Sudan University of Science and Technology

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Determination of Natural and Mineral Ingredients in Sudanese hair Cosmetic Products

تحديد الإضافات الطبيعية والمعدنية في منتجات تجميل الشعر السودانية

A thesis submitted in fulfillment for the degree of Doctor of Philosophy in chemistry

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الآية

بسم الله الرحمن الرحيم

(اللَّهُ لاَ إِلَـهَ إِلاَّ هُوَ الْحَيُّ الْقَيُّومُ لاَ تَأْخُذُهُ سِنَةٌ وَلاَ نَوْمٌ لَّهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الأَرْضِ مَنذَا الَّذِي يَشْفَعُ عِنْدَهُ إِلاَّ بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلاَ يُحِيطُونَ بِشَيْءٍ مِّنْ عِلْمِهِ إِلاَّ بِمَا شَاء وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالأَرْضَ وَلاَ يَؤُدُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ)

سورة البقرة الآية (255)

DEDICATION

To my family and friends

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Abstract

This study focused on the extraction of active natural products, to be used as hair cosmetics from some local herbal products, determination of heavy metals ,antioxidants and proteins in some natural herbal plants and animal fats used as hair cosmetic in Sudan. In the current study, 12 samples of Sudanese hair cosmetic products (3 samples were plant extracts and 9 were animal fats samples) were analysed.

Preliminary phytochemical screeninggave positive results for tannins and flavonoids, which are applied for hair as dye to give excellent color strength and smooth hair surface morphology, Active constituents of Sudanese medicinal plants were successfully extracted using 80% methanol. Hibiscus gave higher content of total tannins (189. 05 mg/dm^3) than that given by colves (48.03mg/dm^3). The latter, however, colves gave higher content of flavonoids (311.86mg/dm^3)than that given by hibiscus(54.86 mg/dm^3).Vitamin C was determined spectrophotometricly. The highest content of vitamin c was found in colves (1.155% w/v) ,the lowest in ginger (0.349% w/v) and the middle in hibiscus (0.855% w/v) .

A very simple HPLC method was performed to estimate niacine, gallic acid and ascorbic acid in ginger, hibiscus and colves samples using aqueous extract. Although ginger gave higher concentration of niacine (11.48 ppm), than that of hibiscus (1.04 ppm) and Colves (0.525ppm) and ginger gave higher concentration of gallic acid (56 ppm), than that of hibiscus (12.05 ppm) and colves (2.265 ppm). However, HPLC chromatograms of herbal samples did not show vitamin C peaks, because the method might not be suitable for its extraction.

A simple, specific, economic, precise and rapid method for spectrophotometric determination of niacin in ginger was developed and validated.
The ICP study measured higher concentration levels of zinc,(from 4.970 ppm to 34.24 ppm), magnesium (from 2813 ppm to 4185 ppm), silicon (from 43.25 ppm to 95.87 ppm) and iron (from 196.21 ppm to 437.91 ppm)in plant extracts samples higher than those of animal fats samples (from 0.000112 ppm to 32.43 ppm); The selenium concentration found to be the same in all samples (0.004993 ppm).

The result of antioxidant for colves, ginger and hibiscus showed high content of phenolic compounds and polyphenols, they gave a strong antioxidant activities. Consequently, they showed a radical scavenging effect of 90.4% ,85.7%, 20.5%, respectively, in their ethanolic extracts.The effect, on Sesame Oil (48.1%),beeswax (26.4%), camel hump fats (14.3%), lamb grease (4%) and bone marrow fats (3.1%), was alsodetermined.

The results obtained the free fatty acid content , protein content and moisture content in Sudanese hair cosmetic products. The highest moisture content and free fatty acid content in this samples were found in Lamb grease 0.9575%, 7.9% respectively. The highest Protein content in this samples was found in Bees wax 3.3667%.

المستخلص

هدفت هذه الدراسة إلي استخلاص المواد الفعالة ،تقدير المعادن الثقيلة وتقدير مضادات الأكسدة والبروتينات في بعض النباتات الطبيعية والدهون الحيوانية المستخدمة في مستحضرات التجميل الشعر السودانية. في الدراسة الحالية، كانت هناك 12 عينة من مستحضرات التجميل للشعرالسوداني (3عينات مستخلصات نباتية و 9عينات من الدهون الحيوانية).
تم إخضاع العينات النباتية (الزنجبيل، الكركدي والقرنفل) لفحص المكونات الأساسية، وأعطى التحليل نتائج إيجابية للتانينات والفلافونويدات التي يتم تطبيقها على الشعر كصبغة لإعطاء قوة لون ممتازة وسطح أملس. تم استخلاص المواد الفعالة من النباتات الطبية السودانية بنجاح باستخدام الميثانول بنسبة 80 ٪.بالإضافة إلى تقدير تركيز الفلافونويد وللتانين الكلى، أظهرت النتائج أن الكركدي أعطى نسبة أعلى للتانين(189.05ملغ/دسم^3) من تلك التي أعطت للقرنفل (48.03 ملغ/دسم^3) ومع ذلك،فقد أعطى هذا النوع الأخير من القرنفل محتوى أعلى من الفلافونويد (311.86 ملجم/ دسم^3) من تلك التي أعطاها الكركدي (54.86 ملغم / دسم3). تم إجراء تقدير طيفي باستخدام مطيافية الأشعة فوق البنفسجية لفيتامين C في هذه النباتات، تم العثورعلى أعلى نسبة من فيتامينC في للقرنفل(1.155)٪وزن / حجم، وأدنى نسبة في الزنجبيل (0.349٪ وزن / حجم) والوسطى في الكركدي (0.855٪ وزن / حجم).

تم إستخدام تقنية الكروماتوغرافيا السائلة عالية الأداء ذات الضغط العالي لتقدير النياسين وفيتامين C وحمض الغاليك في عينات الزنجبيل، الكركدي والقرنفل باستخدام المستخلص المائي، الزنجبيل أعطى تركيزعال من النياسين (11.48 جزء في المليون جزء) ،ثم الكركديه (1.04 جزءفي المليون جزء) والقرنفل (0.525 جزء في المليون جزء). أعطى الزنجبيل تركيزعال من حمض الغاليك (56 جزء في المليون جزء)، ثم الكركدي (12.05 جزء في المليون جزء) والقرنفل (2.265 جزءفي المليون جزء).لكن تسجيلات تقنية الكروماتوغرافيا السائلة عالية الأداء ذات الضغط العالي لتقدير فيتامينC في جميع المستخلصات النباتية لم تظهر، ويعود ذلك غالبا لعدم صلاحية طريقة الإستخلاص.

تم التحقق وتطوير طريقة مقترحة في الدراسة لتكون بسيطة، محددة، اقتصادية، دقيقة وسريعة لتحديد النياسين في الزنجبيل باستخدام مطيافية الأشعة فوق البنفسجية.

باستخدام مطيافية الانبعاث الضوئي بالحث البلازمي المزدوج، قمت بقياس تركيز العناصر في العينات المختلفة. أظهرت النتائج التي أن تركيز الزنك (من 4.970 جزء في المليون جزء إلى 34.24 جزء في المليون جزء) والمغنيسيوم (من 2813 جزء في المليون جزء إلى 4185 جزء في المليون جزء) والسيليكون (من 43.25 جزء في المليون جزء إلى 95.87 جزء في المليون جزء) والحديد (من 196.21 جزء في المليون جزء إلى 437.91 جزء في المليون جزء) في عينات المستخلصات النباتية كانت أعلى من عينات الدهون الحيوانية (من 0.000112 جزء في المليون جزء إلى 32.43 جزء في المليون جزء). وجد أن تركيز السيلينيوم هو نفسه في جميع العينات (0.004993 جزء في المليون جزء).

أظهرت نتائج مضادات أكسدة أن القرنفل، الزنجبيل والكركدي تحتوي على نسبةعالية من المركبات الفينولية والبوليفينول، وأعطت نشاط مضاد للأكسدة قوى. ووجد أن تأثير الجذور الحرة للعينات هو90.4٪، 85.7٪و20.5٪ علي التوالي في مستخلص الإيثانول. وأن تأثيرالجذور الحرة لزيت السمسم كان (48.1٪)، وشمع العسل (26.4٪)، وكانت دهون نخاع العظم (3.1٪) ،دهون سنام الابل (14.3 ٪)، وشحوم الإبل (4 ٪).

نتائج الأحماض الدهنية الحرة، محتوى البروتين ومحتوى الرطوبة في هذه العينات أظهرت أن أعلى محتوى للرطوبة ومحتوى الأحماض الدهنية الحرة في شحم الخروف بنسبة 0.9575 ٪ و 7.9 ٪ على التوالي. تم العثورعلى أعلى نسبة من البروتين في هذه العينات في شمع النحل 3.3667٪.

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List of Abbreviations

Abs	absorbance
°C	degree centigrade
cm^3	cubic centimeters
Conc.	Concentration
g	gram
HPLC	high-performanceliquid chromatography
ICH	International Conference on Harmonization
ICP	Inductively coupled plasma
LOD	limit of detection
LOQ	limit of quantification
M	molarity
Mg	milligram
min	minutes
MS	mass spectroscopy
N.D.	not detected
nm	nanometer
OES	optic emission spectrometry
ppm	part per million

$\bar{X}$	mean, or the average value used
SD	standard deviation
RSD	relative standard deviation
ROS	reactive oxygen species
St	standard
UV	ultraviolet /Visible spectrophotometric
v/v	volume pervolume
λ-max	the maximum absorbance of the sample
µL	microliter
FFA	free fatty acid

List of Publications

Development and validation of spectrophotometric determination for niacin content in ginger

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High-performance liquid chromatographic analysis of the extracts of ginger, hibiscus and colves herbal plant

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Chapter one

Introduction and literature review

1.1Introduction

A cosmetic product refers to any substance or preparation intended to be applied on various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or on the teeth and the mucous membranes of the oral cavity with a view of cleaning them, perfuming them, changing their appearance. correcting body odors protecting them and/or keeping them in good conditions (Pieroni2004). Since ancient times, raw materials for preparing these products have been derived from plants, animals and minerals (Schneider 2005).

Cosmetic beautification outcomes are also associated with nutritional and/or medicinal effects. Hence, emergence of natural cosmetics is a class of health and beauty aid products that combine the benefits of neutral cosmetical ingredients with the elegance, skin feel, and delivery systems .Vitamins A, C and E obtained from vegetables and fruits protect cells and tissues against damaging effect of free radicals (Mukherjee2009). On the other hand, some ingredients from natural products are incorporated in cosmetic preparations owing to their various therapeutic properties, e.g. sunscreen (skin protection effects), antiaging, moisturizing, antioxidant, antiinflammatory and antimicrobialeffects, hair stimulants, etc.(Aburjai2003).

Over the years, herbal products have been used in various civilizations of the world for the cure of human ailments. Advocates of herbal therapies say that these products are safe and efficacious due to long empirical usage and natural origin. Although phytomedicines are often claimed to be beneficial and

free of side effects, there have been reports of acute and chronic toxicity resulting from their use (Hussain 2006) .

Plants have provided mankind with herbal remedies for several diseases for many centuries. In Sudan herbal medicines have been the bases of treatment and cure for various diseases in traditional medicine. The therapeutic potentials of plant and animal origin crude drugs are being used from the ancient times by the simple processes without isolation of pure compounds. The pharmacological action of crude drug is determined by the nature of its constituents. Thus the plant species may be considered as a biosynthesis for chemical compounds such as proteins, carbohydrates, and fats that are utilized as food by animals and humans, but also for a huge number of compounds including alkaloids, terpenoids, flavonoids, glycosides etc. which exert definite physiological effects. These chemical compounds are mostly responsible for the desired beneficial properties (Pulok 2002).

Hair is a protein filament that grows from follicles found in the dermis, or skin. It is one of the defining characteristics of mammals. Hair plays a significant role in body image, and it’s one of the few physical features that we can easily change to create a totally different style. Attitudes towards hair cosmetics vary widely across different cultures and historical periods, but it is often used to indicate a person's personal beliefs or social position, such as their age, gender, or religion.

Cosmetics and techniques have therefore been used to change hair appearance since time immemorial. The cosmetics industry has

developed efficient products that can be used on healthy hair or act on concomitant diseases of the hairand scalp (Sherrow 2006).

1.2 Classification of Hair care cosmetics

Hair care cosmetics include waving, straightening, conditioning and coloring. Conditioning is hair cleansing cosmetics, hair growth promoters and hair grooming cosmetics.

The growth of hair is a complex metabolic process requiring multiple steps, nutrients, enzymes, vitamins and reactions. If any of these nutrients or vitamins are missing, hair won't be able to grow to its full potential.

1.2.1 Hair cleansing cosmetics

Hair cleansing cosmetics remove dirt from the scalp and hair, keep it in a clean condition and make it easy to manage. It is not just enough to remove the dirt: the feeling one has during and after washing the hair and the care given afterward is also very important.

1.2.2 Hair coloring cosmetics

Dye is any substance, natural or synthetic, used to color various materials. A dye can generally be described as a colored substance that has an affinity to the substrate to which it is being applied. Each species contains a large amount of alkaloid , flavonoids and tanning materials (e.g. caffeic acid, chlorogenic acid, ellagic acid, gallic acid). Flavonoids , tannins and ascorbic acid is used traditionally as a colored substance (Szentmihályi 2004).

