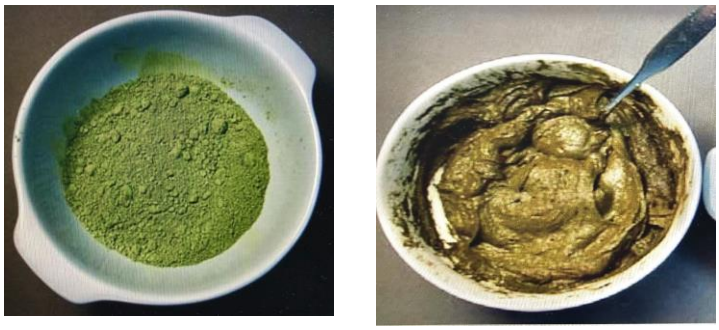


Fig. 3.3.2.1 (a) extract powder (b) semi-liquid mix

3.3.2.2 Color Test

Each powder has been mixed with distilled water to obtain the desired consistency. The mixture has been put immediately into the hair and rested for a different minute. Rinsed the pasta out of hair.

We used different types of hair:

- yak (used as a reference standard for white hair)
- salt and pepper (used as a standard for dark hair)
- bleached hair (used as standard for bleached hair).

Time of developing: The application on the hair was kept for 45 min.

Temperature: The mixture should be kept at 50 °C

Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdryer.

Following this procedure we did other tests increasing the developing time up to two hours to improve the final coloring result.

3.3.2.3 pH Test

All individually tested plants are characterized by an acidic pH of 2.0-3.5. We decided to add an alkaline agent, monoethanolamine, which allows to open the scales cuticle and promote the penetration of the active ingredient on the hair to increase the intensity of the color.

3.3.2.4 Synergy with Other Plants

Four plants such as Barberry, Red Poppy, Hibiscus and Sambucus have been tested adding a small amount of Tea or Coffee powder. We mixed distilled water with powder in order to obtain a semi liquid mixture or pasta, and then we added a small quantity of tea or coffee powder to maintain the desired consistency. The mixture has been put immediately into the hair and rested for 45 minutes. And then, rinsed the pasta out hair.

3.3.2.5 Miscellaneous Test

Three tests for each color were done using different plants to improve the coloring results in different ratios 1: 3, 3: 1, 1:1. For the red color we used Hibiscus and Red Poppy; for the yellow we used Juniper Berry and Rhubarb; for the brown Sambucus and Ratanhia. We mixed distilled water with powder in order to obtain pasta. The mixture has been put into the hair and rested for 2 hours. Here are the test performed, see table below:

Mixing plant	*Exp.1* ***(%)***	*Exp.2* ***(%)***	*Exp.3* ***(%)***
Hibiscus:Red Poppy	75:25	25:75	50:50
Juniper:Rhubarb	75:25	25:75	50:50
Sambucus:Ratanhia	75:25	25:75	50:50

3.4 Results

3.4.1 **Dilution Test**

The results of the dilutions powder extract are shown below, see Table 3.4.1

Plant:Water 1:1 1:1,5 1:2 1:2,5 1:3 1:3,5 1:4 1:4,5 1:6

Plant: Water	Ratio (%)
Uva Ursi : Distilled Water	1:2,5
Rhubarb : Distilled Water	1:3
Sambucus : Distilled Water	1:1,5
Hibiscus : Distilled Water	1:2,5
Barberry : Distilled Water	1:3
Red Poppy : Distilled Water	1:6
Juniper Berry : Distilled Water	1:2
Ginger : Distilled Water	1:2
Ratanhia : Distilled Water	1:2,5
Bilberry : Distilled Water	1:2
Maca : Distilled Water	1:2,5
Cypress : Distilled Water	1:2
Hedge Mustard : Distilled Water	1:4,5
Cacao : Distilled Water	1:2
Plantago : Distilled Water	1:2,5
Potentilla Erecta : Distilled Water	1:3,5
Paprika : Distilled Water	1:2
Cynodon Dactylon : Distilled Water	1:2,5
Spinach : Distilled Water	1:3,5
Rosa Canina : Distilled Water	1:3

Table 3.4.1 Dilution test

3.4.2 **Color Test**

We used different types of hair:

- yak (used as a reference standard for white hair)
- salt and pepper (used as a standard for dark hair)
- bleached hair (standard for bleached hair).

Time of developing: The application on the hair was kept 45 min.

Temperature: The mixture kept at 50 °C.

Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdryer.

We obtained four colors: red, yellow, brown and neutral but we notice that the intensity of the color is not very powerful.

The plants that are able to give a yellow color to different shades are Rhubarb, Barberry, Juniper, Ginger. Hibiscus and Red Poppy give a red color; Sambucus and Blueberry bring a brown color and finally Uva Ursi which gives the hair an emollient effect and a neutral color.

Following this procedure we did other tests increasing the developing time up to two hours to improve the final coloring result. Here are the results of the color test.

Time 45 min

Miscellaneous temperature ≈ 50°C

Types of hair: Yak, bleach, salt and pepper

Rhubarb, Barberry, Juniper, Ginger Yellow Color

Hibiscus and Red Poppy Red Color

Sambucus and Blueberry Brown Color

Uva Ursi Neutral Color

Time 2 hours

Miscellaneous temperature ≈ 50°C

Types of hair: Yak, bleach, salt and pepper

Rhubarb, Barberry, Juniper, Ginger, Hibiscus,

Red Poppy, Sambucus, Blueberry, Uva Ursi

Four Color or Intense Color

The results of the plants in which they have not given any color are: Maca, Hedge Mustard, Cypress, Plantago Afra, Potentilla Erecta, Paprika, Cynodon Dactylon, Spinach, Cacao, Rosa Canina

Time 45 min

Miscellaneous temperature ≈ 50°C

Types of hair: Yak, bleach, salt and pepper

Maca, Hedge Mustard, Cypress, Plantago Afra, Potentilla Erecta, Paprika, Cynodon Dactylon, Spinach, Cacao, Rosa Canina

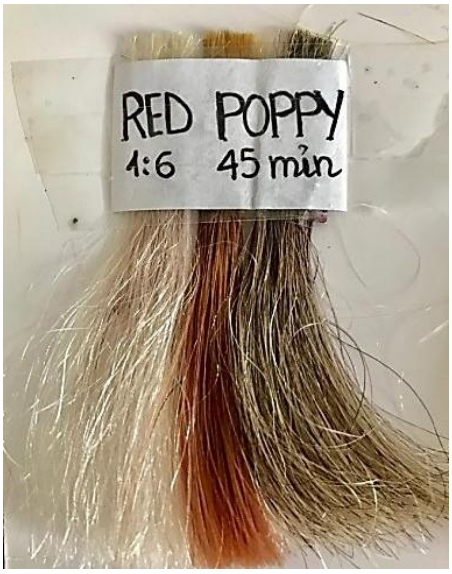
No color or a bland color

3.4.3 pH Test

All individually tested plants are characterized by an acidic pH of 2.0-3.5 to develop the color. When we added an alkaline agent, monoethanolamine, which allows to open the scales cuticle and promote the penetration of the active ingredient on the hair, to increase the intensity of the color in all cases there is a worsening of the result. As we can see from the photos, all the plants work an acid pH.

Acid pH ***Basic pH***

Acid pH ***Basic pH***



Acid pH

Basic pH

Acid pH

Basic pH

3.4.4 Synergy with other plants

Instead, adding a small amount (about 10-15%) of tea or coffee powder to the mixture to be applied enhances the intensity of the color. This effect is due to the presence of tannins, which act as color fixers on the hair. For Rhubarb, Red Poppy, Hibiscus, Sambucus, the color is more powerful than the standard reference, see figures.

Fig.3.4.4.1 (a) Barberry + Thè, (b) Barberry + Coffee

Fig. 3.4.4.2 (a) Red Poppy + Thè, (b) Red Poppy + Coffee

Fig. 3.4.4.3 (a) Hibiscus + Thè, (b) Hibiscus + Coffee

Fig. 3.4.4.4 (a) Sambucus + Thè, (b) Sambucus + Coffee

3.4.5 Miscellaneous test

We noticed that the color is more intense using:

Hibiscus : Red Poppy 75% : 25%
Juniper B : Rhubarb 75% : 25%
Sambucus : Ratanhia 75% : 25%

Yellow

Juniper : Rhubarb
75% : 25%
25% : 75%
50% : 50%

Brown

Sambucus : Ratanhia
75% : 25%
25% : 75%
50% : 50%

Red

Hibiscus : Red Poppy
75% : 25%
25% : 75%
50% : 50 %

3.5 Conclusion

Our research permitted us to discover plants to be used for hair care and color hair. Four colors have been identified: yellow (Rhubarb, Barberry, Juniper, Ginger); red color (Hibiscus, Ratanhia, Red Poppy), brown color (Sambucus, Ratanhia, Blueberry); neutral color (Uva Ursi). The degree of colorfastness is inferior to demi-permanent or permanent colors since these products do not contain peroxide or alkaline amines.

As such, they are, indeed, usually milder to hair and less likely to cause damage to hair fibers. Furthermore they are biodegradable, non-toxic, with low cost and without any side effects. Such plants are easy to find, and bear claims for water bases or natural plants. The plants are characterized with health benefits and derived from various parts of the tree such as flowers, bark, seeds, leaves and roots.

We concluded that the intensity of the color increases with the developing time, the plants work and develop color in acidic pH, Green Tea and Coffee can enhance hair color. Furthermore hair color can be enhanced by mixing plants.

4. PRODUCT FOR OXIDATION HAIR DYEING

The so-called “oxidation” dyes are the only dyes capable of giving permanent hair color in an infinite variety of shades and a perfect coverage of white hair. The formulation of almost all permanent hair dyes products uses oxidation dyes. In this class are all the dyes and color shampoos used in hair salons or for home use, offering the following possibilities:

- Cover-up of white hair in a variety of shades, in particular, the natural shades.
- Simultaneous bleaching and dyeing
- Shading after lightening in all possible tones
- Sufficient durability so that the user only requires one application a month

The different types of dyes described for human hair include, permanent or oxidation dyes, semipermanent dyes, temporary dyes or color rinses and other types of dyes used on human hair. Hydrogen peroxide and different matrix compounds used in hair dyeing are also included. The permanent dyes are by far the most frequently used and hold the dominant share of the market. [68]

4.1 Oxidation hair dyes - Permanent dyes

Oxidation hair dyes consist of dye precursors that form active intermediates, dye couplers that condense with the active intermediates, an oxidizing agent (hydrogen peroxide), and matrix compounds consisting of surfactants, preservatives, and additives for pH adjustment and ingredients for conditioning. These reactions are usually carried out at alkaline pH, generally from 8 to 10. By adjusting the proportions of oxidant, precursors, and couplers, the hair may be made lighter or darker in one process.

Permanent hair dyes should be formulated with two different compositions or parts. The first composition is a precursor-coupler base containing surfactant (to help dissolve the precursors and couplers, to assist in spreading the dye evenly over the hair, and to help thicken the product so it does not run down the face during use), alkalinity (to facilitate the oxidation reaction), a low concentration of a reducing agent (to inhibit oxidation of the precursors by air), the precursors and couplers, and water. The second composition

is an oxidizing base containing oxidizing agent, stabilizer (for the peroxide), and sometimes surfactant (for thickening during use).

4.1.1 Semipermanent, temporary dyes

Semipermanent Hair Dyes:

The term “semipermanent hair dye” refers to those products that dye the hair lasting through four to six shampoos. Table 4.1.1 depicts chemical structures of some dyes currently used in semipermanent hair dye products. To achieve the desired shade, each product contains a combination of up to as many as 18 hair dyes similar to those described in Table. These dyes are generally mononuclear, dinuclear or trinuclear species and are usually aromatic amines, amino nitrobenzenes, or anthraquinone derivatives. These dyes generally diffuse into hair and are retained by weak polar and Van der Waals attractive forces. Therefore, the affinity of the dyestuff generally increases with increasing molecular size. These products do not use hydrogen peroxide to develop the hair color. For this type of product, preformed hair dyes are required.