1.2.2.1 Flavonoids

The flavonoids are a large family of polyphenolic compounds synthesized by plants and structurally derived from the parent substance flavone. Flavonoids present in fruits and leafy vegetables are thought to provide potential and versatile health benefits through radical scavenging and chelating activity. Many studies have suggested that flavonoids like are well-known for its anti- inflammatory, anti-allergic, anti-thrombitic, anti-spasmodic and anticancer properties (Kumar 2015). Each different fruits and leafy vegetables are capable to display different extent of antioxidant activities owing to the presence of varied amount of free phenolic and flavonol contents. Ascorbic acid, a water soluble vitamin is essential nutrient in human diets and found mainly in fruits and vegetables. Due to the remarkable antioxidant properties of this compound, it is widely employed in pharmaceutical and cosmetic industry and also exerted several biological activities (Prabhakar 2010).

Thus, the presence of an appreciable amount of ascorbic acid, flavonoids and phenolic acids in these plants are inferred. The antioxidant activities of the extractive solution represent an important parameter to evaluate the biological property of the plant. Therefore, it is necessary to characterize and quantify the important compounds present in the plant and also to validate the method of separation and identification of active constituents.

The antioxidant activities of the flavonoids are due to their ability to reduce the free radical formation and hence exhibit several biological activities (Trupti 2016).

1.2.2 .2 Tannins

Tannins (commonly referred to as tannic acid) play an important role and have wide applications. Tannins are polymeric phenolic compounds with numerous hydroxyl groups and quite diverse in chemical structure. Literature shows that tannins were extracted by different procedures and techniques. Polyphenols and related

structures are responsible for the antioxidant processes in the human body system (Vijay 2014) .There are water-soluble polyphenols that are present in many plant foods. Tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation(Katie 2006).The anti-microbial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins.

Plant extracts, ascorbic acid, anthocyanins , ferrous sulfate and tannins had excellent dye ability. Plant extracts could be applied for hair dyeing to give excellent colour strength, a smooth hair surface morphology and high-affinity interaction (Panthip2012).

1.2.3 Types of dyes

Types of dyes are: natural and synthetic dyes; sources of natural dyes are plant, vegetable, mineral, insect and animal origin (Panthip2012).

Dyes are divided into types depending on the duration of survival and also on the composition.

(A) There are three types of dyes depending on the period of survival, namely:

i. Temporary dyes: They are easily wiped once shampooing.
ii. Semi - permanent dyes: filter down to the borders inside the hair.
iii. Permanent dyes: They intervene in hair and depth of these pigments. Interact materials shall be large molecules and thus would be unable to get out of the hair when they are washed.

(B) There are three types depending on the installation:

i. Vegetable dyes: Vegetable dyes can usually be recommended to patients sensitized to oxidative dyes, because of their low allergenic power (Garcia 1997). The use of natural dyes on the hair has not made great progress

for several reasons. Firstly, of the fact that natural dyes are not very stable in solution, and are prone to oxidation, discolouration, pH colour shift and fading. Secondly, a single natural dye may not give the right colour, and only henna or walnut seem to be suitable to colour the hair. However, many shades can be obtained by mixing with the leaves of other plants .The leaves of Lawsonia inermis, known as henna, has been applied since ancient times for decorating and dyeing hands and feet, to impart shades of dark red, and for the treatment of certain skin disorders. The compound lawsone, a brown powder isolated from the leaves, is responsible for the red colour in henna. It is used as a staining agent, due to the strong binding of lawsone to the hair, probably upon reaction of thiol groups with keratin (Ali and Sayeed 1997). If the hair is dyed with henna and then treated with a hot decoction of Allium cepa (onion) skin, a coppery colour will be obtained. The incidence of contact dermatitis from using henna appears to be rare but possible (Garcia 1997). A dull golden yellow colour is obtained using apigenin, a flavonoid which occurs widely in plants, but is usually obtained from German chamomile flowers, which is used as a hair rinse for fair hair (Carle 1992). A red colour is obtained from the extract of Hibiscus sabdariffa (Malvaceae), commonly called Karkadeh or red sorrel. Its main red coloured components, anthocyanidins known as delphinidin or cyanidin, can be used, but the intensity of the red colour is a function of pH of the solution (Nour 1993).

ii. Mineral pigments: Salts of heavy metals, including the lead acetate with sulfur precipitator,cause a great damage to the body.

iii. Synthetic organic dyes

One of the broadest colours in which the colours range to meet all aesthetic desires and enter into the hair is a permanent dye lasts several months.

Studying something like hair dyes can be even more complex because it can contain thousands of different chemicals. On top of this, the ingredients in hair dyes have changed over the years. Early hair dyes contained chemicals, including some aromatic amines.

Some people might want to avoid or limit exposure to hair dyes for other reasons. For example, some of the ingredients in hair dyes can cause serious allergic reactions in some people. Hair dyes can also actually cause hair loss in some people. Some doctors advise women to avoid having their hair dyed during pregnancy.

1.2.4 Advantages of natural dyes

- Natural dyes have pharmacological effects and possible health benefits.
- They are obtained from renewable sources.
- Natural dyes cause no disposal problems, as they are biodegradable.

Dyes embellish the hair by bleaching or colouring it briefly, for temporary periods of longer duration, or permanently, depending on the composition of a dye and its degree of penetration of the hair shaft. possible side effects (contact eczema, cancer, increased porosity, brittleness) can extend to an understanding of cosmetic resources that also treat hair and scalp conditions (Guerra2014).

1.2.5 Hair growth stimulants

Hair growth promoters are applied to the scalp to normalize its functions. By increasing the circulation in the scalp, they improve the hair follicle function that promotes hair growth and prevents hair loss. They also help to prevent dandruff and itchiness (Claude 1997).

Recently, various plant extracts have been patented for use in hair-growth or hair-tonic products, and for the prevention of alopecia. The patents claim that the effects are due to stimulation of the hair follicle or scalp metabolism, possibly due to an acceleration of blood circulation, activation of dermal papilla, anti testosterone action, or increased nutrition to the hair follicles through accelerated blood flow, but the mechanisms are not yet clear (Lee 1997, Kameyama 1995).It was discovered that proanthocyanidins extracted from grape seeds promote proliferation of hair follicle cells in vitro and that they possess remarkable hair cycle-converting activity from the telogen phase to the anagen phase in vivo (Takahashi 1998). Studies suggested that Ginkgo biloba leaf extract also promotes hair regrowth, through combined effects on proliferation and apoptosis of the cells in the hair follicle, thus uggesting potential as a hair tonic (Kim 1998, Kobayashi 1993). Other plants such as henna, aloe, rosemary and sage are claimed to have effects on hair growth, but still need more study and clinical trials to substantiate their folk medicine use (Grindlay 1986).

1. 3 Some plants used in hair cosmetics

The use of some natural products in hair cosmetic, due to their low mammalian toxicity, is described including plant parts used, the actives responsible for effect and the benefits of such products. They are used in hair care as hair growth stimulants, hair straightening cosmetics, hair conditioning cosmetics, hair colorants, and for hair and scalp complaints such as dandruff. Essential oils when incorporated into finished products impart many benefits such as a pleasant aroma in perfumery, shine or conditioning effects in hair care products. The use of plant extracts in cosmetic formulation is increasing, mostly because of the poor image that animal-derived extracts have acquired .Plant extracts, are often ill-defined as to the method of extraction, plant-to-solvent ratio and the content of active ingredients. Moreover, the stability of the colour, odour, transparency and/or active ingredients with

time is also often a limiting factor. Plant extracts are different in several respects from purified therapeutic agents. Firstly, they are more dilute than the pure chemicals that are familiar to us; secondly herbs often contain additional active principles that may be closely related both chemically and therapeutically to the constituent primarily responsible for its effects. Herbal‘total extracts’ as well as ‘selective extracts are used in cosmetics. Total extracts are applied mainly according to the historical tradition of their use. On the other hand selective extracts are employed more after investigating their specific activity. Some selective extracts are introduced for different areas of use: licorice for skin irritations, ginkgo as a free radical scavenger, bearberry for skin lightening (complexion);walnut for skin tanning , and wheat germ for stimulating cell proliferation (Marks 1997).

1.3 .1 Hibiscus sabdariffa

In Sudan this plant is used to make a popular beverage known as Karkadia drink .Hibiscus plants grown in Syria and in southern Iraq and Upper Egypt , central and western Sudan and many other Arab countries. The colour of some parts of trees was mainly due to the presence of betacyanins (Tseng 2006).Hibiscus is a typical plant of tropical climates found in the regions of mangroves in significant quantities. Previous pharmacological investigations of the genus Hibiscus plants indicated the presence of species with useful biological activities. The studies conducted to date have demonstrated that plants of the Hibiscus containing anthocyanins that use for hair dyeing (Rosa RM2006).

Hibiscus plant has pharmaceutical and cosmetics benefits. Investigations revealed that the plant is highly rich in vital minerals and nutrients such as iron, copper, calcium, magnesium, manganese required for healthy growth in humans. It is rich also in vitamins, natural carbohydrates, proteins, tannins, gums and other antioxidants including minerals (Salah 2002, Panthip 2012).

The preliminary phytochemical analysis showed that there are some plant chemicals present in the extract such as alkaloids, tannins, saponnins, glycosides, phenols and flavonoids. The intense red-colored Hibiscus sabdariffa flowers are an inexpensive source of anthocyanins with potential to be used as natural, innocuous, and health-beneficial colorants, in hair colouring (Grajeda 2016).The plant is widely cultivated for its well known medicinal properties(Guerra2014).

1.3.2 Henna

Henna or Lawsonia has been cited as a growth accelerator and was used in ancient Egyptian formula to cure the loss of hair (Dweck 1997). Henna leaves (as in figure 1 .1) have been used as a hair dye for thousands of years in north Africa, the Arabian Peninsula, the Levant, and South Asia. Henna leaves have red-orange dyemolecule, lawsone, which penetrates skin and hair and bonds to the keratin. Henna dye blocks UV rays so hair doesn't become sun damaged, strengthens your hair so it won't get split ends, makes your hair glossy and shiny, eliminates dandruff and ringworm, and kills head lice and nits. Allergic reaction to henna is extremely rare.

Figure 1 .1 Henna leaves

The incidence of contact dermatitis appears to be extremely rare with the use of henna(Garcia 1997), since henna leaf extracts have mild anti inflammatory and antiallergic action and analgesic effects

,it is used to stimulate growth of hair (Yonenaga 2001,Ali 1995).When henna paste coats the hair shaft , Lawsone gradually migrates from the henna into the hair shaft and bind with keratin (Figure 1 .2).

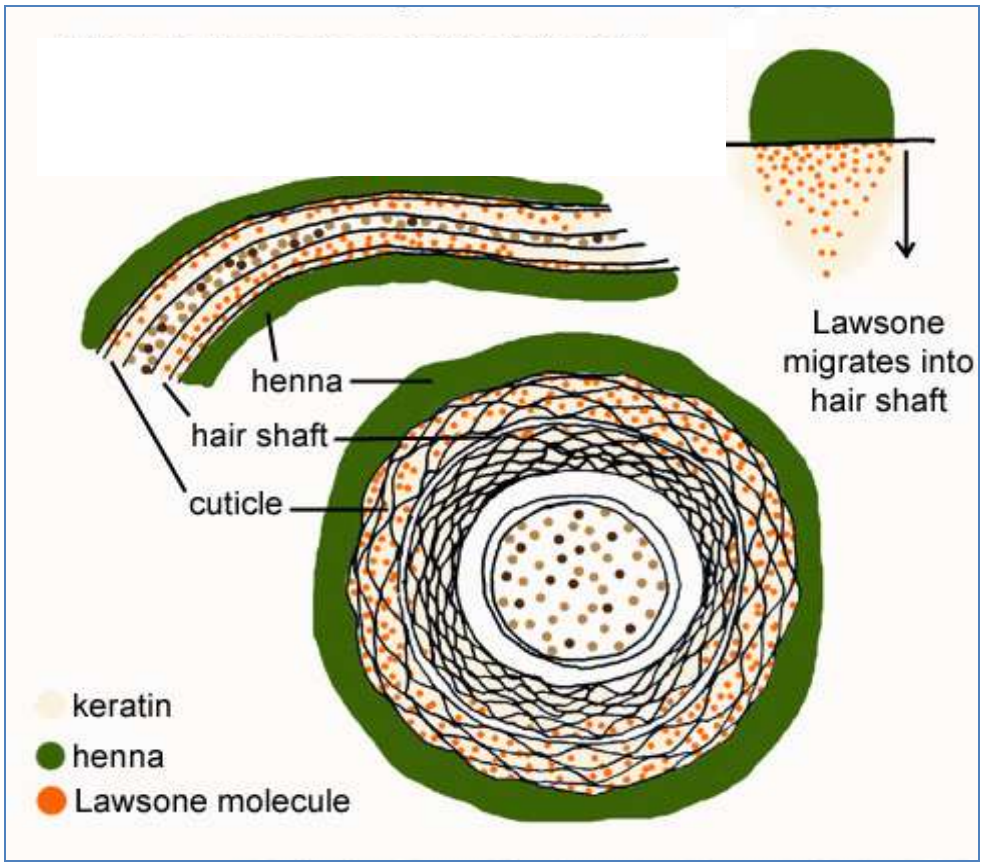


Figure 1 .2A diagram of hair that has been hennaed.

A schematic diagram hennaed hair(figure 1.3) shows that its hennaed hair is the reflection of natural hair colorand Lawsone bound into the keratin, and it slimmers red when light bounces off of Lawsone molecules in the outer layers of the hair shaft.