Therefore, no “major” chemical changes occur to the fibers during this type of dyeing. Other ingredients in these products are matrix compounds, such as solvents (primarily water and glycols or glycol derivatives), surfactants, amide, fragrance and acid or alkali for pH adjustment. Semipermanent hair dyes are generally applied to freshly shampooed hair and allowed to remain on the hair for approximately 20 min. The hair is then rinsed with water.

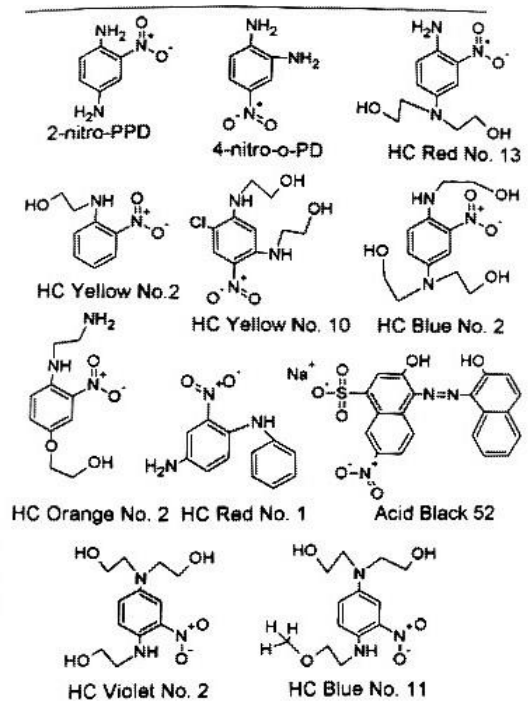


Table 4.1.1 Examples of some dyes used in semipermanent hair dye product

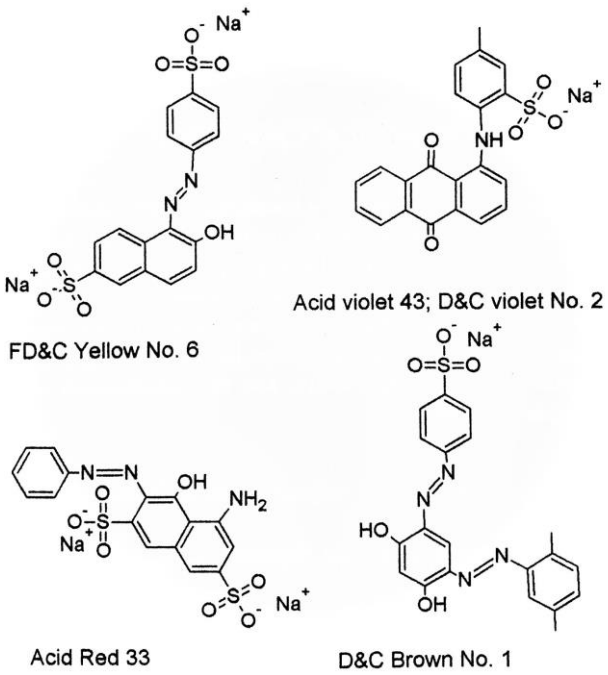
Temporary Hair Dyes:

The objective of temporary hair dyes is to provide color to the hair; color that is capable of being shampooed out of the hair with a single shampooing. Table 4.1.2 depicts structure for a few hair-coloring ingredients previously used in color rinses are described on hair dyes. Each color rinse product consists of a mixture of color, either among those described in Table 4.1.2 or similar FD&C or D&C colors. Generally two to five color ingredients are mixed to achieve the desired shade, because a single ingredient generally will not provide the desired color to the hair. Two dyes are sometimes used to provide tints for gray hair; four to five dyes are generally mixed to achieve reds, browns, or black. These products are usually applied to freshly shampooed hair and combed through. An alternative is to spray the product onto the hair and comb it into the hair to achieve even distribution of the dyes. The hair is then set and dried without rinsing to minimize penetration of dyes into the fibers. The dyes used in color rinses are generally larger molecular species than those used in semipermanent hair dye products. Color rinse dyes are generally anionic or acid dyes.

Temporary hair dye products frequently contain thickeners, a surfactant, sometimes a hair-setting polymer, and a buffer or acid such as tartaric, acetic or citric to provide an acidic medium for application of the dyes to the hair.

Table 4.1.2

Some hair color ingredients used in color rinse products



4.1.2 Chemistry of oxidation dyes

Oxidation dyes precursors are derivatives of aniline (Table 4.1.3). Precursors are difunctional ortho-or para-diamines or aminophenols that are capable of oxidizing to diiminium (IX) or quinoniminium (X) ions. Oxidations dye couplers are electron-rich aromatic species. They are commonly substituted resorcinols or meta-phenylenediamines, usually containing a vacant position para to the amine or phenolic group (Table 4.1.4). Oxidation dye precursors, when oxidized in the absence of couplers, form colored compounds, usually gray or brown-black shades. On the other hand, couplers themselves usually produce little or no color, but in the presence of precursors and oxidizing agents, they modify the color formed by the precursors. Most oxidation dye formulations contain two or three or more ingredients that act as either dye precursors or couplers.

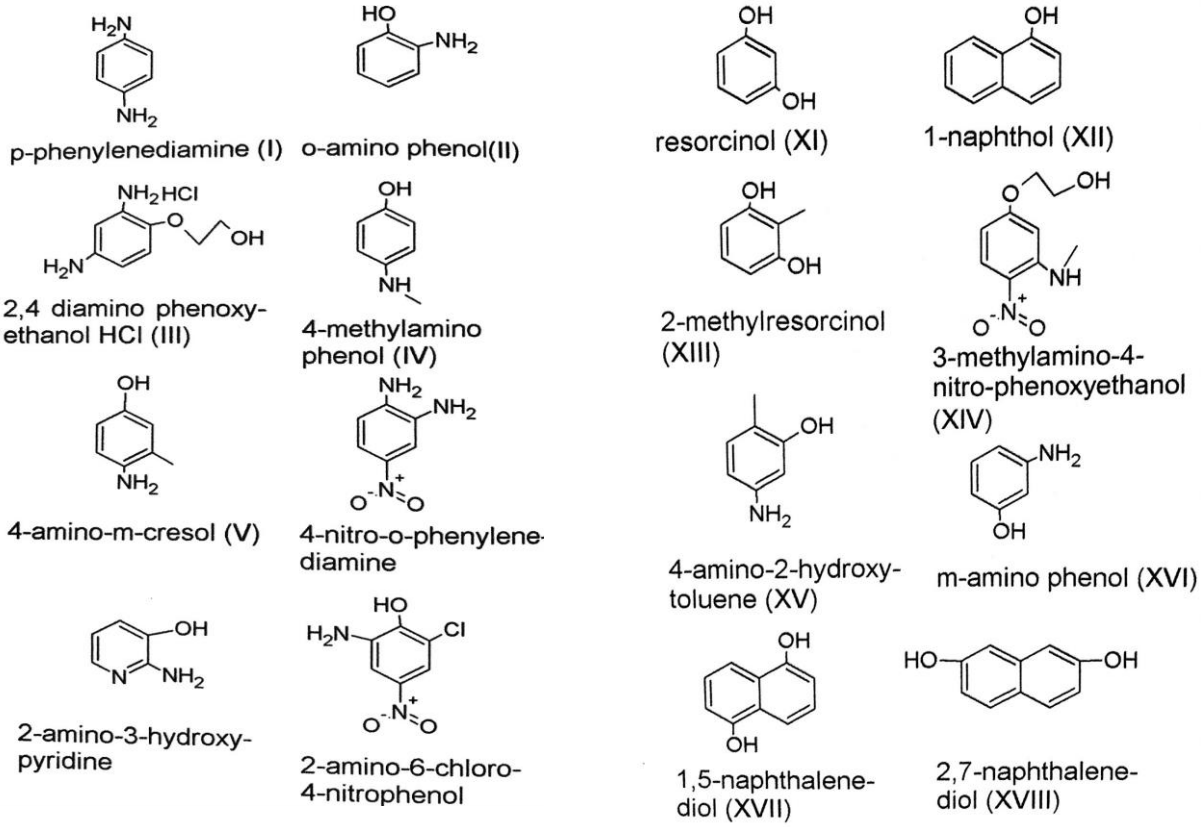


Table 4.1.3 Some oxidation dye precursors **Table 4.1.4** Some oxidation dye couplers

4.1.3 Mechanism of oxidation hair dyeing

The diimine (XXII) has been described as a vital intermediate in oxidative hair dyeing. Subsequently, the protonated diimine (IX) as the reactive species that actually attacks dye coupling agents, ultimately forming indo dyes.

Certainly diiminium are more electrophilic and are therefore more capable of serving as the active species in these reactions than diimines. As such, diiminium ions are described as the active intermediates in the mechanism. By analogy, quinoniminium ions, such as (X), would be the active intermediates formed from ortho- and para-aminophenols. If one assumes that diimines are formed by two one-electron occurs stepwise, then a diiminium ion is formed before diimine.

Most oxidation dye products contain 5-7 or more ingredients capable of acting as either dye couplers or precursors, mixtures of di-, tri-, and polynuclear indo dyes are formed in these reactions. In addition, it is conceivable that nucleophilic groups in hair might even add to the indo dyes, covalently bonding dye molecules to the hair. Penetration of the dye precursors and the couplers can occur, but penetration must be limited to the outer regions of the hair, since the condensation reactions that occur are relatively fast compared with diffusion, and the larger condensation products (at least in the hair) are resistant to shampooing.

A five step reaction mechanism explains the formation of di-, tri-, and polynuclear indo dyes that have been isolated from oxidation dye reactions.

Step 1: Formation of the diiminium ion from the dye precursor.

Step 2: The diiminium ion attacks a coupler (generally para to an amino or phenolic group), forming a dinuclear species.

Step 3: Oxidation of the dinuclear species to a dinuclear indo dye then occurs.

Step 4: Dye precursors or another molecule of indo dye may add by 1,4 addition across the indo dye, forming a trinuclear or polynuclear species.

Step 5: Oxidation of trinuclear or polynuclear species to higher indo dyes occurs.

4.2 Aims of project

In this part of the project we formulated four oxidation formulas Black, Brown, Red, Blonde following the mechanism of oxidation dyes. So the primary intermediates bound to the modifiers intermediate forming a chromogen group that reacts with hydrogen peroxide and develops the color. But these oxidizing formulations contain PPD (Para-phenylenediamine) that is a key ingredient found in the majority of permanent and semi-permanent hair dyes. It is the most common cause of an allergic reaction to hair coloring. Other chemicals in hair dyes, such as ammonia, peroxide, PTDS (para-toluenediamine sulfate), fragrance and pigments can also trigger scalp inflammation and itchiness.

In this chapter we focused on the research of a new raw material, Shield P-17 that allows a reduction of skin irritation and sensitivity reaction due to PPD/PTD and in color formulation with monoethanolamine or ammonia.

4.3 Material and method

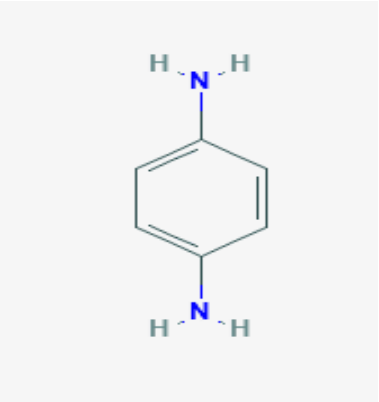
In this part we focused on the research of new raw material to reduce skin irritation and sensitivity reaction due to PPD/PTD and in color formulation with monoethanolamine. Because as we know that the most common products for hair color formulated with synthetic dyes, allow to obtain good results, but such products can damage the structure of hair. It becomes more porous and in many cases can cause allergic reaction, such as itching, redness, desquamation, etc. At the beginning I would like to give you a short description on our research, on raw material used during our project and then on the tests performed. Here is a brief description of some of the more raw material using during our project.