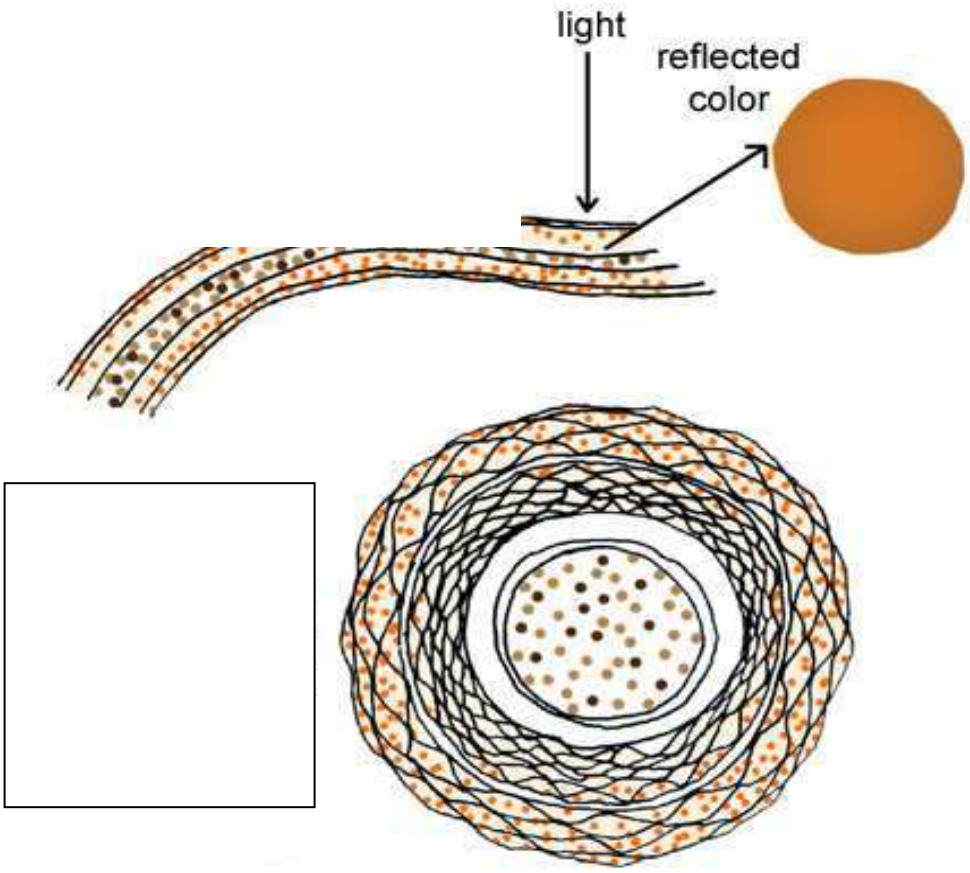


Figure 1 .3 Diagram of henna on hair.

Most cultivated henna has lawsone levels of 1% and is sold to the hair dye industry. Hair quality henna is not finely pulverized or

sifted, does not provide a robust, permanent color, and is hard to rinse from hair. It is cheaply grown, processed and sold. The lower dye content in this henna is altered by the addition of other dyes, metallic salts, and para- phenylene diamine (Mehandi2006).

1.3.3 Salvia officinalis

Salvia officinal or Meramia is used as a lotion to improve the condition of hair and skin also used as a hair dye. Claims of its use, alone or with rosemary, to maintain the sheen of dark curly hair, and to strengthen and stimulate hair growth have been made (Dweck1997).The major constituents responsible for the effect on hair are the tannins, saponins, as well as borneol and camphor (Boiceanu1986).

The genus Salvia, composed of more than 900 species worldwide distributed, is well known for its various uses, including therapeutic ones. Many species within the genus exhibit activities such as antioxidant, anti inflammatory, antimicrobial, etc. The chemical composition of these species includes poly phenols, flavonoids, terpenes, which induces such activities. An extract of sage massaged into the scalp can control dandruff, falling hair or loss of hair if the papilla is dormant and not destroyed. Rosemary is claimed to be a conditioner for greasy hair, a rinse and a tonic that give body and sheen to hair, and infused fresh or dried, rosemary and sage can be used as a daily rinse for dandruff treatment (Cadirci 2012).

1.3.4 Tea

Camellia sinens is (Theaceae) yields both black (red) and green tea. Black tea results from tea leaves that have undergone a fermentation process, while to produce green tea, the leaves are steamed immediately after harvest and then dried. Tea contains more than500 chemical compounds, including tannins, flavonoids, amino acids, vitamins, caffeine and polysaccharides. The amount of vitamin C in 100 g of green tea is about100 mg, which is similar to that found in lemons. In black tea, however, more than 90% of the vitamin C is destroyed during the fermentation process. Green

and black teas contain similar amounts of vitamin B6 (nicotinic acid), vitamin E and vitamin K (Pietta 1998, Lee 1997). Tea flavonoids (poly phenols) have proved to anti inflammatory, antioxidant, antiallergic, antibacterial and antiviral effects, while the tea tannins have antiseptic and antioxidant effects (Katiyar 2001, Elmets 2001, Schreiner 1999).

1.3.5 Clover

Clover extracts from red clover flower applied for hair straightening cosmetic ingredient. It is one of the medicinal plants known for its therapeutic benefits since ancient times, and is used in several forms either fresh or dried or in the form of powder or oil (Widyarini 2001).

Figure 1 . 4 clover flower

1.3.6 Onion

Androgenetic alopecia is an inherited form of hair loss. Onion (Allium cepa) is recognised with healing qualities including their

antibacterial, cleansing, stimulating, and nourishing powers. Onions contain a number of important minerals and vitamins, such as vitamins C and B6, calcium, magnesium, potassium, and germanium. Onion also has high sulphur content. Sulphur is a mineral present in every cell in our body, with its greatest concentration in hair, skin and nails. It has often been called the "beauty mineral" and the "healing mineral" because of its ability to promote circulation and decrease inflammation. These qualities also lend to the theory that adequate amounts of sulphur can jump-start hair growth in people with deficiencies. High amounts of sulphur in onions make them particularly effective in regenerating hair follicles and stimulating hair regrowth (Rahul2012).

1.3.7 Ziziphus spina-christi

Ziziphusspina - christi, (Rhamnaceae) is a multipurpose tree distributed long the entire sahelian area from Senegal to Sudan and from middle to west Asia as well. In Palestine it occurs along the coast and in the Jordan Valley area. It has been used for different disease treatment in traditional medicine such as cholesterol reduction, treating eye inflammation and hair loss.

Figure 1 .5Ziziphusleaves

All parts of Ziziphus spina- christi contain important nutrients and phytochemical compounds. The fruit is rich in carbohydrates

(Saied 2008). The seeds contain 28.5% lipid and 18.6% protein (Nazif 2002). The leaves(figure 1 .5) are rich in iron, calcium, and magnesium .The plant extract of Ziziphus uses as cleanses, prevents dandruff infection and bacteria (Ali 2012).

1.3.8Ginger

Ginger is one of the medicinal plants known for its therapeutic benefits since ancient times, and is used in several forms either fresh or dried or in the form of powder or oil. Ginger contains many minerals, vitamins and oils as well as antioxidants and so it has many medical and aesthetic benefits also, It makes hair more manageable, softer and shinier(Shady2013).
It improves blood circulation in the scalp, which promotes hair growth. Free radicals fight by containing antioxidants, and this protects the hair cells from laminating and falling and provides softness (Minaz 2017). It treats the dandruff because it contains disinfectant against germs. It also reduces inflammation of the scalp owing to its antiseptic properties. It has antibacterial and anti-inflammatory properties which fight many different scalp problems caused by harmful microbes (Megan 2016). It makes the scalp healthy and clean and protects it from dehydration (Minaz 2017). It thickens, helps increase its length and prevents hair shattering(Melh 2017).

1.3.9 Garlic

Garlic or Allium sativum (L. Liliaceae) (Figure 1 .6) can help to control dandruff. It has been used since ancient times as a vegetable with many properties, including antiseptic, tonic, antioxidant, antiinflammatory (Agiga 2000), anti bacterialand antifungal effects (Ankri 1999, Hughes 1991). Garlic should not be placed directly on the skin since it may cause blisters and a burning sensation in some people or contact dermatitis and allergic reactions in others (Siegers 1992). Each species contains a large amount of flavonoids and tanning materials (e.g. caffeic acid, chlorogenic acid, ellagic acid, gallic acid) (Szentmihályi 2004).

Figure 1 .6Garlic

1.4 Useful ingredients in hair cosmetic products

There some useful constituents such as:

1.4 .1 Anthocyanin pigments

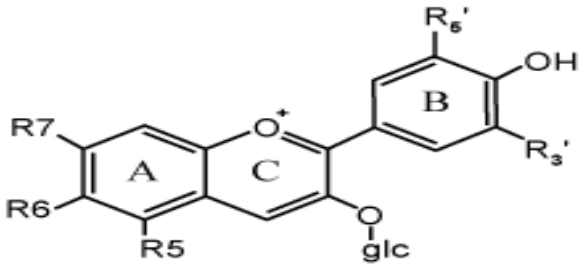


Figure 1 .7 Structure of Anthocyanidins

Anthocyanins are derivatives of anthocyanidins (figure 1 .7) which include pendant sugars .The common sugars found in anthocyanin are glucose , galactose and rhamnose .The differentshadeoftheflowerareduetothepresenceofsomeanthocyanini ndifferentmedia(i.e.theiracidicsaltsarered,alkalisaltsareblueandfree anthocyanins are violet.).

1.4 .2 Principle of natural dyes

i. Most natural dyes need both a plant extract and a mineral mordant to make a permanent colour.

ii. The stronger the dye extract , the more plant used , the deeper the colour.
iii. Mineral (metal salt).
iv. Time, Temperature , Concentration are the variables involved in any chemical reaction. Higher temperature means less time needed for dyeing, as does higher concentration of dyestuff. Mordants are those compounds which bind the natural dyes and prevent the colour from either fading with exposure to light or washing out Hence mordant is a chemical which enables the fabric to take up the colour of the dye.

Metallic salts, tannins (tannic acid) and oils are also used as mordants. Generally cotton mordanted with these mordants. These mordant impart affinity for basic dye.

1.4 .3 Betacyanins

Some useful hair Colouring cosmetics are Betacyanins pigments in hibiscus flowers. The colour of some parts of trees was mainly due to the presence of betacyanins. Betacyanins were identified by HPLC-PDA-MS as betanin, isobetanin, betanid in, isobetanidin, and phyllocactin (Obedosorio 2011).

1.4 .4 Ginseng

Ginseng refers to a wide spectrum of distinct species with different appearances and medicinal qualities, which grow or are cultivated in different geographical locations. Several classes of compounds have been isolated from ginseng root. They include tri terpene saponins, essential oil-containing poly acetylenes and sesquiterpenes, polysaccharides, peptidoglycans, nitrogen-containing compounds and various ubiquitous compounds suchas fatty acids, carbohydrates and phenolic compounds (Nadezhd 2007).Ginseng is an important traditional drug used for more than 2000 years, It has been shown to promote hair growth in several recent studies , and its extract is used as traditional medicine prescription (Shahnaz 2014).Conventional liquid chromatographic methods coupled with ultraviolet detection with low-wavelength range are sensitive to determine ginsenosides. Individual

ginsenosides can be identified by TLC using ,for example, n-butanol: ethylacetate: water (4:1:5, v/v/v). The ginsenosides appear as brown spots after spraying with sulphuric acid: water (1:1, v/v) and heating at 105°C in an oven Wuj (2001) and Ludwiczuk et al. (2005) investigated the separation of ginsenosides by TLC, HPLC.

1.4 .5 Tannic acid

It is a specific form of tannin, a type of polyphenol. Its weak acidity is due to the numerous phenol groups in the structure. Commercial tannic acid is usually extracted from any of the following plant parts.

Tannic acid, however, is a specific type of tannin (plant polyphenol), the two terms are sometimes used interchangeably. This is particularly widespread in relation to green tea and black tea, both of which contain tannin but not tannic acid. Tannins are a basic ingredient in the chemical staining of wood, and it is also found in the seeds, bark, cones, and heartwood. Tannic acid is a common mordant used in the dyeing process for hair (Pettinga 1979).

1.4 .6 Gallic acid

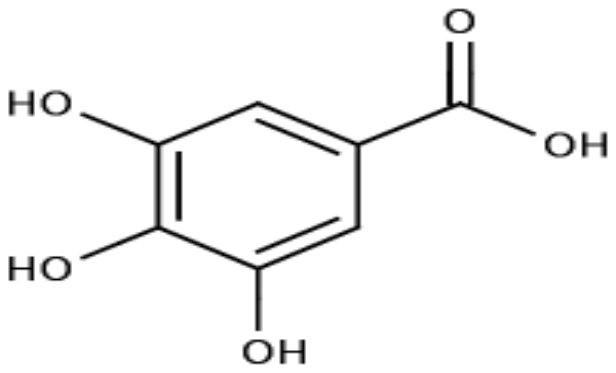


Figure 1 .8 Structure of Gallic acid

Figure (1.8) shows the chemical formula of gallic acid. It is white, yellowish-white, or pale fawn-colored crystals (Andrew 2004).

Gallic acid is commonly used in the pharmaceutical industry(Fiuza 2004) as a standard for determining the phenol content of various analytes by the Folin- Ciocalteau assay; results are reported in gallic acid equivalents(Andrew 2008). Gallic acid is used for hair growth This is achieved through a composition which comprises a gallic acid ester and in some plants extract which is applied on the hair or scalp for the desired benefits (Unilever 2014).

Gallic acid is found in a number of land plants, (Zucca2013,Nakai 2000). Many foodstuffs contain various amounts of gallic acid, especially fruits including strawberries, grapes bananas. (Pandurangan 2015, Koyama 2007), as well as teas (Hodgson 2000, Pathak 2004) and cloves .Historical context and use is that ,Gallic acid is an important component of iron gall ink(Gálvez 1994) .