4.3.1 Material

Here is a brief description of raw material using during our project:

4.3.1.1 p-Phenylenediamine:

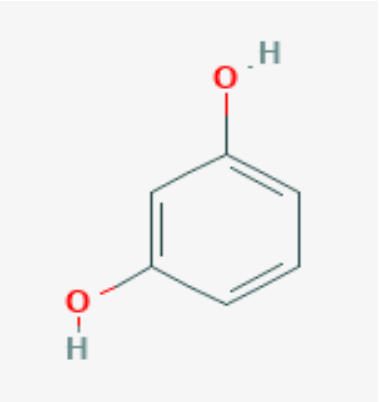
IUPAC name: benzene-1,4-diamine.



P-phenylenediamine appears as a white to purple crystalline solid (melting point 234 F) that turns purple to black in air. p-Phenylenediamine is primarily used as a dye intermediate and as a dye (e.g., hair dyes and dyes used for dyeing furs), as well as a chemical intermediate. Soluble in hot water, in alcohol, ether, chloroform. Toxic by skin absorption, inhalation or ingestion. Eczematous contact dermatitis may result from chronic (long-term) exposure in humans.[40]

4.3.1.2 Resorcinol

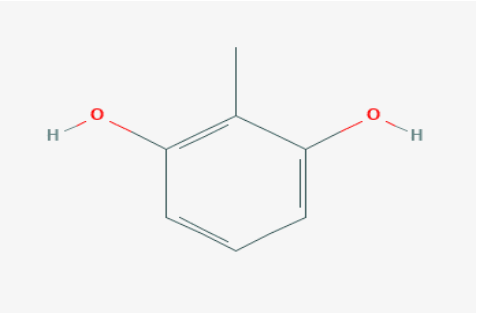
IUPAC name: Benzene-1,3-diol



Resorcinol is a very white crystalline solid that becomes pink on exposure to air and light if not completely pure. Resorcinol is a 1,3-isomer (or meta-isomer) of benzenediol with the formula C6H4(OH)2. It is a secondarily used dye. Density approximately 1.28 g/cm3. Very soluble in carbon tetrachloride; soluble in ethanol, ethyl ether; slightly soluble in benzene, chloroform, hot water 80°C. It is used as an antiseptic and disinfectant in topical pharmaceutical products in the treatment of skin disorders and infections such as acne, seborrheic dermatitis, eczema, psoriasis, etc. Irritating to skin and eyes. Toxic by skin absorption.[41]

4.3.1.3 2-Methyl-Resorcinol

IUPAC name: 2-methylbenzene-1,3-diol



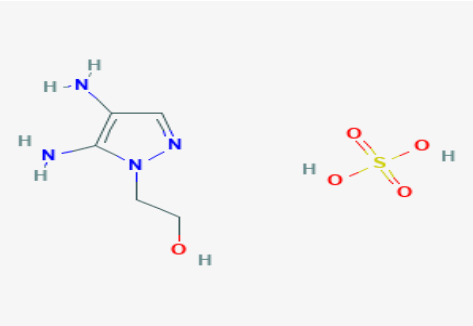
2- Methyl-Resorcinol is a very white crystalline solid. It's secondarily used as a dye intermediate and as a dye. Soluble in ethanol, ethyl ether, hot water 80°C; slightly soluble in benzene, chloroform.

It is used in products related to the hair (hair tools, hair salons, shampoo, conditioner, hair dye).[42]

Irritating to skin and eyes. Toxic by skin absorption.

4.3.1.4 4,5 Diamino-1-(2-hydroxyethyl) Pyrozol Sulphate

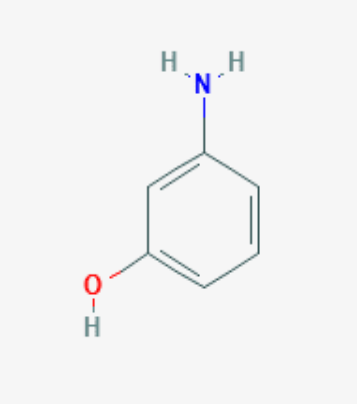
IUPAC Name: 2-(4,5-diamino pyrazole-1-yl)ethanol; sulfuric acid [46]



Used in hair colors and dyes products characterized as permanent color. Very soluble in carbon tetrachloride; soluble in ethanol, ethyl ether, benzene, chloroform, hot water 80°C. Toxic by skin absorption. Irritating to skin and eyes.

4.3.1.5 m-Aminophenol

IUPAC NAME 3-aminophenol

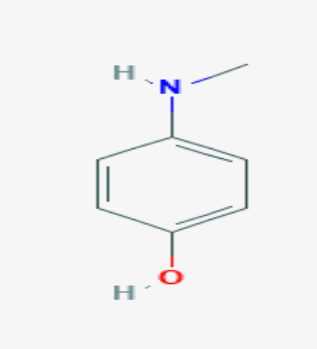


3- aminophenol is an aminophenol that is one of three amino derivatives of phenol which has the single amino substituent located meta to the phenolic -OH group.

M-aminophenol appears as white crystals or off-white flakes. Slightly soluble in benzene, toluene, chloroform; soluble in cold water; very soluble in acetonitrile, diethyl ether, ethyl acetate, acetone, ethanol, dimethyl sulfoxide, hot water

It is used for colorants, dyes, or pigments; includes colorants for drugs, textiles, personal care products (cosmetics, tattoo inks, hair dye, shampoo). [43]

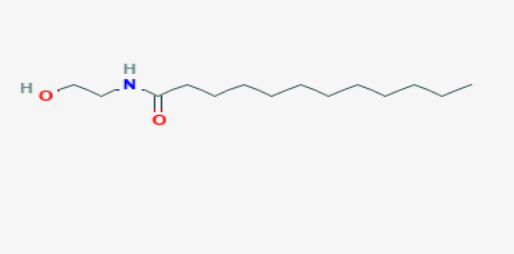
4.3.1.6 p-Methyl-Aminophenol



IUPAC 4-(methylamino)phenol

4- methylaminophenol is the phenol that is the N-methyl derivative of 4-aminophenol.[44] It is a colourless crystalline solid. It's primarily used as a dye intermediate and as a dye. Soluble in alcohol, ether, hot water. Toxic by skin absorption.

4.3.1.7 Cocamide MEA

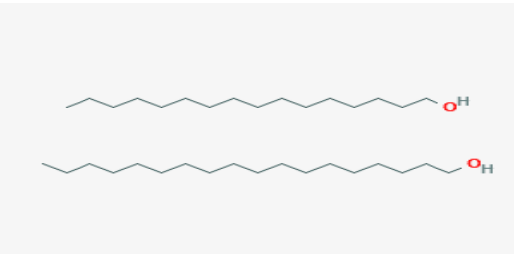


IUPAC NAME: N-(2-hydroxyethyl)dodecanamide

N-Lauroyl Ethanolamine, Lauramide MEA, Lauryl Diethanolamide are the synonyms.

N-(dodecanoyl)ethanolamine is an N-(long-chain-acyl)ethanolamine resulting from the formal condensation of the carboxy group of dodecanoic acid (myristic acid) with the amino group of ethanolamine. It is a N-(long-chain-acyl)ethanolamine and a N-(saturated fatty acyl)ethanolamine. It is fatty acyls and derives from a dodecanoic acid. Insoluble in water. Used in cosmetics as antistatic, emulsifying, emulsion stabilizing, foam boosting, surfactant, viscosity controlling, emollients, thickening agents. [53]

4.3.1.8 Cetyl Stearyl Alcohol

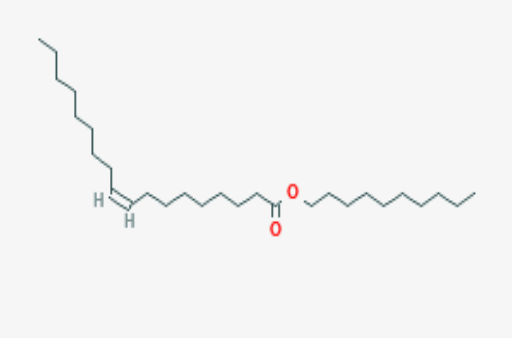


IUPAC NAME: Hexadecan-1-ol

Waxy white solid with a mild soapy odor made from cetyl alcohol and stearyl alcohol, both fatty alcohols. They usually have an even number of carbon atoms, with a single alcohol group (–OH) attached to the last carbon. Cetyl alcohol has 16 carbon atoms. Stearyl alcohol has 18. Cetearyl alcohol is a combination of the two, so it has 34 carbon atoms. Its molecular formula is C34H72O2. They are used in personal care products, mainly skin lotions, hair products, and creams as emulsifying, surfactant, viscosity controlling.[54]

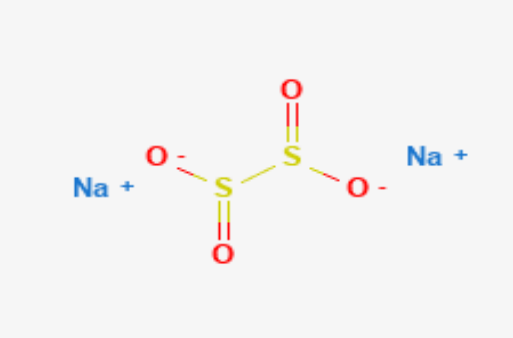
4.3.1.9 Decyl Oleate

IUPAC NAME: decyl(*Z*)-octadec-9-enoate



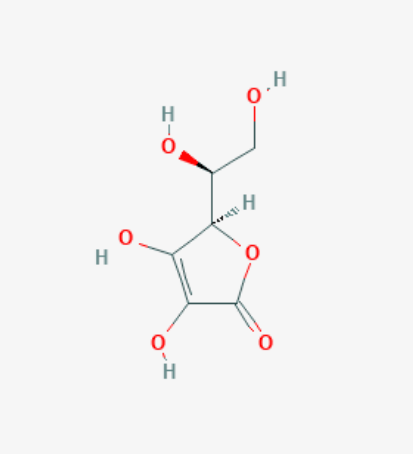
Decyl oleate is a wax ester obtained by the formal condensation of the carboxy group of oleic acid with the hydroxy group of decanol. It derives from an oleic acid and a decan-1-ol. It is used as emollient in personal care products, including cosmetics, shampoos, perfumes, soaps, lotions, toothpastes, etc. [55]

4.3.1.10 Sodium Hydrosulfite



Sodium dithionite is a whitish to light yellow crystalline solid having a sulfur dioxide-like odor. It is used in dyeing products as an antioxidant. Sodium dithionite is an inorganic sodium salt that is the disodium salt of dithionous acid. It contains a dithionite(2-). Slightly solubility in alcohol; very soluble in water; insoluble in acids. Strong reducing agent. It has a role as a reducing agent and a bleaching agent.[47]

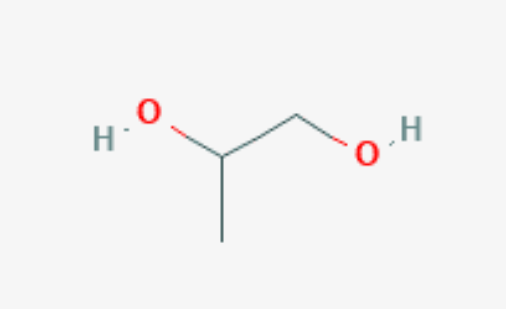
4.3.11 Ascorbic Acid



Ascorbic Acid is a natural water-soluble vitamin (Vitamin C). Found in citrus and other fruits, and in green vegetables, and deficiency of which is the cause of scurvy. Vitamin C cannot be produced or stored by humans and must be obtained in the diet. L-ascorbic acid is a white to very pale yellow crystalline powder with a pleasant sharp acidic taste. Almost odorless. Insoluble in ether, chloroform, benzene, petroleum ether, oils, fats, fat solvents. Solubility in water. [50] Ascorbic acid is

a potent reducing and antioxidant agent that functions in fighting bacterial infections, in detoxifying reactions, and in the formation of collagen in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries.

4.3.1.12 Propylene Glycol



Propylene Glycol is a propanediol that consists of propane where the hydrogens at positions 1 and 2 are substituted by hydroxyl groups. Other names for propylene glycol are 1,2-dihydroxypropane, 1,2-propanediol, methyl glycol, and trimethyl glycol.

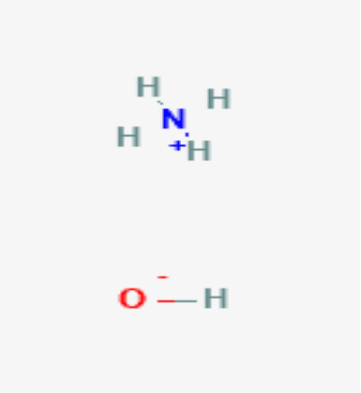
Propylene glycol is a synthetic liquid substance that absorbs water. It is clear, colorless, slightly syrupy liquid at room temperature, practically odorless and tasteless. Propylene glycol is used in the chemical, food, and pharmaceutical industries. The Food and Drug Administration (FDA) has classified propylene glycol as an additive that is “generally recognized as safe” for use in food. It is used to absorb extra water and maintain moisture in certain medicines, cosmetics, or food products. It is a solvent for food colors and flavors, and in the paint and plastics industries. Propylene glycol is used as an organic solvent that dissolves other substances (solutes), generally solids and diluent in pharmaceuticals and many other industrial applications and topical pharmaceutical preparations.

As an antiseptic it is similar to ethanol, and against molds it is similar to glycerin and only slightly less effective than ethanol. It is a better general solvent than glycerin and dissolves a wide variety of materials, such as corticosteroids, phenols, sulfa drugs, barbiturates, vitamins (A and D), most alkaloids, and many local anesthetics.

Propylene glycol is used as a skin-conditioning agent-humectant, solvent, viscosity-decreasing agent, and humectant in cosmetic formulations. It is also used in non-ionic detergents and as a humectant in the pharmaceutical, cosmetics, animal foodstuffs and tobacco industries. Propylene glycol is commonly used as a plasticizer in aqueous film-coating formulations. [51]

It is also used in cosmetics and in the food industry as a carrier for emulsifiers and as a vehicle for flavors in preference to ethanol, since its lack of volatility provides a more uniform flavor.

4.3.1.13 Ammonium Hydroxide



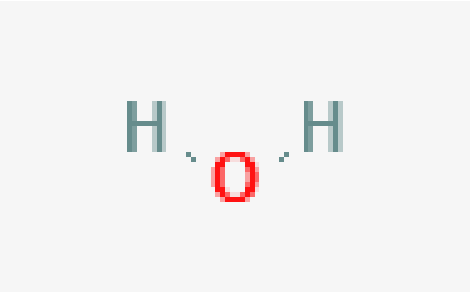
IUPAC name azanium hydroxide

Ammonium hydroxide appears as a colorless aqueous solution having an exceedingly pungent, characteristic odour. It is a solution of ammonia in water. Concentration of ammonia ranges up to approximately 30%. It has a role as a food acidity regulator. Solubility in water: miscible. Ammonia vapors (which arise from the solution) irritate the eyes.[52]

4.3.1.14 Water

IUPAC name: oxidane

Water is an oxygen hydride consisting of an oxygen atom that is covalently bonded to two hydrogen atoms. It is an oxygen hydride, a mononuclear parent hydride and an inorganic hydroxy compound. It is a conjugate base of an oxonium. It is a conjugate acid of a hydroxide. Water (chemical formula: H_2O) is a liquid at standard ambient temperature and pressure, but it often co-exists with its solid state,



ice; and gaseous state, steam (water vapor). The polarity of water is an important factor in determining its solvent properties. [49]

Very soluble in ethanol, methanol, acetone. Water dissolves some amount of virtually every solid or gas with which it comes in contact.

4.3.1.15 Shield P-17

The INCI Name is Polyacrylamide, Acrylates Copolymer, Polyvinyl Alcohol, Aloe, Barbadensis Leaf Juice, Edta, Ascorbic Acid.

Shield P17® is a clear liquid, can be added directly into the dye's preparatory formula after dyes have been added and around 60°C. Soluble in water, insolubile in oils.

SHIELDP17® represents a revolution on the hair dyes market, it' s formula allows a significant reduction of skin irritations and sensitivity reactions due to PPD/PTD and in color formulation with monoethanolamine, easy to use and does not affect the color result. Recommended dosage: 1-2 %.

Mechanism of action: The Shield P-17 (INCI Name is ***polyacrylamide, acrylates copolymer,*** **polyvinyl alcohol**, aloe barbadensis leaf juice, edta, ascorbic acid) have affinity to the keratin and inhibit the penetration of irritant hair dyes because the functional group of Shield P-17 such as polyacrylamide, acrylates copolymer, polyvinyl alcohol bond with functional group such as disulfide (-S-S), amino ($-NH_2$) and carboxylic acid (-COOH) of keratin forming a film on the skin.[56]

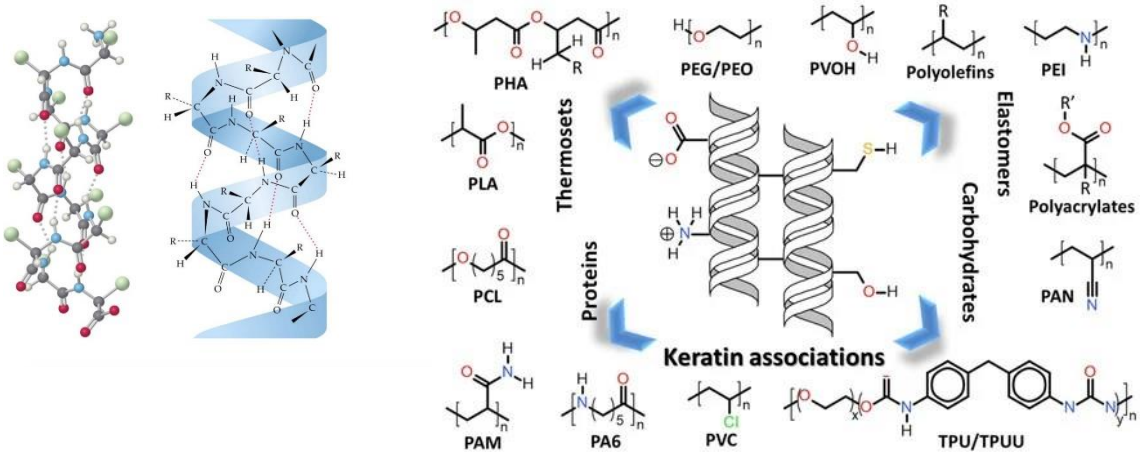


Fig. 4.3.1 The keratin affinity of some functional group

4.3.2 Method for oxidation dyes

In this part of the project we formulated four formulas Black, Brown, Red, Blonde following the mechanism of oxidation dyes. So, the primary intermediates bound to the modifiers intermediate forming a chromogen group that reacts with hydrogen peroxide and develops the color.

The study in vivo and salon test was done to evaluate scalp irritation, skin discomfort and performance during and after the application of a hair color formulated with Shield P-17.

In the end we selected four formulas for each color to verify the product stability, meaning how long the product can maintain its original form without any visible changes, its intended physical, chemical and microbiological qualities as well as functionality under appropriate conditions.

We followed this method for the realization of our project:

- Emulsification test
- Application test
- Inserting dyes
- Inserting actives
- Salon test
- Study in vivo
- Stability test

4.3.2.1 Emulsification Test

Oxidation hair dyes should be formulated with two different compositions or parts. The first composition is a precursor-coupler base containing surfactants (to help dissolve the precursors and couplers, to assist in spreading the dye evenly over the hair, and to help thicken the product so it does not run down the face during use), alkalinity (to facilitate the oxidation reaction), a low concentration of a reduction agent to inhibit oxidation of the precursors by air), the precursors and couplers, and water. The second composition is an oxidizing base containing oxidizing agent, stabilizer (for the peroxide), and sometimes surfactant (for thickening during use).

At the beginning we focus on the formulation of a base composed: water, emulsifier, thickening agents, oils, colorants, antioxidants, solubilizing, oxidizing agents, functional additives. Here is a tincture formula, see Table below:

TINCTURE FORMULA	***w/w***
WATER *(Distilled water)*	q.s to 100
EMULSIFIERS *(Cetearyl Alcohol)*	5.0-20.0
THICKENING AGENT *(Cocamide MEA)*	1.0-20.0
OILS *(Decyl Oleate)*	1.0-10.0
COLORANTS *(Precursors and couplers)*	1.0-5.0
ANTIOXIDANTS *(Ascorbic Acid, Sodium Hydrosulfite)*	1.0-5.0
SOLUBILIZING AGENTS *(Propylene Glycol)*	5.0-15.0
ALKALINITY AGENT *(Ammonium Hydroxide 30%)*	3.0-10.0
FUNCTIONAL ADDITIVES *(Shield P-17)*	0.1-5.0

Table 4.3.2.1 *Formulation*

Method of preparation:

1. To formulate the base, add the solubilizing agent to distilled water with stirring at room temperature. Add the antioxidant, reducing agent-stabilizer, dye precursors, couplers stirring until they dissolve completely and then heat at 70°C (Fase A).

2. Separately, add thickening agent, oil to emulsifiers and heat at 70°C (Fase B).

3. Add Fase B to Fase A homogenizing.

4. Then, add the alkalinity under 40°C and the functional active.

5. Cool to room temperature.

We used an emulsifier Cetearyl Alcohol and a thickening agent Cocamide MEA for the realization of a base in different ratio 3:1 2:1 1:1 1.5:1 1:1,5 1:2 in order to obtain a good structure of cream and it was easy to apply. We decided to introduce in formula an oil, Decyl Oleate to increase the hydration in different ratios from 1:1:1/2 to 1:1:1. To evaluate the structure or viscosity of the product we put a score:

- *Score 1*: Liquid product
- *Score 2*: Low viscosity
- *Score 3*: Medium viscosity
- *Score 4*: High viscosity

COCAMIDE MEA : CETEARYL ALCOHOL 3:1 2:1 1.5:1 1:1 1:1,5 1:2

COCAMIDE MEA (gr)	15	10	7,5	10	10	10
CETEARYL ALCOHOL (gr)	5	5	5	10	15	20
RESULTS (score)	2,75	2,5	2,2	3,25	3,5	4

DECYL OLEATE: COCAMIDE MEA : CETEARYL ALCOHOL 1:1:1/2 1:1:1

COCAMIDE MEA (gr)	10	10
CETEARYL ALCOHOL(gr)	10	10
OIL (gr)	5	10
RESULTS (score)	3,25	3

Evaluation test

Value	1	2	3	4
Description	Liquid	Low viscosity	Medium viscosity	High viscosity

4.3.2.2 Application Test

The tincture formula has been mixed with an oxidizing agent 20 volume in a ratio 1:1 and then the mixture has been put immediately into the yak hair and rested for a different minute.

Time of developing: The application on the hair was kept for 30 min.