1.4 .7 Caffeic acid

Caffeic acid: a potential use in medications and cosmetics ,and its seucture shows (figure 1.9)

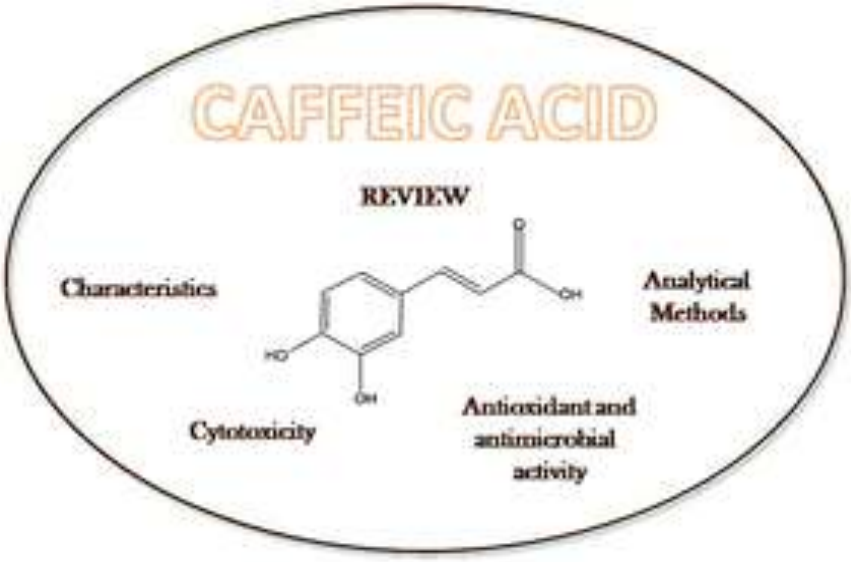


Figure 1 .9 Structure of Caffeic acid

1.5 Essential oils for Hair care

Essential oils when incorporated in a hair care product will impart shine and conditioning effects. They are used in apatented permanent waving systems This helps to provide not only hair

conditioning and improvement in the hair texture, but also a longer lasting pleasant aroma, which eliminates the negative odour of the perm lotion (Purohit 1994). The hair can also be enhanced by the use of a few drops of essential oils in the final hair rinse or added straight to a mild shampoo. Oils such as rosemary, West Indian bay and chamomile all help to condition and encourage healthy hair growth; lavender can be used to repellice and fleas; while bergamot and tea tree can help to control dandruff.

In addition to vitamins, there are many other nutrients should have in diet to promote healthy hair growth. For instance, essential fatty acids, which can be found in flaxseed oil, salmon oil and primrose oil, will help to improve the texture of hair and keep it from becoming brittle. Kelp has minerals that help to promote hair growth (Sato2002).

1.6 Essential Vitamins for Healthier Hair

When it comes to hair, the three most important are proteins, vitamins and minerals. Protein hair masks are used for hair growth ,This is another effective tip on how to grow hair. Addition to that, the nutrients found in this hair mask also offer hair shine and smoothness (Meenal 2017) .

For healthy hair growth, enough vitamin C, A, E, D and B vitamins such as Biotin (also known as Vitamin B7 or Vitamin H) and Niacin (Vitamin B3) should be taken .The Deficiencies may Lead to Thinning Hair. Hair follicles need to have certain vitamins in order to be healthy and allow hair to grow.

A vitamin is an organic compound necessary for the proper biochemical functioning of an organism in very small amounts even though it cannot be produced in sufficient amount in the organism and has to be taken from diets and supplements. The body can produce vitamins from their precursors in food (for example, vitamin A from beta carotene). Some other vitamins have special sites of syntheses, for example vitamin K is produced by the microorganisms in the gut and vitamin D is produced in the skin

through the catalysis of the ultraviolet radiation of sunlight. vitamins have different important functions in the body. While vitamin C and vitamin E are known antioxidants, B vitamins are necessary for enzyme functioning in biochemical reactions.

Vitamin deficiencies are classified into: primary and secondary. Primary deficiency describes a situation where less than required amount of a vitamin is obtained from diet.

Secondary deficiency describes an inability to absorb adequate amount of a vitamin due to underlying disease state, drug interaction or lifestyle.

There are also compounds called anti-vitamins that inhibit the absorption or activity of specific vitamins. For example, avidin, a protein found in egg whites is known to reduce the absorption of biotin or Vitamin B7 ,while pyrithyamine competes with thiamine or Vitamin B1 since their structures are similar. These called anti-vitamins can also lead to vitamin deficiencies.

In fact, each vitamin deficiency produces a disease complex which can be detected by certain early signs. Even before the full manifestation of certain vitamin deficiencies, specific signs can mark the progression of a vitamin deficiency. One such important sign is hair loss. Since the hair and skin are external, they provide an important early representation of systemic anomalies. When your hair starts falling off, vitamin deficiencies should be one of the first causes you consider.

1.6 .1 Vitamin A

This vitamin helps to keep hair moist. Vitamin A supports hair growth by helping to keep the skin scalp in healthy condition. It aids in the secretion of sebum into the scalp as it is needed, which keeps the hair from becoming dry and brittle, which can often be the start of hair loss. Vitamin A is also an anti-oxidant. Care should be taken with Vitamin A supplementation (especially retinoid) for hair loss since excessive amounts of vitamin A, a state called hyper vitaminosis A for hair loss.

1.6 .2 Vitamin D

The two most important forms of this vitamin are D2, which is obtained from plant foods, and D3, which is made by the skin when exposed to sunlight. While the main role of vitamin D is to maintain healthy levels of calcium and phosphorous in the blood, vitamin D also strengthens hair follicles. Recent research suggests it may also to help stimulate the growth of new hair follicles. Symptoms of vitamin D deficiency include depression, muscle weakness, and hair loss .

Hair follicles are highly sensitive to hormones, and vitamin D is a hormone that plays an important role in calcium homeostasis, immune regulation and cell growth differentiation.

Vitamin D deficiency is rare since that the sunlight is all that is required for the synthesis of vitamin D. To reverse hair loss due to Vitamin D deficiency, you need more exposure to sunlight especially early in the morning.

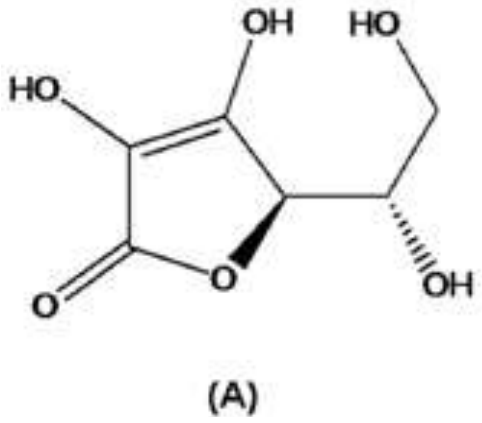
1.6 .3 Vitamin E

Vitamin E or alpha-tocopherol is a fat-soluble antioxidant that is predominantly found in green leafy vegetables, nuts and seeds, seafood, and fruits. Topical application of Vitamin E can be in the form of an oil extract used on your skin and in your hair for growth and nourishment. This vitamin increases blood circulation, especially near the scalp. This is necessary for healthy hair follicles, as it provides them with enough oxygen. The follicles are better able to regenerate, and hair will continue to grow. A deficiency of vitamin E can cause the opposite to happen. Not only do we need to have vitamins for our overall health, we need to have them for the health of our hair. There are many vitamin deficiencies that can lead to hair loss

Hair benefits stimulates hair growth, prevents premature graying, gives lustrous hair and repairs split ends. For instance, without enough Biotin in our diets, hair growth can be inhibited. Enough Biotin can prevent further hair loss (Rahi 2017).

1.6 .4 Vitamin C

Also called Ascorbic acid is a naturally occurring organic compound with antioxidant properties. It is a white solid but impure samples can appear yellowish(PadayattyS2004).Figure 1 .10 shows the chemical formula of vitamin C.



(A)

Figure 1 .10 Structure of ascorbic acid

Ascorbic acid, a water soluble vitamin is essential nutrient in human diets and found mainly in fruits and vegetables. is known to be important to skin health. Due to the remarkable antioxidant properties of this compound, it is widely employed in pharmaceutical and cosmetic industry and also exerted several biological activities. Phenolic compounds are ubiquitous in plants and these are secondary metabolites which shield the plants against UV- radiation or resist the pathogenic aggression(Prabhakar, 2010) .

Vitamin C assists in the production of collagen, a connective tissue responsible for keeping body's tissues and organs together.

vitamin C deficiency may play a role in weak, dry breaking and split ends hair. Along with other nutrients, vitamin C can help support hair growth by assisting with collagen production and with the absorption of from plant sources . Because this is a water-soluble vitamin, body cannot store it since it is washed out via the

digestion food storage processes, so it must replenish daily via diet(Minaz 2017).

Owing to the remarkable antioxidants properties of ascorbic acid, it is widely employed in pharmaceutical and cosmetic industry and also it contains several compounds (Tapan 2016).

1.6 .5 B Complex Vitamins

There are some types of B vitamins that we need in order to have healthy hair. These are Vitamin B3 also known as Niacin, biotin, para amino benzoic acid and vitamin B6. B-complex deficiency may cause dizziness and fatigue as well as hair loss and weak, brittle hair. B vitamins, particularly biotin (B7), may support hair growth by strengthening the keratin structure of the hair shaft, the part the hair structure that is visible.

1.6 .5 .1 Niacin

Niacin, or vitamin B-3, is a water-soluble vitamin known to dilate the blood vessels(Figure 1.11). When taking supplements, the result is better circulation of blood to nourish scalp and hair follicles. Flushing in most cases is harmless. However, it may indicate toxicity, occurring when niacin levels are too high, a risk factor of supplements. The effect of niacin on hair growth is it not only stimulates hair growth, but also to minimize the accumulation of cholesterol.

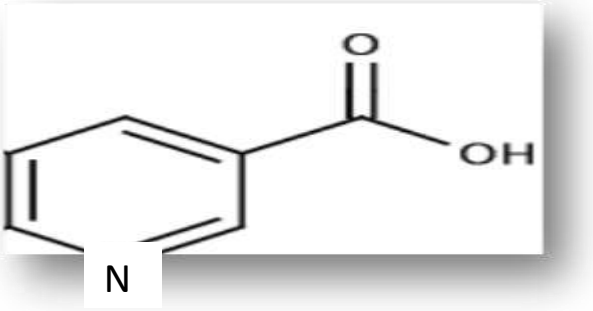


Figure 1 .11 Structure of Niacin

The main function of Niacin is to transform carbohydrates in to energy. Niacin also helps to maintain the structure of the blood cells and improves blood circulation. That's why niacin brings more blood flow to the scalp, bringing more oxygen and nutrients to the hair follicles. That's why experts tout the benefits of niacin for hair growth. Niacin accelerates blood circulation, and thus maintains the growth of hair growth and non-falling hair loss significantly. Of course, the simplest way to ensure that you get adequate niacin and all other needed nutrients for hair growth is a complete, clinically proven hair growth supplement for women(Eshe 2017) .

1.6 .5 .2 Biotin

Vitamins are essential to healthy hair and sustained growth. They can relieve dryness, thicken your hair shaft, eliminate split ends, stop breakage and prevent premature balding and graying. Vitamin supplements or foods enriched with vitamin A and C, biotin and niacin can help to transform dull, stressed and weakened hair into shiny, lustrous, glossy and faster-growing locks. Biotin, or vitamin B-7, is a B-complex vitamin useful in preventing hair loss and premature graying. Biotin encourages elasticity within the hair's cortex, according to an article on the Nutro Vita website, while thickening cuticles to eliminate breakage. The article also reported that biotin deficiencies could lead to seborrhea dermatitis, making scalp itchy, dry and flaky.

Just like any other part of the body, hair roots require proper nutrition, including vitamins, to grow healthy hair strands. Vitamin deficiencies can cause slow hair growth and even hair loss. However, if you are not deficient, there is little evidence that taking additional vitamins will increase hair growth. You can use vitamin supplements to increase the amount of hair-growing vitamins in your body, but you must follow the daily international unit dosage recommendations to avoid overdose.

Biotin, also known as vitamin B7, is essential to the growth of nails and hair. A biotin deficiency can result in weak, brittle hair and hair loss. Biotin supplements are effective at promoting hair growth when there is a biotin deficiency, but there is little evidence that taking more than necessary increases hair growth. (Esheasale 2017).Over time, poor metabolism of nutrients can contribute to undernourished hair follicle cells. Although rare, a biotin deficiency results in skin rashes and hair loss. A study conducted at Harvard University suggests that biotin is one of the most important nutrients for preserving hair strength, texture, and function(Jlongevity 2000).

1.7 Omega-3 Fatty acids and Protein

Omega-3 fatty acids are essential fatty acids. Although they are necessary for human health, the body does not make them, so they must be obtained from outer source to reduce inflammation, Omega-3 fatty acid and protein supports hair growth by supporting a healthy inflammatory response and improving the flow of oxygen and nutrients to hair follicles. All forms of protein, whether animal or vegetable sourced, can encourage healthy hair development. Because hair is made up of protein, fat, water and carbohydrates, a protein deficiency may cause weak hair or hair loss (Sonali 2017).

1.8 Main Problems of hair and scalp

1.8.1 Hair loss

Hair loss can be caused due to different reasons, such as genetic tendencies, environmental triggers, exposure to chemicals, medicines, nutritional deficiency, extreme stress or long illness etc. Hair loss is embarrassing, and not always something that is easy to treat, but the truth of the matter is, hair loss is something that millions of people all over the world experience. There are many reasons why people can start losing hair, and one of these reasons is that they have vitamin deficiencies, they are not getting the

nutrients they need for healthy hair growth(Kaushi 2011). Also damage to the hair structure (Milan 2011).