Temperature: The mixture should be kept at 30 °C

Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdryer.

4.3.2.3 Inserting Dyes

For the realization of our project we formulated four colors: black, brown, red and blonde.

- We used PPD and RES to obtain the black color in different percentages.
- We used PPD and 2MR to obtain the brown color in different percentages.
- We used HPS and MAP to obtain the red color in different percentages.
- We used PMS and RES to obtain the blonde color in different percentages.

	BLACK 1	***BLACK 2***	***BLACK 3***	***BROWN 1***	***BROWN 2***	***BROWN 3***	***RED 1***	***RED 2***	***RED 3***	***BLOND 1***	***BLOND 2***	***BLOND 3***
PPD	1	0,5	1,5	0,75	0,5	1						
RES	1	1	1							0,3	0,15	0,45
2MR				0,75	0,75	0,75						
HPS							0,75	0,5	1			
MAP							0,25	0,25	0,25			
PMS										0,3	0,3	0,3

Water	q.s to 100
Oil fase	30,00
Sodium Hydrosulfite	0,15-0,3
Ascorbic Acid	0,15-0,3
Propylene Glycol	5,00-8,00
Ammonium Hydroxide 30% solution	5,00-8,00

Method of preparation:

To formulate each color formula, add the solubilizing agent (Propylene Glycol) to water with stirring at room temperature. Add the antioxidant, reducing agent-stabilizer (Ascorbic Acid, Sodium Hydrosulfite) and then the dye precursors and couplers, stirring until they dissolve completely and then heat at 70°C (Fase A) Separately, add thickening agent to emulsifiers (Oil fase) and heat at 70°C (Fase B). Add B to A homogenizing. Then, add the alkalinity (Ammonium Hydroxide 30%) at 40°C.

4.3.2.4 Inserting Actives

We selected four formulas which gave the best results and then we added 1% and 2% of Shield P-17 on each formulation.

4.3.2.5 Salon Test

Salon test: Evaluate the product performance when used in formula.

Test method:

Number of models with sensitive skin: 10

Hair color type: 5 with ammonia and 5 ammonia free

Score: 0 => not performing Score: 10=> highest performance

4.3.2.6 Study in Vivo

We did a study in vivo to evaluate scalp irritation and skin discomfort during and after the application of a hair color formulated with Shield P-17.

Test method:
Number of model with sensitive skin: 26
Number of applications: 54
Number of shades used for testing: 13

4.3.2.7 Stability of Products

We selected four formulas with 1% to verify the product stability for three months, so it means how long the product could maintain its original form without any visible changes, its intended physical, chemical and microbiological qualities as well as functionality under appropriate conditions.

Products

Black Color + 1%Shield P17

Brown Color + 1% Shield P17

Red Color + 1% Shield P17

Blonde Color + 1% Shield P17

Conditions of test:

Room (temp = 25°C)

Incubator (temp = 42°C)

Fridge (temp = 4°C)

Parameters:

Aspect (visiv)

Color (visiv)

Odour (olfative)

Viscosity (score)

4.4 Results

4.4.1 Emulsification Test

We noticed that the viscosity is the best at a 1:1 ratio, Cocamide MEA (10 gr) and Cetearyl Alcohol (10gr) but we decided to introduce in formula an oil, Decyl Oleate to increase the hydration. We choose at a 1:1:1 ratio, Cocamide MEA (10 gr) and Cetearyl Alcohol (10gr) and Decyl Oil (10gr) for our formulation and we obtained a good structure of cream and very easy to apply on the hair.

4.4.2 Application Test

Mixture: One part of tincture with one part of Hydrogen Peroxide 20 volume

Time of developing: The application on the hair was kept for 30 min.

Temperature: The mixture should be kept at 30 °C

Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdryer.

4.4.3 Inserting Dyes

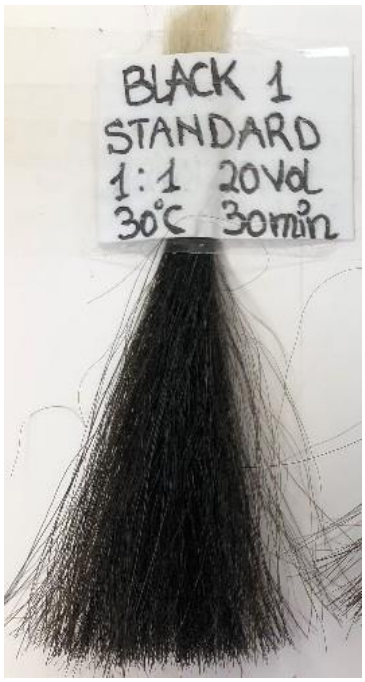
We formulated four formulas in this part of the project: Black, Brown, Red, Blonde.

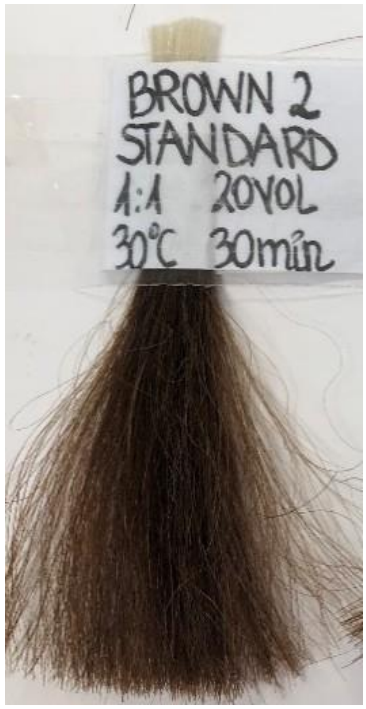
- We used PPD (as primarily) and RES (as coupler) to obtain the black color in different percentages.
- We used PPD (as primarily) and 2MR (as coupler) to obtain the brown color in different percentages for both hair dyes.
- We used HPS (as primarily) and MAP (as secondarily) to obtain the red color in different percentages.
- We used PMS (as primarily) and RES (as secondarily) to obtain the blonde color in different percentages.

	BLACK 1	***BLACK 2***	***BLACK 3***	***BROWN 1***	***BROWN 2***	***BROWN 3***	***RED 1***	***RED 2***	***RED 3***	***BLOND 1***	***BLOND 2***	***BLOND 3***
PPD	1	0,5	1,5	0,75	0,5	1						
RES	1	1	1							0,3	0,15	0,45
2MR				0,75	0,75	0,75						
HPS							0,75	0,5	1			
MAP							0,25	0,25	0,25			
PMS										0,3	0,3	0,3

As you can see from the photos when we increased the percentage of primarily (PPD, PMS, HPS) hair dyes the color is more intensive. And when we increase the percentage of secondarily (RES, 2MR, MAP) the nuance of color change. Here are the photos of the test performed.

***Parameters**: dilution 1:1, 20 volume, temp 30°C for 30 minutes on yak hair.*

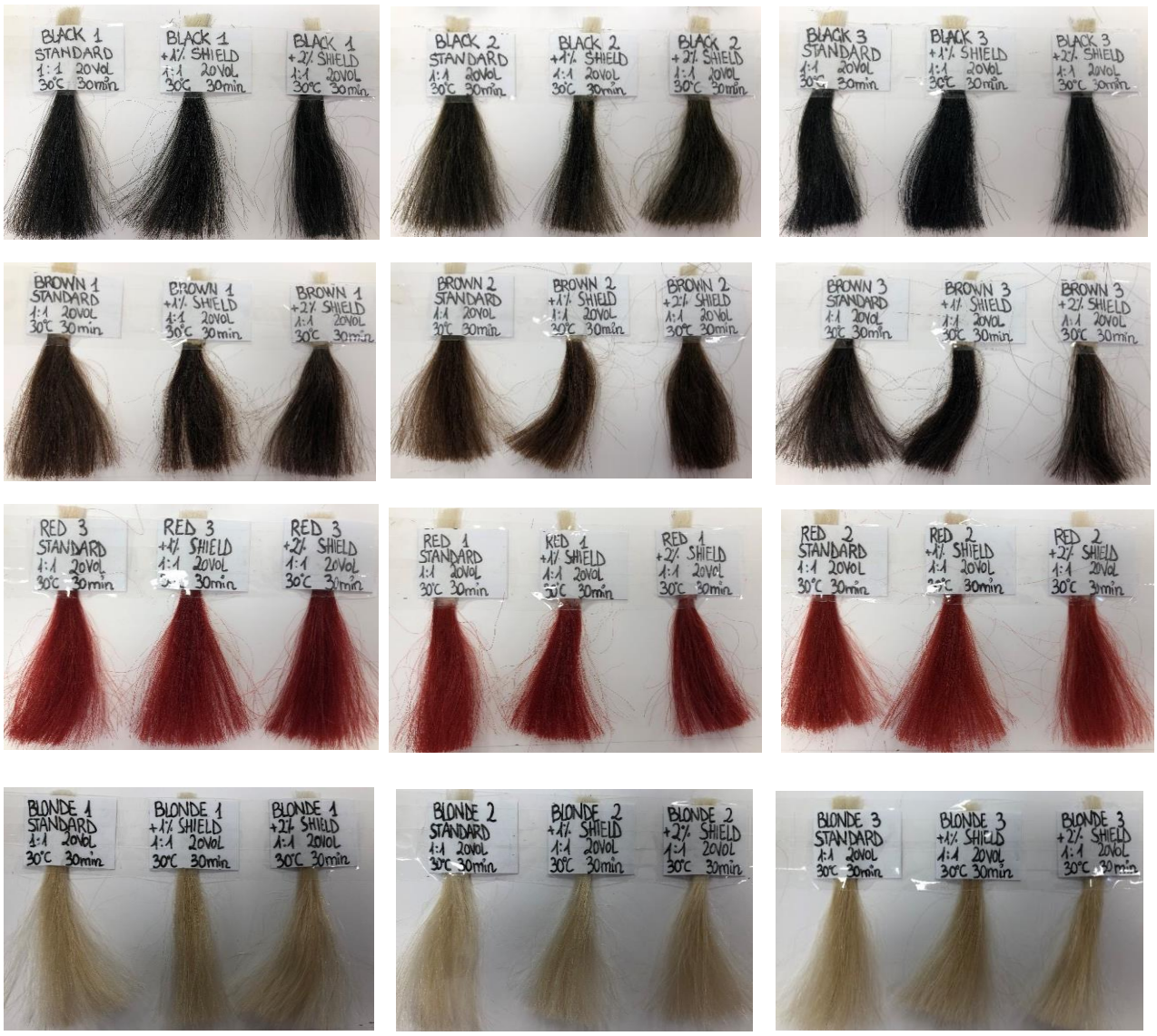




Parameters: dilution 1:1, 20 volume, temp 30°C for 30 minutes on yak hair.

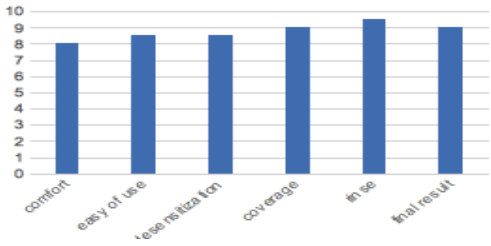
4.4.4 Inserting Actives

As you can see from the photos, we conclude that the Shield P17 used at 1% the color did not affect compared to 2%. Furthermore, we noticed that the luminosity has increased. So we recommend using the Shield P-17 at 1%.



4.4.5. Salon Test

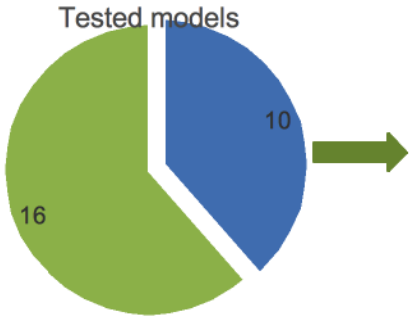
Salon test shows a good performance of Shield P-17 when used in formula, see graphic.