1.8.1.1 The normal cycle of hair growth and loss

Hair is composed of protein called keratin. Keratin is an extremely strong protein which makes hair resistant to wear and tear. This is the same kind of protein that makes up the nails and the outer layer of skin.

The hair follicle is basically the root of the hair. At the base of the hair follicle is a bulb shaped structure called the dermal papilla. The dermal papilla is fed by the bloodstream which carries nutrients to produce new hair. The dermal papilla produces the pigment called melanin which gives the hair colour. The dermal papilla is a very important structure to hair growth because it contains receptors for male hormones and androgens. Androgens regulate hair growth in scalp hair. Androgens may cause the hair follicle to get progressively smaller and the hairs to become finer in individuals who are genetically predisposed to male pattern hair loss. The cycle of hair growth lasts for 2 to 3 years. Each hair grows approximately 10 cm per annum. Each cycle of hair growth can be broken down into three phases. These are the growth phase, the transitional phase and the rest phase. Each hair goes through each phase, independent of neighbouring hairs.

About 85% of hairs are growing at any one time meaning they are in the growth phase. The growth phase can vary from 2 to 6 years and a hair would continue to grow to about 1 metre if uncut. At the end of the growth phase, the hair enters the transitional stage which only lasts about two weeks. Hair then enters the resting phase which lasts about 5 to 6 weeks. During the resting phase the hair is still attached to the hair follicle but is not growing. After the resting phase the hair is shed and a new hair starts to grow in its place thus starting a new hair growth cycle. Resting hairs are randomly spread throughout the scalp so that no bald spots are seen.

The average person has 100,000 hairs on their scalp. It is normal to shed some hair each day as part of the hair growth cycle described above, the average person loses about 50 to 100 hairs per day. However some people may suffer from excessive hair loss for different reasons. This can affect men, women or children. Kaushi (2011) describe the most common causes and possible treatments of hair loss .

1.8.2 Alopecia areata

Androgenic alopecia is characterized by progressive loss of hair from the scalp.Alopecia areat is an autoimmune condition. Our immune system normally only attacks infections such as bacteria and viruses, but in the case of alopecia areat, it attacks the hair follicles instead. White blood cells attack the hair follicle causing the hair to stop growing and enter the resting phase. The cause is not fully known. The hair follicles are not permanently damaged, and in many cases the hair grows back within a few months. There is a genetic link to alopecia areat with evidence that it runs in families in about one in five cases.

It initially appears as a rounded about an inch in diameter and affects men and women equally. It often starts in childhood. Alopecia areata is patchy hair loss, alopecia to talis total scalp hair loss and alopecia universalis is the loss of all body and scalp hair(Eamonn2017).

1.8.3 Aging of hair

Hair aging comprises weathering of the hair shaft, and aging of the hair follicle. The former involves progressive degeneration of the hair fiber from the root to the tip, while the latter manifests as decrease of melanocyte function or graying, and decrease in hair production in androgenetic and senescent alopecia. The scalp is subject to intrinsic or physiologic aging, and extrinsic or premature aging due to external factors. Intrinsic factors are related to individual genetic and epigenetic mechanisms with inter individual

variation. Prototypes are familial premature graying, and androgenetic alopecia . Extrinsic factors include ultraviolet radiation, air pollution, smoking, nutrition, and lifestyle. Experimental evidence supports the hypothesis that oxidative stress plays a major role in premature skin and hair aging (Milan 2011).

1.8.4 Graying

Hair graying is a natural age-associated feature. The hair graying trait correlates closely with chronological aging and occurs to varying degrees in all individuals. This graying incidence appears irrespective of sex and hair colour. In men, graying usually begins at the temples and in the sideburns. Women will usually start around the perimeter of the hairline. Gradually, the gray works its way back through the top, sides, and back of the hair. The rate at which an individual turns gray depends on genetics. It is not uncommon to observe kinships with marked early graying throughout. Although graying is understood as a loss of pigment in the shaft, its cellular and molecular origins are incompletely understood. The colour of hair mainly relies on the presence or absence of melanin pigment.

This interpretation is supported by the observation that melanocytes in graying hair bulbs are frequently highly vacuolated, a common cellular response to increased oxidative stress. The extraordinary melanogenic activity of pigmented bulbar melanocytes, continuing for up to 10 years in some hair follicles, is likely to generate large amounts of reactive oxygen species via the hydroxylation of tyrosine . If not adequately removed by an efficient antioxidant system, an accumulation of these reactive oxidative species will generate significant oxidative stress. It is possible that the antioxidant system becomes impaired with age leading to damage to the melanocyte itself from its own melanogenesis-related oxidative stress (Tobin 2001).

1.8.5 Dandruff

Plant materials can be used as hair growth stimulation ,hair colorants and dyes, and in a number of hair and scalp complaints such as dandruff. Dandruff is a major problem, yet little is known about the underlying mechanism and subsequent biochemical changes that occur in the scalp skin and lead to its manifestation. The characteristic flaking and scaling of the scalp experienced by dandruff sufferers suggests that the desquamation process is impaired. Dandruff is also associated with a dramatic decrease in free lipid levels, with significant decreases in ceramides, fatty acids and cholesterol. Thus the epidermal water barrier is impaired in the scalp of dandruff sufferers, and the perturbed barrier leaves sufferers more prone to the adverse effects of microbial and fungal toxins, and environmental pollutants, thus perpetuating the impaired barrier (Harding 2002).

1.9 Photoprotection of hair and scalp

Awareness of sun protection has become imperative as a consequence of direct exposes to the sun. The chemicals that act as sun protectors are widely utilized and offer the most convenient means of protecting the skin and hair against acutesunburn and chronic pathologic effects of ultraviolet radiation. Their use on the hair-bearing scalp is problematic for cosmetic reasons, unless complete baldness is present. Although hats provide the best protection of the scalp from ultraviolet radiation, not all patients find them convenient or acceptable for this purpose. The protection of the hair against photodamage has been extensively studied .It has been found that hair dyes may protect hair against photodamage (Pande 2001).

Recent experimental work indicates that cinnamid propyl trimonium chloride, is suitable for photoprotection of hair, while simultaneously providing an additional conditional benefit on hair

(Gao 2001) .Solid lipid nanoparticles have been developed to offer photoprotection on their own by reflecting and scattering ultraviolet radiation (Wissing 2001). Systemic photoprotection has been the focus of more recent investigation, in as much as this would overcome some of the problems associated with the topical use of sunscreens. Studies illustrate photoprotective properties of supplemented antioxidants, particularly beta-carotene (pro-vitamin A), α-tocopherol (vitamin E), and L-ascorbate (vitamin C)

1.9.1 Anti-aging compounds

Recent advances in the care of aging hair and scalp are "anti-aging" compounds. Owing to water dilution and short contact time, anti-aging compounds do not have any effect in shampoos. Antioxidants in shampoos, such as vitamin C and E, protect fatty substances in the shampoo from oxidation. Topical anti-aging compounds of current interest are green tea polyphenols, selenium, copper, phytoestrogens, melatonin (Bangha1996).

1.10 Heavy metals in some natural hair Cosmetics

Healthy hair not only makes you feel confident, but it also shows everyone else that you care about your health and well-being. Hamad et al. (2010), found that nutritional deficienciesor anemia might play a role in increasing hair loss due to the weakening of the hair shaft that causes damage to the hair and slows its growth. Hair problems caused by nutritional deficiencies can be corrected with a proper diet, or by taking natural products for hair growth. Natural products help nourish the body more efficiently. It contains some ingredients of natural origins, to nourish hair from within and promotes existing hair growth. In the last few decades, the determination of minerals and trace elements has been important to enhance production efficiency in plants and food (Rodrıguez 2011).Zinc, magnesium, silicon, sulphur, selenium andiron are the best minerals that are necessary to keep hair healthy, strong and

shiny and prevent hair loss. These are all naturally occurring substances , which are often present in the environment at low levels.

Elements have many important roles in the growth and health of hair such as copper, iron and zinc. Hair loss can be one of the visible symptoms of anemia, or iron deficiency. But even if you are not anemic, you may have low iron levels that are affecting your health and may result in thinning hair.

Iron and zinc are two minerals that promote healthy hair growth. Iron is an essential mineral that has several important roles in the body including helping to make red blood cells, which carry oxygen around the body to all the cells. But even if you are not anemic, you may have low iron levels that are affecting your health and may result in thinning hair. A diet rich in the minerals iron and zinc, will help nourish hair from the inside out. If you do not have enough of the minerals in your diet to promote hair growth, consider taking dietary supplements for hair growth; vitamin C helps boost iron absorption (Gene2009).

Zinc is an essential mineral and its presence in adequate amounts in your body, is required for the division of cells in your hair follicles. Zinc is involved in the synthesis of proteins and nucleic acids, and plays a role in various metabolic pathways and cellular functions. Zinc is involved in almost every metabolism that occurs in the body, and affects hair growth. It also plays an important functional role in hair follicle cycle. Dealing with hair loss, zinc is a potent inhibitor of the regression of the hair follicle, and accelerates the healing process of the hair follicles. Studies showed that it might also help to promote hair re-growth in some cases of alopecia as well (Kil MS2013).Zinc deficiency causes hair loss (Heyneman 1996 , Maret 2006).

This trace mineral is necessary to balance the calcium ingested as dietary supplements and as a consequence has a key role to play in the growth of healthy hair. Its deficiency is one of the main factors that causes hair loss that affects the functions of thyroid, metabolism, heart nervous system, muscular and digestive system. Healthy circulation in the scalp ensures that hair follicles get the nutrients they need to grow. Because of magnesium's role in supporting blood flow it helps support optimal nutrient delivery to the hair follicles.

Silicon is fundamental for your health, including that of your skin and scalp. This mineral helps to strengthen your blood vessels and improve blood flow, which can stimulate the blood flow to your scalp and encourage growth. It is also a necessary component of the skin's connective tissues and helps to strengthen your bones, nails and hair.

Human hair is made from an important protein called keratin, which is high in sulphur content. The presence of sulphur gives healthy hair its strength and elasticity; conversely, the absence of enough sulphur leads to brittle hair that is easily broken. If you have fragile hair, nails and/or dry skin, this could indicate a lack of this mineral in your diet. Including the right amount of sulphur can certainly help to bolster the overall health of your hair (Giampaolo 2016).

Selenium boosts hair growth and is included in many anti-dandruff shampoos to fight against dandruff. In combination with zinc, selenium are advised by doctors for hair loss. This helps in proper functioning of hormones and increased growth of hair. They can be used to treat many hair problems as gray hair, hair loss, dandruff, etc... (Jayshree 2017).

1.11 Antioxidants hair cosmetic products

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms (Dabelstein2007). Free radicals are highly reactive molecules with unpaired electrons that can directly damage various cellular structural membranes, lipids, proteins, and DNA. The damaging effects of these reactive oxygen species are induced internally during normal metabolism and externally through exposure to various oxidative stresses from the environment. The body possesses endogenous defence mechanisms, such as antioxidative enzymes (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidative molecules (vitamin E, vitamin C, glutathione, ubiquinone), protecting it from free radicals by reducing and neutralizing them (Shindo 1994).

Radicals induced by environmental factors, such as smoke, air pollution, ozone and sunlight cause skin to age prematurely. Thus, the use of antioxidants and radical scavengers in hair care is very important and widespread. In addition, antioxidants are essential components in cosmetic formulations to increase the shelf life of the products by reducing the oxidative degradation of sensitive ingredients. Oxygen based radicals are predominantly generated in the water phase. They attack unsaturated lipids, proteins and nucleic acids in the cell. All living organisms protect themselves against them with a combination of lipid-soluble antioxidants, such as vitamin E and carotenoids and water-soluble antioxidants, such as vitamin C, glutathione and different enzymes (Mayhew 2007).

In the case of failure of the antioxidant defence system, antioxidants need to be supplemented from outside sources, like synthetic one . However, studies conducted subsequently have demonstrated that synthetic antioxidants have toxic effects and, consequently, restrictions have been imposed on their use.

Therefore, researchers have focused their studies on plant-derived natural antioxidants (Kulisic 2004).

Human hair is exposed to a number of chemical and physical hazards, such as combing, brushing, heating, drying and different chemical treatments which render it brittle and dull. Moreover, UV-light irradiation , causes significant hair damage. The culprits are called ROS (reactive oxygen species). Most of these ROS are generated in the water phase; thus wet hair is particularly susceptible. Attention should be paid to the sulfur-containing amino acid cystein which is located in the rigid outer layer of the hair structure, the cuticula. But also the aromatic amino acids tryptophan and tyrosine are easily degraded by light. Damaged hair has a porous surface and is thus more sensitive to free radicals.

Obviously, drying wet hair with a conventional dryer damages the hair structure significantly. This damage is not primarily caused by the heat itself but is induced by reactive oxygen species generated in the water film on wet hair. Thus, the application of antioxidants should offer adequate protection against this particular hazard hair is subjected to every day (Zulli 2000, Johon 2013).

A number of methods are used to determine the radical scavenging effects of antioxidants. The DPPH method is a preferred method because it is fast, easy and reliable and does not require a special reaction and device. DPPH is a stable, synthetic radical that does not disintegrate in water, methanol, or ethanol. The free radical scavenging activities of extracts depend on the ability of antioxidant compounds to lose hydrogen and the structural conformation of these components (Shimada 1992, Fukumoto2000). The DPPH free radical, which is at its maximum wavelength at 517 nm, can easily receive an electron or hydrogen

from antioxidant molecules to become a stable diamagnetic molecule (Soares 1997). Owing to the DPPH radical's ability to bind H, it is considered to have a radical scavenging property. A solution of DPPH radicals prepared in methanol is converted into DPPH-H (diphenyl hydrazine) molecules in the presence of an antioxidant agent, as shown in the following equation. Discoloration occurs due to the decreasing quantity of DPPH radicals in the environment. The discoloration of the DPPH therefore reflects the radical scavenging activity of the analysed extract (Guo 2007, Molyneux 2004).

$$DPPH + A\text{-}H \rightarrow DPPH\text{-}H + A$$

1.12 Fats and oils as hair cosmetics

Fats and oils are water-insoluble substances of plant or animal origin that consist predominantly of triglycerides. Those that are solid or semisolid at room temperature are normally called fats, while those that are liquid under the same conditions are called oils.

Oils are obtained by different techniques from the fruit of some vegetables or some animal organs. They are principally constituted by triglycerides; they also contain another lypophilic substances in low proportions, such as fatty alcohols, fatty acids, vitamins, phytosterols, etc. These last components determine in many cases their cosmetical and pharmaceutical activity.

The principal constituents of vegetable oils are esters of glycerol and fatty acids along with partially glyceridic material such as lecithin and substances such as tocopherol. Their composition will vary according to the species and the use will depend especially upon the variety, type and proportion of fatty acids (Antonio2000).

In the earliest history of hair cosmetics there is naturally occurring waxes, animal fats and vegetable oils. Animal fats were used alongside plant oils in cosmetics to provide grooming effects on head and beard hair (Parichart 2017).

1.12.1 Lamb grease

Lamb grease, or so-called scientifically known as lanolin, is a fatty substance taken from lamb fat and wool; after separating from the skin after slaughter. Locally in Sudan known as(Wadak). It treats the problem of dryness, flattening, and destructions. It gives the hair a soft and a natural touch to look as if it has been softened all the time. The hair is fully greased, helping to protect it from surrounding factors such as weather fluctuations and heat, moisturizes the scalp, prevents dryness and a layer of crust in it ,stops the fall and stimulates the growth of new hair (Crystal 2014).

1.12.2 Sesame oil for hair

The sesame oil contains many benefits for the hair because it contains a lot of amount of nutrients and proteins that nourish the hair and strengthen the roots. The problem of greying as a natural hair colour to make it darker, enhances hair growth, stimulates blood circulation in the scalp, nourishes it and protects hair from the harmful UV rays of the sun as it is a natural protection of the harmful sun rays. The hair eliminates the problem of head lice because it contains a lot of natural antibacterial and antifungal substances. It helps to treat dandruff problem, smoothes hair and protect hair from damage that can be caused by heat (Lily 2018).

1.12.3 Beeswax for hair

It is considered one of the best types of physiotherapy which contributes to stimulating and strengthening hair growth. Also

keeps hair shiny and smooth, treats curly hair problems, and protects hair from environmental conditions and damage so it enters into the preparation of some hair care products such as conditioner and shampoo.

It contains many chemicals that are divided into saturated hydrocarbons, aromatic and coloured materials that give wax its natural scent and distinctive colour in addition to minerals. It also contains fatty alcohols and some of the most important vitamins ,vitamin C (Hayel 2016).

1.12.4 Bone marrow fats

The bone marrow is a white and butter like gel, which is found inside the bones of cows, goats, and sheep, known in Sudan as (Dehn Elsag). It contains a high percentage of proteins that nourish the entire body, which is also a material that enters into a lot of cosmetic recipes for hair to increase its softness and eliminates the problem of entanglement. Also it makes hair brighter and healthier, restores its vitality and freshness, prevents its fall, nourishes it and increases its length (Wala 2017).

1.12.5 Camel hump fats

Hump is a fatty mass carried by camel over the back. Locally it is known as (Sanam Elebel) and consists of grease and fat. Studies have shown that the camel hump fats have a lot of benefits, such as, intensifing the hair to give it the super smoothness and removing its wrinkles, increasing its length, giving it the nutrition it needs, preventing dehydration and breakage, strength eningit is follicles and preventing falling (Aatikha 2018).

A majority of antioxidants naturally present in plants occurs in phenolic structures and especially in flavonoids structures.The

extracts of ginger, hibiscus and colves herbal plant must contain antioxidants component (Youssef 2015).

1.13 Proteins as hair care product

Proteins are polymers of amino acids. Twenty different types of amino acids occur naturally in proteins. Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. As a result they have different molecular structures and physiochemical properties. Proteins are important constituents of cosmetics for a number of different reasons. They are containing essential amino-acids which are essential to human health, but which the body cannot synthesize. cosmetic analysts are interested in knowing the total concentration, type, molecular structure and functional properties of the proteins in cosmetics.

Every single individual strand of hair on head is 88-90% comprised of the protein keratin. In fact, protein makes up 20% of our body. So, you need to get a healthy dose of protein to ensure that not only hair growth, but also all your other body functions are regulated effectively.

Since hair growth and tissue repair is not regarded as an essential function for your body's regular functioning and survival, the supply of protein to hair follicles is cut off. Therefore, if protein deficiency persists for an extended period of time, the dry and brittle hair will present. So, major protein deficiency-related hair loss can solve with a protein treatment.

Chemical treatments such as coloring and relaxing these factors cause chemical degradation of lipids and proteins,as well as abrasi on of the protein layers (Milan 2011).

1.14 Sudanese hair cosmetic products

In Sudan we have unique local hair Cosmetic, such as; (Karkar) oil, henna (Abdalla 2016), (Seder)Ziziphuss pinachristi, (Karkadia) Hibiscus, ginger and colves. (Karkar) oil is mixture of vegetable Oils for example Sesame Oil with beeswax, Lamb grease and colves exracts. It used as a hair grooming product for preventingmany problems of hair and scalp.

1.15 Instrument used

1.15.1 Ultraviolet /Visible (UV) spectrophotometer

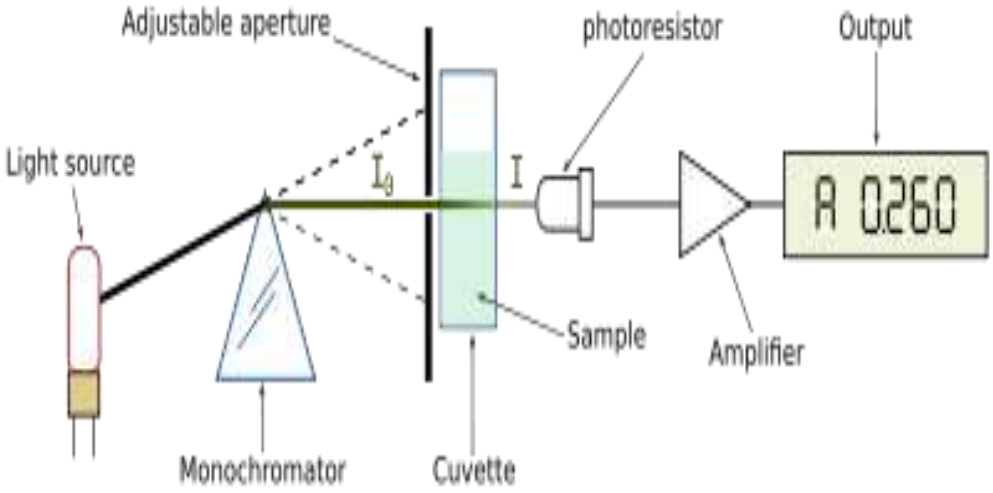


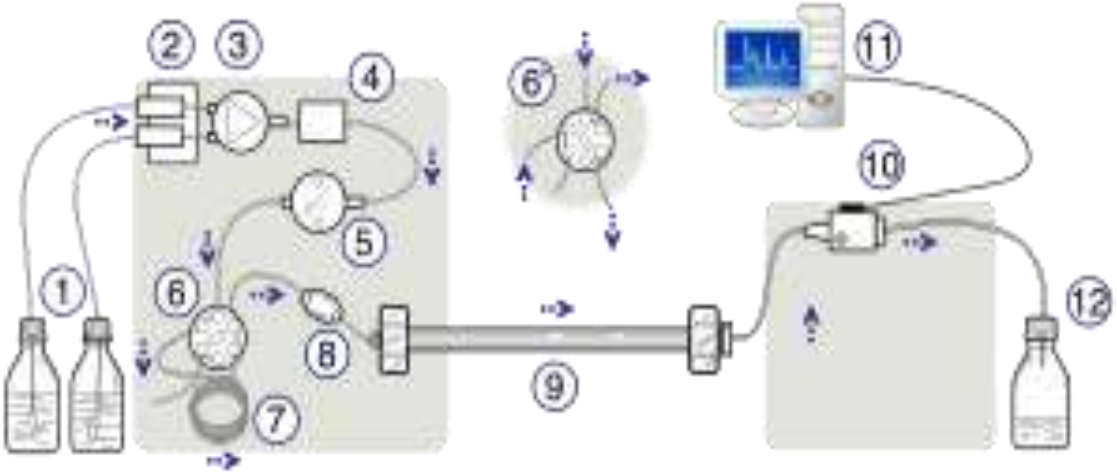
Figure1.12 the UV-VIS instrument

Spectrophotometers use a monochromator containing a diffraction grating to produce the analytical spectrum(figure 1.12). The grating can either be movable or fixed. If a single detector, such as a photomultiplier tube or photodiode is used, the grating can be scanned stepwise so that the detector can measure the light intensity at each wavelength.

When making transmission measurements, the spectrophotometer quantitatively compares the fraction of light that passes through a reference solution and a test solution, then electronically compares

the intensities of the two signals and computes the percentage of transmission of the sample compared to the reference standard. The bandwidths are transmitted through the test sample. Then the photon flux density of the transmitted or reflected light is measured with a photodiode, charge coupled device or other light sensor. The transmittance or reflectance value for each wavelength of the test sample is then compared with the transmission or reflectance values from the reference sample. Most instruments will apply a logarithmic function to the linear transmittance ratio to calculate the 'absorbency' of the sample, a value which is proportional to the 'concentration' of the chemical being measured (Ganguli 2006).

1.14.2 High- performance liquid chromatography (HPLC)



(1) Solvent reservoirs, (2) Solvent degasser, (3) Gradient valve, (4) Mixing vessel for delivery of the mobile phase, (5) High-pressure pump, (6) Switching valve in "inject position", (6') Switching valve in "load position", (7) Sample injection loop, (8) Pre-column (guard column), (9) Analytical column, (10) Detector (i.e. IR, UV), (11) Data acquisition, (12) Waste or fraction collector.

(HPLC)is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on

pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

The active component of the column, the adsorbent, is typically a granular material made of solid particles 2–50 μm in size. The components of the sample mixture are separated from each other owing to their different degrees of interaction with the adsorbent particles. The pressurized liquid is typically a mixture of solvents and is referred to as a "mobile phase". Its composition and temperature play a major role in the separation process by influencing the interactions taking place between sample components and adsorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, most often a combination.

The schematic of a HPLC instrument typically includes a degasser, sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components. A digital microprocessor and user software control the HPLC instrument and provide data analysis. Some models of mechanical pumps in a HPLC instrument can mix multiple solvents together in ratios changing in time, generating a composition gradient in the mobile phase. Various detectors are in common use, such as UV/Vis. Most HPLC instruments also have a

column oven that allows for adjusting the temperature at which the separation is performed (Gerber 2004).

1.15.3Inductively Coupled Plasma-Optic Emission Spectrometry (ICP-OES)

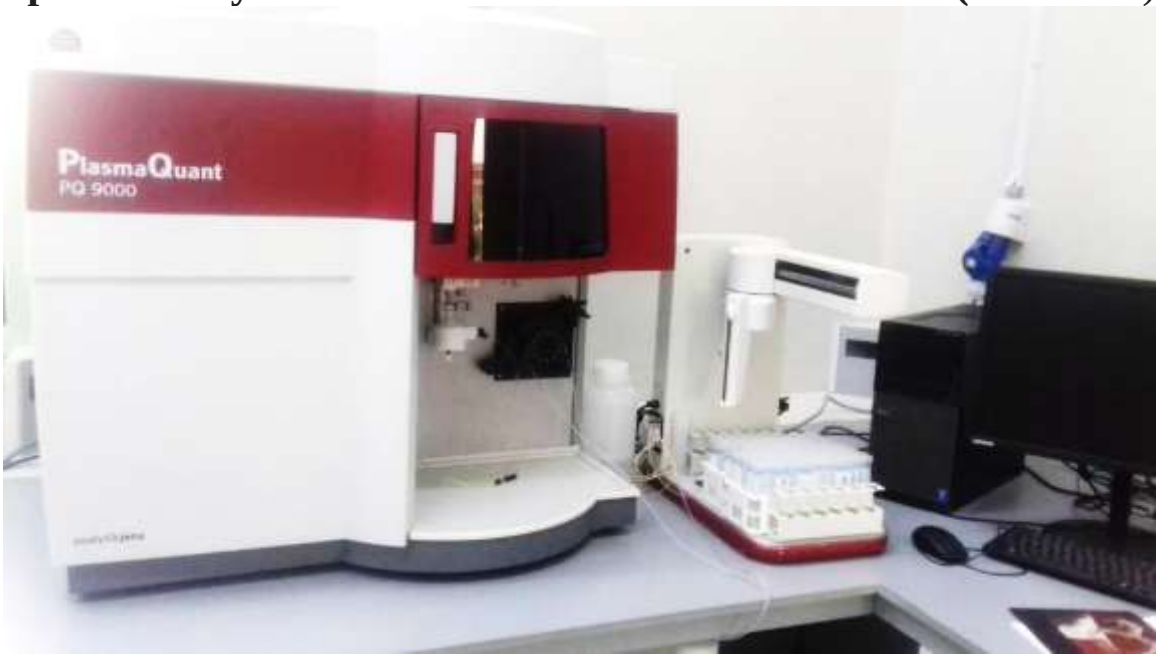


Figure 1.14 Perkin-Elmer 2001 Model Inductively Coupled Plasma-Optic Emission Spectrometry (ICP-OES)instrument

Calibration was carried out on a daily basis using the appropriate program options of the apparatus. For each element being analyzed, one straight calibration line with at least four concentrations was determined. One reagent blank solution was also measured in each case. For each measurement, at least three measurements were carried out automatically by the unit and then averaged. The quality of the calibration function within the working range is verified. The correctness of the calibration was verified with an aqueous certified reference sample. During analysis, an internal standard is added online to each sample and standard solution (Heinz2011).