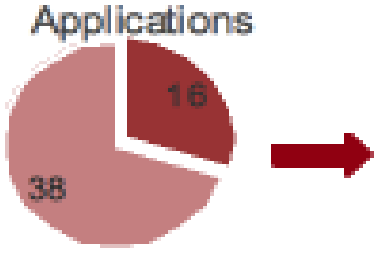
4.4.6 Study in Vivo

In vivo study shows a significant reduction of scalp irritation and skin discomfort.

- 40% of models had benefits from the application of the hair dye formulated with Shield
- Improvement of skin discomfort and irritation was observed in 30% of the applications carried out with Shield.



40% of models had benefits from the application of the hair dye formulated with Shield P17



Improvement of skin discomfort and irritation was observed in 30% of the applications carried out with Shield P17®

4.4.7 Stability

We concluded that after three month the physical and chemical parameters of our formulations didn't change, so the products were stable. The tables showed the stability of products.

Products

Black Color + 1%Shield P-17

Brown Color + 1% Shield P-17

Red Color + 1% Shield P-17

Blonde Color + 1% Shield P-17

Conditions of test:

Room (temp = 25°C)

Incubator (temp = 42°C)

Fridge (temp = 4°C)

Parameters:

Aspect (visiv)

Color (visiv)

Odour (olfative)

Viscosity (score)

Evaluation of viscosity of products

Value	1	2	3	4
Description	Liquid	Semi-liquid	Cream	Very creamy

BLACK FORMULA WITH 1% SHIELD P-17

Black Formula	**T=0**	**T=30 days**		
Characteristic		**T=Amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	Light beige	Light beige	Light beige	Light beige
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3	3	3	3

Black Formula	**T=0**	**T=60 days**		
Characteristic		**T=Amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	Light beige	Light beige	Light beige	Light beige
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3	3	3	3

Black Formula	**T=0**	**T=90 days**		
Characteristic		**T=Amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	Light beige	Light beige	Light beige	Light beige
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3	3	3	3

BROWN FORMULA WITH 1% SHIELD P-17

Brown Formula	**T=0**	**T=30 days**		
Characteristic		**T=Amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	White	White	White	White
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3	3	3	3

Brown Formula	**T=0**	**T=60 days**		
Characteristic		**T=Amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	White	White	White	White
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3	3	2,75	3

Brown Formula	**T=0**	**T=90 days**		
Characteristic		**T=amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	White	Light beige	Light beige	White
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3	3	2,75	3

RED FORMULA WITH 1% SHIELD P-17

Red Formula	T=0	T=30 days		
Characteristic		**T=Amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	Light beige	Light beige	Light beige	Light beige
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3	3	3	3

Red Formula	T=0	T=60 days		
Characteristic		**T=Amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	Light beige	Light beige	Beige	Light beige
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3	2,75	2,75	3

Red Formula	T=0	T=90 days		
Characteristic		**T=Amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	Light beige	Light beige	Beige	Light beige
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3	2,75	2,75	3

BLONDE FORMULA WITH 1% SHIELD P-17

Blonde Formula	T=0	T=30 days		
Characteristic		**T=Amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	White	White	White	White
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3,25	3,25	3,25	3,25

Blonde Formula	T=0	T=60 days		
Characteristic		**T=Amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	White	White	White	White
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3,25	3,25	3,25	3,25

Blonde Formula	T=0	T=90 days		
Characteristic		**T=Amb**	**T= 40°C**	**Fridge**
Aspect	Cream	Cream	Cream	Cream
Color	White	White	White	White
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Viscosity	3,25	3,25	3	3,5

4.5 Conclusion

We formulated four formulas in this part of the project: Black, Brown, Red, Blonde.

- We used PPD (as primarily) and RES (as coupler) to obtain the black color.
- We used PPD (as primarily) and 2MR (as coupler) to obtain the brown color.
- We used HPS (as primarily) and MAP (as secondarily) to obtain the red color.
- We used PMS (as primarily) and RES (as secondarily) to obtain the blonde color.

When we increased the percentage of primarily (PPD, PMS, HPS) hair dyes the color was more intensive. And when we increased the percentage of secondarily (RES, 2MR, MAP) the nuance of color change.

Shield P-17 can be added directly into the dye's preparatory formula after dyes have been added and around 60°C. We concluded that the shield P-17 used at 1% the color did not affect compared to 2%. Furthermore, we noticed that the luminosity has increased. So we recommended using the Shield P-17 at 1%.

In vivo study showed a significant reduction of scalp irritation and skin discomfort.
- 40% of models had benefits from the application of the hair dye formulated with Shield
- Improvement of skin discomfort and irritation was observed in 30% of the applications carried out with Shield.

Salon test showed a good performance of Shield P-17 when used in formula.

After three month the physical and chemical parameters don't change, so the products are stable.

5. PRODUCT FOR BLEACHING COLOR

Hydrogen peroxide has been used to lighten the natural shade of hair for almost 150 years. In order for this chemically induced hair bleaching to occur it is necessary to break down the melanin granules in the hair cortex that are responsible for the natural colour of the hair. This poses a significant challenge when the structure of hair fibres is considered. Hydrogen peroxide is the principal oxidizing agent used in bleaching composition, and salts of persulfate are often added as “accelerators”. The pH of these products is generally from 9 to 11. Stabilizers (e.g., sequestrants) and separate containers are often used to reduce the rate of decomposition of the peroxide and to provide satisfactory shelf life.

5.1 Hair bleaching products

A maximum hair lightening product for either stripping or frosting hair will generally consist of three different parts, the hair lightener base (alkalinity), the lotion developer (containing the peroxide) and the booster powder or accelerator containing salts of persulfate. The solution applied to the hair will be prepared just prior to use by mixing the lightener base with the lotion developer and with the booster powder. [57]

The types of hair bleaching: *Hydrogen Peroxide:* there are three types of hydrogen peroxide (solution/emulsion/cream). Professional trade 6%, 9% and 12% (20, 30 and 40vol). Stabilizers added to prevent decomposition (Sodium stannate, Chelant EDTA)

Liquid products containing alkali: Accelerates decomposition of hydrogen peroxide and the bleaching of hair. Ammonia is most effective (pH around 10).

Bleaching powders: Essential for lightening and used with peroxide and ammonia containing products as above. Often contains ammonium persulfate and sodium persulfate with fillers eg.silica. Consider toxicological properties of persulphates as they are respiratory sensitizers.

5.1.1 Chemistry of bleaching agents

The primary purpose in bleaching human hair is to lighten the hair. This goal is most readily accomplished by oxidation. However, because of the severe reaction conditions required for destruction of the chromophoric groups of hair pigments, side reactions with the hair proteins occur simultaneously. Hydrogen peroxide, the principal component of hair bleach systems, reacts faster with melanin than with hair proteins. However, since hair is primarily proteinaceous it contains a large percentage of oxidizable groups. For example hair contains thioester bonds of the cortical matrix and of the cuticle proteins. Because these groups are in the structural proteins of hair, degradation of these proteins also occurs during bleaching.

Chemicals bleaching with either alkaline peroxide or alkaline peroxide-persulfate first attacks the thioester groups that bind 18-methyl eicosanoic acid to the surface proteins. This reaction partially removes the hydrophobic surface barrier and it creates sulfur acids (primarily sulfonate groups) on and in the fiber surface. These actions provide an acidic, hydrophilic hair surface with a lower isoelectric point.[58]

Chemical bleaches weaken the cell membrane complex by oxidizing thioester between cuticle cells. Bleaches also oxidize cysteine residues of the matrix in the cortex and other hair regions rich in cysteine such as the A-layer and the exocuticle inside cuticle cells. These reactions result in breakdown of the cell membrane complex, the cuticle and cortex components and ultimately dissolves protein in these regions.

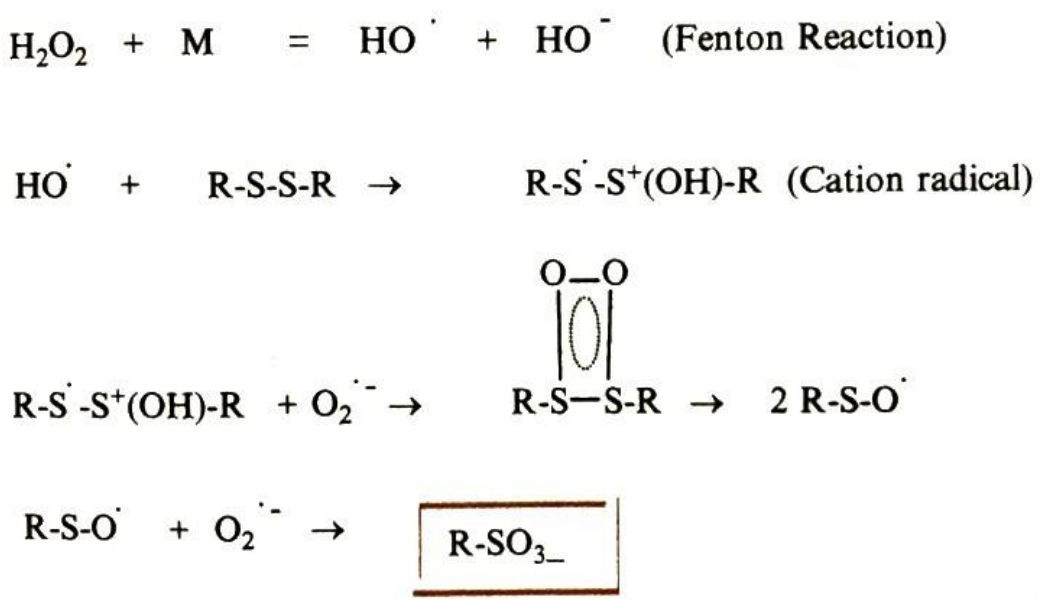
The first demonstrated that the primary reaction of oxidizing agents with the proteins of human hair occurs at the disulfide bond of cysteine. Small amounts of degradation also occur to the amino acid residues of tyrosine, threonine, and methionine during severe bleaching. The main site of attack, however, is at the disulfide bonds of the cystyl residues in the fiber. 15-25% of the disulfide bonds in human hair are degraded during “normal” bleaching, however, as much as 45% of the cystine bonds may be broken during severe “in practice” bleaching. This latter amount of damage may occur while frosting hair, or while bleaching hair from black or brown-black to light blond. [59][60]

5.1.2 Mechanism of hair bleaching

When virtually no metals are present to generate free radicals, the primary mechanism for oxidation of the disulfide bond in hair with alkaline hydrogen peroxide occurs through the monoxide and dioxide primarily via electrophilic oxygen transfer and nucleophilic oxygen insertion according to the following pathway through S-S fission.[61]

When metals like iron II or copper I are present hydrogen peroxide can react with these to form free radicals by Fenton's reaction and the oxidation mechanism follows a different pathway but still lead to sulfonate via the S-S fission pathway as summarized below:

Mechanism for the oxidation of disulfide by alkaline peroxide with Metals (Fe++ or Cu++)



The formation of the hydroxyl and perhydroxyl radicals and molecular oxygen that result in the hair by the decomposition of alkaline hydrogen peroxide in the presence of transition metal ions like iron and copper. These Free radicals induce formation of cysteic acid from disulfide in the F-layer, and other regions of the fiber during the oxidation dye process when transition metals like copper or iron are present. However the inclusion of certain chelants into oxidation dye formulations can inhibit or reduce the formation of cysteic acid at or near the hair fiber surface. These effects are explained by

the chelants binding low levels of copper known to be in some tap waters. This action by chelants (in alkaline peroxide) inhibits the known metal induced free radical formation and the resultant formation of sulfonate by the oxidation of disulfide at or near the fiber surface.