1.16 Objective of the study

Natural herbal products extracted from plants are used in many treatments worldwide. The World Health Organization (WHO)recommends that herbal products should be evaluated for efficacy, potency as well as safety to protect public health(Hussain 2006).

1.16.1 General objectives

The main objectives of this research work are:

1. Extraction of active natural products to be used as hair cosmetics from some local herbal products.
2. Determination of heavy metals in natural herbal cosmetic products.
3. Determination of antioxidants and Proteins in natural herbal plants and animal fats used as cosmetic products.

1.16.1 Specific objectives

1. Extraction and cleanup of active ingredients of hair cosmetics in ginger ,hibiscus and colves natural herbal products .
2. Phytochemical screening and evaluation of the total flavonoids and tannins contents in the extract of these hair cosmetics by Ultraviolet (UV) spectrophotometry.
3. UV spectrophotometric determination of vitamin C in these extracts of hair cosmetics.
4. Determination also of niacin , vitamin C in addition it gallic acid but using High-performance liquid chromatography-mass spectroscopy (HPLC-MS).

5. Determination of zinc, magnesium, silica, sulphur , selenium and iron heavy metals content in these natural hair cosmetics using inductively coupled plasma optical emission spectrometry (ICP-OES).
6. Development and validation of spectrophotometric determination of niacin content in ginger.
7. Determination of antioxidants and proteins in ginger ,hibiscus and colves, fats, bees wax and sesame oil used as hair cosmetic in Sudan.

Chapter two

Materials and methods

2. Materials and methods

2.1 Materials

2.1.1 Chemicals and reagents

All chemical materials and reagents used in this work were of analytical grade. The samples of hibiscus, colves and ginger were collected in June, 2017, from the Khartoum market. Distilled water was used throughout the experiments.

- Acetic acid HPLC-grade, Merck (Germany).
- Acetic anhydride.
- Aluminium chloride.
- Ammonia.
- Ammonium molybdate.
- Boric acid.
- Chloroform.
- Copper sulphate
- Deionized water from a high purity water system, quality: specific resistance > 18.2 MΩ cm.
- Dimethyl sulfoxide (DMSO).
- 2.2-Di(4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH).
- Element stock solutions, commercial solutions with certificate were used.
- Ferric chloride $FeCl_3$.
- Gallic acid standard.
- Hydrochloric acid.
- Hydrogen peroxide.
- Meta phosporic acid with acetic acid solution.
- Methanol HPLC-grade, Merck (Germany).
- Methyl red indicator.
- Niacin standard.
- Nitric acid (65 %).
- Oxalic acid.

- Petroleum ether.
- Phenolphthalein.
- Potassium ferro cyanide.
- Potassium hydroxide.
- Propyl gallated standard.
- Sodium nitrite .
- Sodium hydroxide.
- Sodium sulphate anhydrous.
- Sulphuric acid.
- Vitamin C standard.
- Whattman filter paper no 41.

2.1.2 Instruments used

2.1.2.1 Ultraviolet /Visible (UV) spectrophotometer,(Thermo scientific multiskan spectrum,SN1500-722, Finland).

2.1.2.2 Ultraviolet /Visible (UV) spectrophotometer,(Shimadzo,1650PC, Japan) with 1cm quartz cell.

2.1.2.3High- performance liquid chromatography (HPLC) equipped with an analytical column A C-18 LUNA (5 micron 25 cm×4.6 mm). prominence UFLC Shimadzu corporation **2.1.2.4 Inductively Coupled Plasma-Optic Emission Spectrometry (ICP-OES**) **:**Perkin-Elmer 2001 Model Inductively Coupled Plasma-Optic Emission Spectrometry (ICP-OES) as shown in figure 2.4Vessels of plastics quartz glass were used. They were rinsed with nitric acid when they were reused.

2.2 Methods

2.2.1 Determination of active ingredients of hair cosmetics in ginger, hibiscus and colves natural herbal products

2.2.1.1 Extract preparation

Air-dried samples weresmashed using mortar and piston, thenextracted with 80% methanol by shaking at room temperature for 48 hours. The samples were filtered and the residual material rinsed with additional solvent (two portions each of 100 cm^3). Extracts were transferred to a round-bottomed flask and

concentrated under vacuum by rotatory evaporator to form a thick extract. The sample extracts were then dried at 60 ^{0}C until a solid material was obtained. The dried powder samples were, however, used for the various analyses.

2.2.1.2 Phytochemical analysis

The methanolic extract of the samples were subjected to preliminary phytochemical screening to identify the chemical constituents. Test for the presence of tannins, flavonoids, saponin, steroid, alkaloids, Cumarin and terpenoids, glycosides, was done using Okereke (2015) method with some modifications.

Tests for alkaloids :to the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, the following tests were performed.

(Mayer's reagent test)To 3 Cm^3 of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of creamy precipitate indicates the presence of alkaloids.

Tests for flavonoids (Alkaline reagent test): the extract was treated with few drops of sodium hydroxide solution separately in a test tube. Formation of intense yellow color, which becomes colorless on addition of few drops of dilute acid indicates the presence of flavonoids.

Tests for tannin and phenolic compounds (ferric chloride test):a small amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

Test for saponin: froth test the extract was diluted with distilled water and shaken in a graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Test for steroid and triterpenes :in dry tube extract was washed with petroleum ether and chloroform then filteration was done.

Adropsof acetic anhydride andconcentratedsulphuric acidwere added on the wall of tube. Formation of green color indicates presence of steroid, or brown color indicates presence of triterpenes.

Test for anthraquinone glycoside : extracts were dissolved in mixture of potassium hydroxide solution and 3%hydrogen peroxide neutralized with hydrochloric acid,thenchloroform andamrnonium solutionwere added.Formation of pink color in middle oftube indicates presence ofanthraquinoneglycoside.

2.2.2 Ultraviolet /Visible spectrophotometric determination of total flavonoids and total tannins

2.2.2.1 Determination of tannins Content

About 1 cm^3 of hibiscus and colves extract samples (1mg/cm^3)was transferred to vilals, 1cm^3 of 1% $K_3Fe(CN)_6$ and1cm^3 of 1% $FeCl_3$were added, and the volume was made up to 10 cm^3 with distilled water .After 5 min absorbance was measured at 510 nm against the reagent blank. A set of reference standard solutions of tannic acid (20, 40, 60, 80 and 100 μg/cm^3) were also prepared in the same manner. Absorbances for test and standard solutions were measured against the blank at 510 nm by the UV/Visible spectrophotometer .

2.2.2.2 Determination of total flavonoids content

Aliquots of each extract were pipetted out in series of test tubes and the volume was made up to 2cm^3 with distilled water , 0.3cm^3 of 5% sodium nitrite was added to each tube and incubated for 5 minutes at room temperature , and 0.3 cm^3 of 10 % aluminium chloride was mixed. After 5 minutes, 2 cm^3 of 1M Sodium hydroxide was added and diluted to 10 cm^3 with distilled water. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 μg/cm^3) was prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 415 nm with an UV/Visible spectrophotometer.

2.2.3 Ultraviolet /Visible spectrophotometric determination of vitamin C inhibiscus , colves and ginger samples

Standard Ascorbic acid0.1 %w/v solution

0.1g of ascorbic acid was weighted accurately and dissolved in freshly prepared0.05M oxalic acid solution and diluted to 100 $cm^3$with distilled water.

2.2.3.1 Preparation of different standard solution

Aliquots of 0.5, 1, 2,3 ,4 and4.5 cm^3 of 0.1 %w/v standard ascorbic acid solution were taken in separate 25cm^3 volumetric brown flasks into which4.5 ,4 ,3,2 and 0.5cm^3 of 0.05M oxalic acid solution was then added .Then 0.5 cm^3metaphosporic acid with acetic acid solution,1cm^3of5%v/v sulphuric acid solution, and 2$cm^3$of 5%w/v ammonium molybdate solution were added in each flask, and the volume was completed to the mark with distilled water.

2.2.3.2 Preparation of sample solutions

1g of each sample was accurately weighed and transferred to25 cm^3 conical flask into which 10cm^3 of 0.05M oxalic acid solution and the sample was placed under shade for extraction of vitamin C content . After 24 h each sample was filtered through 0.45μm

filter paper using pump. Then 2.5cm^3 of each sample was transferred to separate 25cm^3 volumetric brown flask , 2.5cm^3 of 0.05M oxalic acid solution , meta phosporic acid with0.5cm^3 acetic acid solution were then added to each flask followed by 1$cm^3$of 5%v/v sulphuric acid solution and 2cm^3 of 5% m/v ammonium molybdate solution , and the volume was completed to the mark with distilled water.

Each sample was then analysed for vitamin C at 760 nm compared with standard vitamin C (Iqbal 2010).

2.2.4 HPLC Method for determination of niacine, ascorbic acid and gallic acid in ginger , hibiscus and colves

2.2.4.1 Preparation of aqueous extract

About 5 g of samples powder was weighed accurately, transferred to falcon tube, extracted with 30% of methanol and 70% with distilled water and acidified to pH 3 with phosphoric acid, by shaking for 30 min .The samples then filtered with 0.45 μL filter paper using pump, and subjected to determination of niacine, ascorbic acid and gallic acid in ginger , hibiscus and colves .

2.2.4.2 Preparation of mobile phase

The mobile phase was prepared by mixing methanol and formic acid 0.1% in water in as gradient system.Table2.1 shows the chromatograph operating conditions.

Table2.1: HPLC instrument operation conditions

Time min	Methanol(B)	formic acid 0.1% in water(A)
0.01	5	95
5	90	10
9	5	95
10		

Flow rate :0.38 cm^3/min
Column Temp. :40^o C
The injection volume :10μL

Mass spectroscopy (MS) Conditions
Instrument: MS 2020(Shimadzu corporation).
Ionization :ESI positive (DUIS PROBE)
Dl :400
Nebulizing gas :1.5 L/min
Dry gas :5 L

2.2.5 Method of determination of niacin in ginger

A method for the determination of niacin was developed and applied to analyze in ginger and some plants extract . 0.5 g of ginger was powdered and dissolved into 25 cm^3water by shaking for one hour for niacin extraction. The solution was then filtered through Whattman filter paper no 41.This filtrate was diluted suitably with distilled water. The absorbance of this solution was measured and the amount of niacin was read from the calibration curve.

2.2.5.1Determination of wavelength of maximum absorbance(λ-max)

A standard stock solution of niacin (100 μg/cm^3) was prepared using distilled water as solvent, and 0.2cm^3 was diluted to 10 cm^3 with the same solvent to obtain 2 μg/cm^3standard solution. The standard solution was scanned in the wavelength region of 200-400 nm.

2.2.5.2 Method validation

Validation of the developed method was done following the guidelines laid down in International Conference on Harmonization (ICH) guidelines Q2 (R1) (2005).The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents

preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc. Literature survey has revealed various analytical methods for determination of niacin in pharmaceutical formulations in combination with other drugs (Narayankar 2015).The following parameters were evaluated:

2.2.5.2.1 Linearity and range

Linearity of any analytical method is its ability, within a given range, to get test results that are directly, or through a mathematical transformation, proportional to concentration of analyte. Six different concentrations (5- 30μg/cm^3) of niacin were scanned on UV spectrophotometer in UV-range (i.e., 200-400 nm). The spectrum was recorded. Least square regression analysis was done by constructing the calibration plot between concentration and absorbance (Jain 2011).

2.2.5.2.2 Sensitivity

Sensitivity of the developed method was determined with respect to limit of detection (LOD) and limit of quantification (LOQ). A series of varying drug concentrations (5-30 μg/cm^3) were analyzed to find LOD and LOQ. LOD is the lowest detectable amount of an analyte in a given sample that may or may not be quantified, under the stated experimental conditions, whereas LOQ is the lowest quantifiable amount of analyte in any sample. LOD and LOQ were computed by using standard deviation (σ) and slope value (s) obtained from calibration curve (Revathi 2014).

Equations: LOD = 3.3 σ/s LOQ = 10 σ/s The LOD and LOQ were calculated according to the 3.3 σ/S and 10σ/S criterions, respectively, where σ is the standard deviation of the y- intercept of the regression line and s is the slope of the calibration curve.

2.2.5.2.3 Accuracy

Accuracy is the percentage of analyte recovered by assay from known added amount. Solutions were prepared at levels 75%, 100% and 150% of 20 μg/cm^3 test concentration of the sample solution using standard working solution as per the test

method and absorbance was noted down. The whole procedure was done in triplicate (Sethuraman 2013) .