The cuticle is protected by the hydrophobic layer consisting of lipids, including 18-methyleicosanoic acid. This may help to prevent the diffusion of oxidants within aqueous dye baths into the hair cortex. Additionally, the structure of the cuticle itself forms a barrier, which also helps prevent the diffusion of substances into the hair cortex.

On immersion in water, hair fibres are known to swell. This is due to the disruption of hydrogen bonds within proteins inside the hair. However, Wolfram has shown a relationship exists between the pH and the extent of swelling that occurs. This could be rationalised by the deprotonation of protein side-chains at high pH, leading to a build-up of negative charges that repel each other. Additionally, the cleavage of disulphide bonds may contribute to the expansion of fibres. Swelling of hair fibres facilitates the diffusion of hydrogen peroxide through the cuticle layers. Therefore, ammonia can be used to assist diffusion, as it will result in an alkaline pH that leads to significant hair swelling. A combination of ammonia and hydrogen peroxide has also been shown to break up and solubilise heterogeneous melanin granules effectively. This occurs as melanin is converted into melanin free acid (MFA), which is a more soluble form of melanin. Conversion of melanin into MFA is accompanied by a slight structural change. It does not result in substantial colour change of the hair. However, solubilisation of the pigment is sometimes a prerequisite for bleaching to occur. The oxidative breakdown of melanin that follows during bleaching has been proposed to lead to voids within the hair fibre, which also contribute to hair fibre swelling.

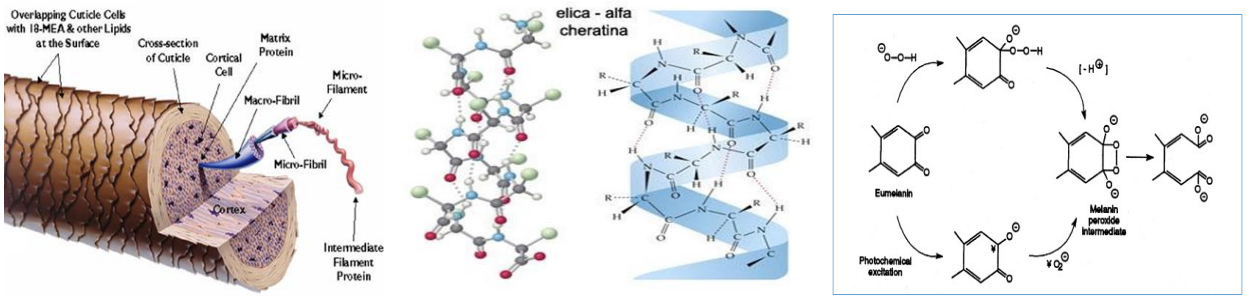
Furthemore, methionine, tyrosine, lysine, and histidine, in addition to cysteine, are degraded to the greatest extent. Methionine is also sensitive to oxidation and is probably oxidized to its sulfoxide and possibly to methionine sulfone. Tyrosine, with its electron-rich phenolic ring, is also sensitive to oxidation. The amine salts of lysine and

histidine should be resistant, although the free amines of these species may be slowly oxidized in the bleach medium, see Fig. 5.1.2.1

Since bleaching compositions are usually formulated between pH 9 and 11, the hydrolysis of peptide and amide bonds and the formation of lanthionyl residues in hair are possible side reactions during bleaching. The hydrolysis of amide groups of the residues, will increase the ratio of acidic to basic group in the fibers; i.e.,amide hydrolysis will decrease the isoelectric and isoionic points of the fibers.

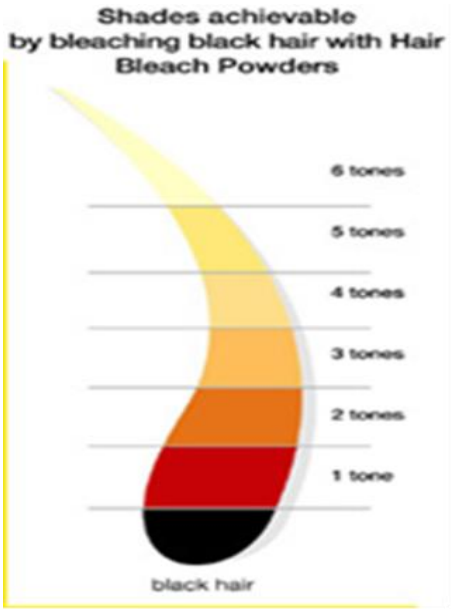
Peptide bonds are the major repeating structural unit of polypeptides and proteins. Hydrolysis of peptide bonds can occur at high pH and is most likely to occur during frosting or bleaching from black or brown-black to light blond, where long reaction times and higher concentrations of alkalinity and oxidizing agents are employed.

Fig.5.1.2.1 Mechanism of hair bleaching



5.2 Aims of project

In this part of the project we focused on the hair bleaching process. As anticipated the most common products for hair bleaching are formulated with ammonium persulphate and potassium persulphate which allow to obtain good results, but such products can damage the structure of hair. It becomes more porous and in many cases can cause irritating, redness, desquamation, allergic reaction etc.



Keeping in mind these factors we did a research of new raw materials that have the capacity to improve the bleach hair process and the sensoriality. This new raw material allows binding heavy metals by cation exchange. So it behaves as a very strong magnet for heavy metals facilitating their removal.

5.3 Material and Method

At the beginning I would like to give you a short description on our research, on raw material used during our project and then on the tests performed.

5.3.1 Materials

During our research we found a raw material of natural origin to improve the bleaching process and to improve the sensoriality of the hair. Here is a brief description of raw materials found during our research.

5.3.1.1 Zeosafe Cl-17

Zeosafe CL-07 (INCI Name Zeolite) is a zeolite clinoptilolite a natural substance which has a great scientific interest in the cosmetic world. It comes from the Greek words "zeo" boil and "lithos" stone, so it means “the stone that boils” because when it is heated, it releases water without changing the structure and it seems like a bubble. This is a mineral of volcanic origin, with a regular and microporous crystal structure, characterized by a huge amount of void volumes inside the crystals. Chemically, zeolite is a hydrated aluminosilicates with three dimensionally structures formed by SiO_4 and ALO_4, with regular channels and interconnected pores of 4° diameter, contains water and cations inside the structure (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} ..).

There are more than 100 different zeolite forms, of which 52 different types of natural zeolites, including Clinoptilolite, Mordenite and Aussie are used for their ion exchange and for absorption properties. There are about 150 forms of zeolite which have been synthesized for specific applications. Natural zeolite clinoptilolite is available in extra fine powder, without additives, preservatives or other substances. It is extracted as it is, crushed and subjected to multiple processes of sieving to obtain a dense and homogeneous powder.[66]

Properties: reduce free radicals in skin tissue, draw out impurities, protect against radiation and UV lights, regenerate cells, counteract inflammation, boost the immune system, antioxidant, immunostimulant, rigenerating, mineralising, bulking, etc

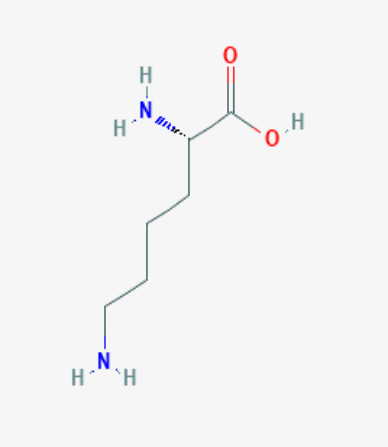
Mechanism of action: Molecular sieve characteristics, Absorbent and cation exchange capacity, High selectivity.

Its crystalline structure allows it to bind, by cation exchange, heavy metals. So zeolite behaves as a very strong magnet for heavy metals facilitating their removal.

Zeolite Clinoptilolite is not toxic and has a very long stability. The final assessment report on the safety of use of Zeolite, estimated by the CIR (Cosmetic Ingredient Review) concludes that the Zeolite is safe in cosmetic products.

5.3.1.2 L-Lysin

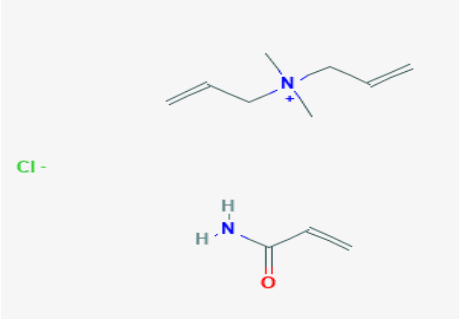
IUPAC Name: (2S)-2,6-diaminohexanoic acid



L-lysine is an L-alpha-amino acid; the L-isomer of lysine. It is a conjugate acid of a L-Lysinate. It is an enantiomer of a D-lysine. It is a tautomer of a L-lysine zwitterion. L-Lysine is a white crystals or crystalline powder odourless. Insoluble in ethanol, ethyl ether, acetone, benzene but it is soluble in water.

L-Lysine is a nutritional supplement containing the biologically active L-isomer of the essential amino acid lysine, with potential anti-mucositis activity. It has a role as a micronutrient, a nutraceutical, an anticonvulsant, a plant metabolite, a human metabolite and an algal metabolite. It is an aspartate family amino acid, a proteinogenic amino acid, a lysine and a L-alpha-amino acid. Upon oral intake, L-lysine promotes healthy tissue function, growth and healing and improves the immune system. L-Lysine appears to have antiviral, anti-osteoporotic, cardiovascular, and lipid-lowering effects, although more controlled human studies are needed. It is used as Antistatic; Hair conditioning; Skin conditioning; Anti hair loss.[62]

5.3.1.3 Polyquaternium-7



Polyquaternium-7 is an organic compound in the polyquaternium class of chemicals and used in the personal care industry. It is the copolymer of acrylamide and the quaternary ammonium salt diallyldimethylammonium chloride. The DADMAC monomer is highly hydrophilic. Absorption of moisture from the air lends "conditioning" properties to the products that contain the copolymer such as shampoos, hair and skin conditioners and other personal care products including some bar soaps. Polyquaternium-7 is used as a modifier, for example in shampoo, hair conditioner, hair spray, mousse, soap, gel, styling agent, shaving product, deodorant and antiperspirant. In cosmetics it is used as an antistatic and film forming.[63]

5.3.1.4 Rise starch

Starch is the major constituent of milled rice and its characteristics differ widely among cultivars, as reflected in properties such as the amylose/amylopectin ratio and final gelatinization temperature. Starch is composed of essentially linear amylose and highly branched amylopectin, normally 15–25 g amylose and amylopectin being 75–85 g/100 g of starch. It is well established that the amylose/amylopectin ratio is a major factor influencing the physicochemical and functional properties of starch. It is used for conditioning, restructuring, protective, etc. [64][65]

5.3.1.5 Shield P-17

The INCI Name is polyacrylamide, acrylates copolymer, polyvinyl alcohol, aloe, Barbadensis Leaf Juice, Edta, Ascorbic Acid. Shield P17® is a clear liquid. Soluble in water, insolubile in oils. Shield P-17® represents a revolution on the market, it’ s formulas have affinity to the cheratin forming a film on the skin and inhibit the

penetration of irritant components due to the functional group of Shield P-17 that it bonds with the functional group of keratin. Properties: film forming, conditioning, improved hair brightness. Recommended dosage: 1-2 %. [67][69]

5.3.1.6 Bleaching Powders

We used four different hair bleaching powders containing a high concentration of peroxide and silicate responsible for bleaching. [67] Some characteristic of bleaching powders:

- Bleaching level up to 8 tones
- Compact powders
- Formula conceived with no ammonia or low ammonia concentration
- Creamy texture
- Available in different colors
- Available in different fragrances

Benefits:

- Easy and fast application
- Excellent bleaching outcome

5.3.2 Method for Bleaching

We are following this method for the realization of our project. The bleaching products have been diluted with 6%, 9% or 12% (20, 30 or 40 volumes) Hydrogen Peroxide (developer) according to the following ratio: mixing 1 part of powder with 2 parts of developer until a creamy consistency is obtained. Leave on hair until the desired lift level is obtained. The lift level depends on the strength of Hydrogen Peroxide. *Application:* We used different types of hair: black, dark brown and medium brown hair. The application on the hair was held for 20min, 45 min and 90 min.
Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdryer.

The study: our study consists of five phases: 1) application of the bleaching powder with a new chelant; 2) the research of new actives to improve the final sensoriality; 3) the application of bleaching powder with the active; 4) the application of bleaching powder containing chelant and active substance; 5) Other test temperature, dilution, time, volume of H_2O_2.

- **Step 1** - *Comparison* Bleaching powder & Innovative Chelant
- **Step 2** - *Research Actives*
- **Step 3** - *Comparison* Bleaching Powder & Active
- **Step 4** - *Comparison* Bleaching powder & Chelant & Active
- **Step 5** - *Other test* Temperature, Dilution, Time, Volume of H_2O_2

5.3.2.1 Bleaching with Innovative Chelant Test

Four bleaching products have been diluted with 12% (40 volumes) of Hydrogen Peroxide (developer) according to the following ratio and parameters.

Mixing: Mix 1 part of powder with 2 parts of the developer until a creamy consistency is obtained and leave on hair for some minutes.

Application: The tests are performed on dark brown hair.

Time: the application on the hair was held for 45 min.

Temperature: The tests are performed at 30°C

Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdryer.

Following this method, we added 1% and 2% of zeolite in bleaching formulations to evaluate the efficacy of zeolite on the bleaching hair process and each one is compared with the standard formulations.

5.3.2.2 Research and Bleaching with Active Test

During the hair bleaching process the four products formulated with ammonium persulphate and potassium persulphate allow to obtain good results, but the hair becomes more porous. Keeping in mind these factors we did a research of new raw materials that have the capacity to improve the sensoriality of the hair and then we did some tests using each active in different percentages on bleaching powders.

The actives that we used on bleaching formulations are four:

- *1% L-Lisina,*
- *2% Polyquaternium,*
- *2% Shield P-17,*
- *3% Amido di Riso*

Mixing: Mix 1 part of bleaching powder + actives with 2 parts of developer, hydrogen peroxide 40 volumes, until a creamy consistency is obtained and leave on hair for some minutes.

Application: The tests are performed on dark brown hair.

Time: The application on the hair was held for 45 min.

Temperature: The tests are performed at 30°C.

Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdryer.

5.3.2.3 Bleaching with Innovative Chelant and Actives

Some tests are performed using the active and zeolite at 1% on bleaching formulations to evaluate if these ingredients modified the bleaching hair. Four tests are performed and each one is compared with the bleaching formulation containing actives.

- *1% Zeolite + 1% L-Lisina + Bleaching Powder*
- *1% Zeolite + 2% Polyquaternium + Bleaching Powder*
- *1% Zeolite + 2% Shield P-17 + Bleaching Powder*
- *1% Zeolite + 3% Amido di Riso + Bleaching Powder*

Method

Mixing: Mix 1 part of bleaching formulation (bleaching powder + actives + zeolite) with 2 parts of the developer, hydrogen peroxide 40 volumes.

Application: The tests are performed on dark brown hair.

Time: The application on the hair was held for 45 min.

Temperature: The tests are performed at 30°C.

Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdryer.

5.3.2.4 Other test

We did some tests by changing the temperature, the time, the volume of Hydrogen Peroxide and dilution manteing the same parameter to notice the bleaching process.

5.3.2.5 Temperature

Four tests are performed by changing the **temperature** from 30°C to room temperature and each one is compared with the standard bleaching formula.

- *1% Zeolite + 1% L-Lisina + Bleaching Powder*
- *1% Zeolite + 2% Polyquaternium + Bleaching Powder*
- *1% Zeolite + 2% Shield P-17 + Bleaching Powder*
- *1% Zeolite + 3% Amido di Riso + Bleaching Powder*

Method

Mixing: Mix 1 part of bleaching formulation (bleaching powder + actives + zeolite) with 2 parts of the developer, hydrogen peroxide 40 volumes.

Application: The tests are performed on dark brown hair.

Temperature: The tests are performed at **room temperature**.

Time: The application on the hair was held for 45 min.

Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdryer.

5.3.2.6 Time

Four tests are performed by changing the **developing time** from 45 min to 90 minutes and each one is compared with the standard bleaching formula.

- *1% Zeolite + 1% L-Lisina + Bleaching Powder*
- *1% Zeolite + 2% Polyquaternium + Bleaching Powder*
- *1% Zeolite + 2% Shield P-17 + Bleaching Powder*
- *1% Zeolite + 3% Amido di Riso + Bleaching Powder*

Method

Mixing: Mix 1 part of bleaching formulation (bleaching powder + actives + zeolite) with 2 parts of the developer, hydrogen peroxide 40 volumes.

Application: The tests are performed on dark brown hair.

Temperature: The tests are performed at 30°C.

Time: The application on the hair was held for **90 minutes**.

Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdryer.

5.3.2.7 Volume

Four tests are performed by changing the **volume** of oxygen: We used hydrogen peroxide at 20 volumes and 30 volumes and each one is compared with the standard bleaching formula.

- *1% Zeolite + 1% L-Lisina + Bleaching Powder*
- *1% Zeolite + 2% Polyquaternium + Bleaching Powder*
- *1% Zeolite + 2% Shield P-17 + Bleaching Powder*
- *1% Zeolite + 3% Amido di Riso + Bleaching Powder*

Method

Mixing: Mix 1 part of bleaching formulation (bleaching powder + actives + zeolite) with 2 parts of the hydrogen peroxide **20 volumes and 40 volumes**.

Application: The tests are performed on dark brown hair.

Temperature: The tests are performed at 30°C.

Time: The application on the hair was held for 45 min.

Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdryer.

5.3.2.8 Dilution

Four tests are performed with a different **dilution** ratio, from 1: 2 to 1:1 and each one is compared with the standard bleaching formula.

- *1% Zeolite + 1% L-Lisina + Bleaching Powder*
- *1% Zeolite + 2% Polyquaternium + Bleaching Powder*
- *1% Zeolite + 2% Shield P-17 + Bleaching Powder*
- *1% Zeolite + 3% Amido di Riso + Bleaching Powder*

Method

Mixing: Mix **1 part of bleaching formulation** (bleaching powder + actives + zeolite) with **1 part of the hydrogen peroxide** 40 volumes.

Application: The tests are performed on dark brown hair.

Temperature: The tests are performed at 30°C.

Time: The application on the hair was held for 45 minutes.

Rinse and drying test: The rinsing of the samples was carried out with warm water, and drying was done by a hairdrye

5.4 Results

5.4.1 Bleaching with Innovative Chelant Test

As you can see from the photos, we concluded that the zeolite used at 1% bleaches more, compared to 2%. So we recommend using the zeolite at 1%. Furthermore, we noticed that the sensoriality effect is reduced.

*Bleaching powder + Innovative chelant (**1% , 2%**)*
***Parameter:** dil.1:2 , 40 vol, 30°C, 45 min*

5.4.2 Research and Bleaching with Active Test

We selected four active: L-Lysine with texturizing properties, anti-hair loss, constituent of the hair; Polyquaternium as film forming and conditioning agent; Shield-P-17 with film forming, conditioning, improved hair brightness properties; Rice Starch as conditioning, restructuring, protective, softness and improved hair brightness. We concluded that the actives improved the sensoriality of hair and became more soft and brighter. Below are the tests of bleaching powder with the active ingredients.

Bleaching Powder & Active
(1% L-Lisina, 2% Polyquaternium, 2% Shield, 3% Amido di Riso)
Parameter: *dil.1:2 , 40 vol, 30°C, 45 min*

5.4.3 Bleaching with Innovative Chelant and Actives

Here are the tests performed using the active and zeolite at 1% on bleaching formulations and each one is compared with the bleaching formulation containing actives. As we see from the photos, the four conditions didn't change the bleaching process.

Bleaching powder & Active & 1% Zeolite

Parameter: *dil.1:2 , 40 vol, 30°C, 45min*

5.4.4 Other Test

Changing the temperature, the time, the volume of hydrogen peroxide and dilution we noticed that the bleaching process changed.

5.4.5 Temperature

Lowering the temperature from 30°C to room temperature has been reduced the bleaching effect compared with the standard bleaching formula.

Other test - ***Temperature***

Parameter: dil.1:2, 40 vol, 45 min, Room temperature (**23°C**)

5.4.6 Time

Increasing the developing time the bleaching effect has been increased in comparison with the standard bleaching formula. Here are the results of the test performed.

*Other test – **Time***

Parameter: dil.1:2, 40 vol, 30°C, **90 min**

5.4.7 Volume

As you can see from the photos, we conclude that the bleaching effect reduces with the lowering water volume.

Step 5 – *Other test – **Volume***

Parameter: dil.1:2, **30 vol, 20 vol**, 30°C, 45 min

5.4.8 Dilution

From tests performed we conclude that the bleaching effect is improved using the dilution 1:1, but the product was too pasty and the application on the hair was very difficult.

Step 5 – *Other test* – ***Dilution***

Parameter: dil.1:1, 40 vol, 30°C, 45 min

5.5 Conclusion for bleaching

Zeosafe CL-07 (INCI Name Zeolite) is a zeolite clinoptilolite a natural substance which has a great scientific interest in the cosmetic world. This is a mineral of volcanic origin, with a regular and microporous crystal structure, characterized by a huge amount of void volumes inside the crystals. Chemically, zeolite is a hydrated aluminosilicates with three dimensionally structures formed by SiO_4 and AlO_4, with regular channels and interconnected pores of 4° diameter, contains water and cations inside the structure (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} ..). Natural zeolite clinoptilolite is available in extra fine powder, without additives, preservatives or other substances. It is extracted as it is, crushed and subjected to multiple processes of sieving to obtain a dense and homogeneous powder.

Its characteristics as molecular sieve, absorbent and cation exchange capacity with high selectivity. Due to these characteristic permits it has a lot of use in cosmetic products as: reduce free radicals in skin tissue, draw out impurities, protect against radiation and UV lights, regenerate cells, counteract inflammation, boost the immune system.

Zeolite Clinoptilolite is not toxic and has a very long stability. The final assessment report on the safety of use of Zeolite, estimated by the CIR (Cosmetic Ingredient Review) concludes that the Zeolite is safe in cosmetic products.

Furthermore, during our project we conclude that the zeolite used at 1% bleaches more, compared to 2% on hair bleaching products. So we recommend using the zeolite at 1%.

In addition mixing zeolite with actives such as *L-Lysine* with texturizing properties, *Polyquaternium* as film forming and conditioning agent, Shield P-17 with film forming, conditioning, Rice Starch as conditioning, restructuring, protective improved the sensoriality of hair and became more soft and bright and the bleaching process didn’t change.

Changing the temperature, the time, the volume of hydrogen peroxide and dilution we noticed that the bleaching process changed.

- Lowering the temperature from 30°C to room temperature has been reduced the bleaching effect compared with the standard bleaching formula.
- Increasing the developing time the bleaching effect has been increased in comparison with the standard bleaching formula.
- Lowering the volume of hydrogen peroxide we concluded that the bleaching effect has been reduced.
- The bleaching effect is improved using the dilution 1:1, but the product is too pasty and the application on the hair is very difficult.

To reassume, Zeolite could be used to increase bleaching; Lowering the temperature reduces the bleaching effect; The bleaching effect increases with the developing time; Lowering the water volume reduces the bleaching effect; Using the dilution 1:1 improves the bleaching effect

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