2.2.5.2.4 Precision

Precision of an analytical method is the degree of repeatability under the normal operating conditions. The precision was determined with standard quality control samples prepared in triplicate at same concentration covering the entire linearity range. The precision of assay was determined by intra-day and intermediate, i.e., inter-day precision (comparing the assay conducted on 3 different days) and were recorded as % RSD for a statistically significant number of replicate measurements. From the resulting absorbance the mean, SD (standard deviation) and RSD (relative standard deviation) were calculated (Bhavar 2015) .
The average value used was the arithmetic mean, (usually abbreviated to the mean),which is the sum of all the measurements, , divided by the number of measurements, n.
Equations:

The $mean\ \bar{X} = \frac{\sum X_i}{n}$(2.1)

WhereX_i is mean, n is number of measurements

The standard deviation, $SD = \sqrt{\frac{\sum(X_i - \bar{X})^2}{(n-1)}}$(2.2)

Relative standard deviation $RSD = \frac{100\ X\ SD}{\bar{X}}$(2.3)

2.2.5.2.5 Repeatability

Repeatability analysis was performed by analyzing samples of same concentrations (six times) of standard niacin (0.8µg/cm^3). The resulting absorbance used to calculate the mean, SD and RSD.

2.2.5.2.6 Robustness

The robustness of any analytical method is the measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Robustness is an indicative of reliability of a method during normal usage. Robustness was tested by varying detection wavelength (±1 nm) of optimized conditions from the standard detection wavelength (262 nm) (Desai P2013) .

2.2.6 Inductively coupled plasma optical emission spectrometry (ICP-OES)

The Method was used to screen the contents of trace elements. The study covered six different samples of Sudanese hair cosmetic products. Four of them were plant extracts of hibiscus, colves, ginger and alshia butter; the rest were animal fats marked (Wadak and camel hump) .

Seeds were brought from seed wholesalers and local markets in the Khartoum.The samples was grinded in a grinder. One gram of each dry powdered samples were weighed into 100 cm^3 glass beakers. 3 cm^3 of 65% HNO_3 and 5 cm^3 of 35% H_2O_2 were added to the samples and allowed to react overnight. At the following morning, the beakers were carefully heated until clear solutions were obtained. Care was taken to ensure that the samples would not dry. A mixture of 3 $cm^3$of 65% HNO_3 and 9 $cm^3$of 37% HCl was added and gently heated until a small volume of acid remained. The residue was filtered and the solutions were precisely transferred to 100cm^3 plastic standard flasks and made to volume with deionized water. Element contents were analyzed with a Perkin-Elmer 2001 Model inductively coupled plasma-optic emission spectrometry (ICP-OES) (Umran2011).The concentration levels of the various metals in some Sudanese hair cosmetic products are summarized in Table 5.1.

2.2.7 Method for determination of antioxidant activity

Shimada(1992) reported that a number of methods and modifications were proposed to determine antioxidant activity including total antioxidant activity. In this study, radical scavenging effect was determined for different samples of Sudanese hair cosmetic products .Such as Sesame Oil, Beeswax, Bone marrow fats, Camel hump fats, Lamb grease and ethanolic extracts of ginger , hibiscus and colves.

Antioxidants content was determined according to the method of Shimada et.al.(1992),with some modification. A 5mg of samples was dissolved in 1cm^3 of dimethyl sulfoxide . In 96-wells plate, the test samples were allowed to react with 2.2-Di(4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. DPPH was prepared in methanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage of antioxidants was determined in comparison with that of a propyl gallate as standard. All tests and analyses were run in triplicate.

2.2.8 Analysis of proteins in the samples

Crude protein of thesamples was determined using method according to(AOAC 1990) ,as follows :

2.2.8.1 Digestion1 :

0.2 g of each sample was accurately weighed and placed in small digesting flask (50cm^3). About (0.4g) catalyst mixture (96% anhydrous sodium sulphate and 3.5% copper sulphate)was added.3.5cm^3 of approximately98%of sulphuric acid was added. The contents of flask were then heated for 2 hours till the color changed to blue-green. The tubes were then removed from digester and allowed to cool.

2.2.8.2 Digestion 2 :

The digested samples were transferred to the distillation unit and 20$cm^3$of sodium hydroxide(40%)were added. Ammonia was received in 100cm^3 conical flask containing 10$cm^3$of 2% boric acid plus 3-4 drops of methyl red indicator.The distillation was continued until the volume reached 50cm^3.

Titration :thecontent of flask were titrated against 0.02M hydrochloric acid. The crude protein was calculated.

2.2.8.3 FFA content

Acid value: about 5g of the sample were weighed accurately in to 250cm^3conicalflask. 50$cm^3$of mixture of 95% alcohol and diethyl ether solvents(1%)were added.The solution was neutralized after addition of 1cm^3 phenolphthalein as indicator.The content of flask were heated with caution until oil was completely dissolved , then titrated with0.1M potassium hydroxide with constant shaking.The acidity is frequently expressed as free fatty acid for which calculation should be using equation:

Free fatty acid = acid value / 2 (2.4)

Chapter three

Results and discussion

3. Results and discussion

3.1 Results of phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, flavonoids, triterpenoids, steroids, tannin, Saponin, Cumarin, Anthraquinone glycoside and phenols. The methanolic extract of the samples were subjected to preliminary phytochemical screening to identify the chemical constituents.The results are shown in Table 3.1.

Table3.1 :Qualitative Phytochemical Analysis of hibiscus and colves

No	Group of compounds	Colves	Hibiscus	Ginger
1	Saponin	+	+	+
2	Cumarin	++	-	-
3	Tannins	++	+	+
4	Flavonoids 1	+	+	+
4	Flavonoids 2	++	++	++
5	Alkaloids	-	+	+
6	Steroid	-	+	+
7	Tri terpenes	++	-	+
8	Anthraquinone glycoside	-	-	+

(+) indicates presence while (–) indicates the absence of the components.

The phytochemical Analysis of hibiscus and colves was carried out. Investigation revealed that these plants are highly rich with component required for hair healthy growth. The preliminary phytochemical Analysis showed that there are some plant chemicals present in the methanolic extract such as tannins, flavonoids, saponin, steroid, alkaloids, cumarin and terpenoids and glycosides.

3.2 Results of spectrophotometic determination of total tannins and flavonoids

The methanolic extract of the samples were prepared to examine the total tannins content and the total flavonoid scontent.The absorbance of total tannins and flavonoidsin colves and hibiscus was measured at 510nm and 415 mn respectively (Table 3.2.).

Table3.2 : UV absorbance of total tannins and flavonoids

`

Samples	Absorbance of total tanninsat 510nm	Average	Absorbance of total flavonoids at 415 mn	Average
Hibiscus	0.9175	0.9202	0.1843	0.1842
	0.9277		0.1842	
	0.9153		0.1841	
Colves	0.3608	0.3561	1.3590	1.3567
	0.3572		1.3603	
	0.3504		1.3508	

Table3.3 shows the concentration of total tannins and flavonoids contents found in colves and hibiscus.

Table3.3:Tannins and flavonoids contents in colves and hibiscus

Samples	Concentration of total tannins (mg/dm^3)	Concentration of total flavonoids(mg/ dm^3)
Colves	48.03	311.86
Hibiscus	189. 05	54. 86

Numerous studies have examined the solubility of tannins in solvents, but no solvent system has been found to be completely satisfactory. Solubility of tannins depend on many factors including the structure of the tannins themselves. Pure solvents

were insufficient extraction media for the recovery of phenolics and particularly tannins (Isam Eldin 2016).

In this study, the extraction was made by shaking the samples in the 80% methanol solvent for 48 hours at room temperature. Hibiscus gave a higher content of total tannins(189. 05 mg/dm^3) than that given bytotal colves (48.03mg/dm^3). Colves, however, colve sgave higher content of flavonoids (311.86mg/dm^3)than that given by hibiscus (54. 86 mg/dm^3) .

3.3 Results of UV spectrophotometric determination of vitamin C samples

In the present study ,an investigation was carried out to analyze three different types of medicinal plants such as ginger , colves and hibiscus for their vitamin c content to generate a scientific database for the users to know the daily uptake of vitamin C.

Table3.4 Results of spectrophotometric determination of vitamin C content in some medicinal herbal plants

Samples	Absorbanceof vitamin C	Percentage % w/v of vitamin C
Hibiscus	0.1234	0.855
Colves	0.4166	1.155
Ginger	0.1258	0.349
Standard	0.6454	-

vitamin C concentration from the three different medicinal plants was spectrophotometrically determined at 760nm .It can be seen from (Table3.4) that the highestcontent of vitamin c was found in colves (1.155% w/v) ,the lowest in ginger (0.349% w/v) and the middle in hibiscus (0.855% w/v).

3.4 Results for determination of niacine, vitamin C and gallic acid in ginger, hibiscus and colves using high-performance liquid chromatography/mass spectroscopy (HPLC/MS)

Aqueous extract of ginger, hibiscus and colve splants \.,is rich in many chemical compounds, for example, ascorbic acid, niacin and gallic acid. Hair growth is associated with niacin to improve blood flow to the scalp. A healthy scalp is crucial for healthy hair growth.

Column chemistry, solvent type, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so there was no interference from solvent and other compounds. For HPLC analysis, initially various mobile phases were tried in attempts to obtain the best separation and resolution between Niacine, vitamin C and gallic acid. The mobile phase consisting of gradient elution of methanol and formic acid 0.1% in HPLC-grade water was found to be an appropriate mobile phase allowing adequate separation using C-18 column at a flow rate of $0.38cm^3$/min.

Niacine, vitamin Cand gallic acid were chromatographically determined in aqueous extract samples of ginger ,hibiscus and colves .The calibration curves of niacin vitamin C and gallic acid standards are shown in figures 3.1 , 3.2and 3.3, respectively .

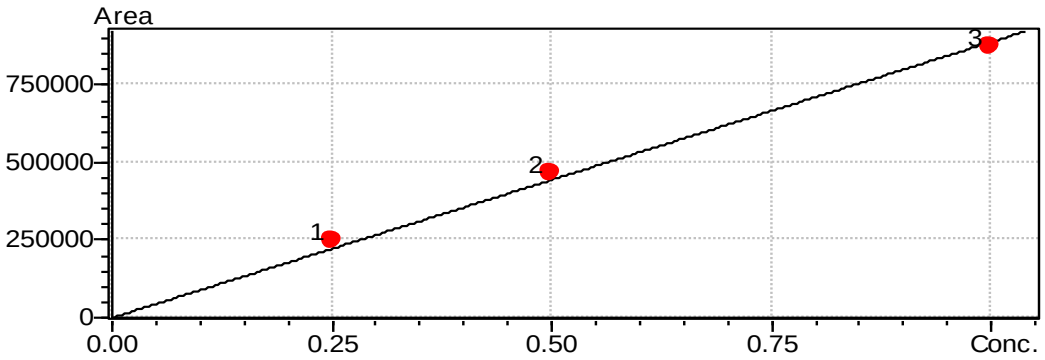


Figure 3.1: Calibration curve of niacin standards

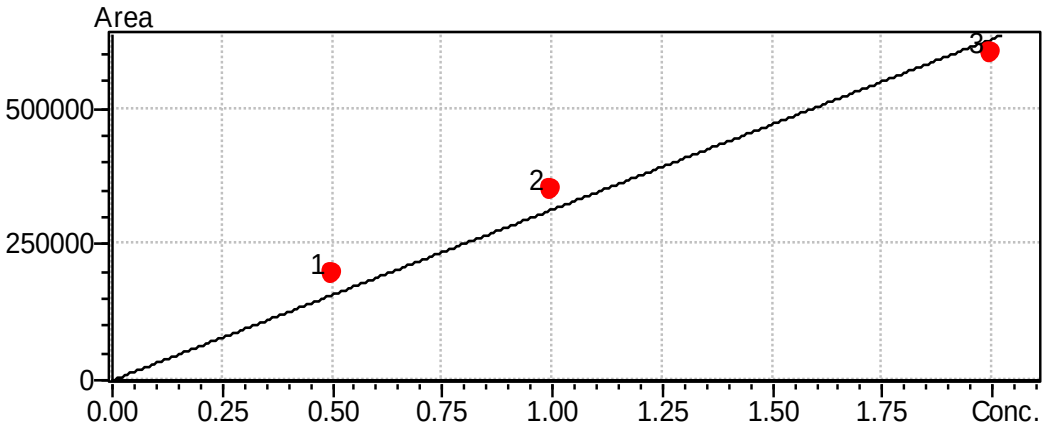


Figure 3.2: Calibration curve of gallic acid standards

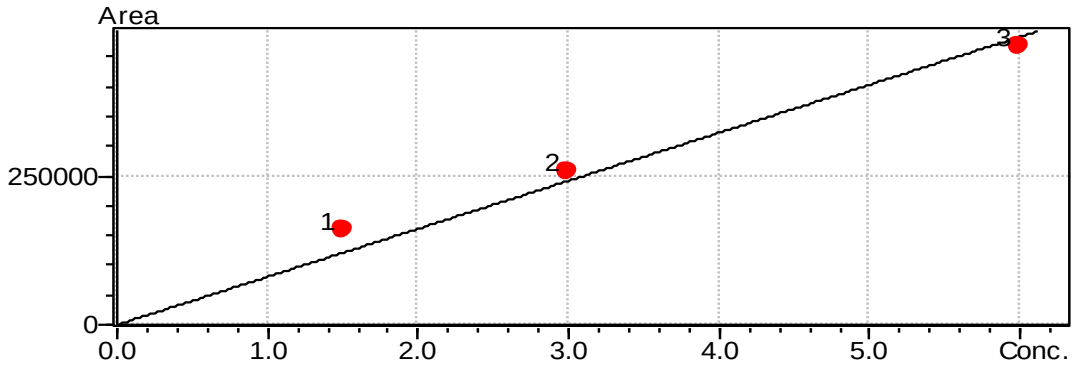


Figure 3.3: Calibration curve of vitamin C standards

Figure 3.4, 3.5and 3.6, shows theHPLC chromatogram of hibiscus, ginger and colves level 1, level 2, level 3of standards, respectively.

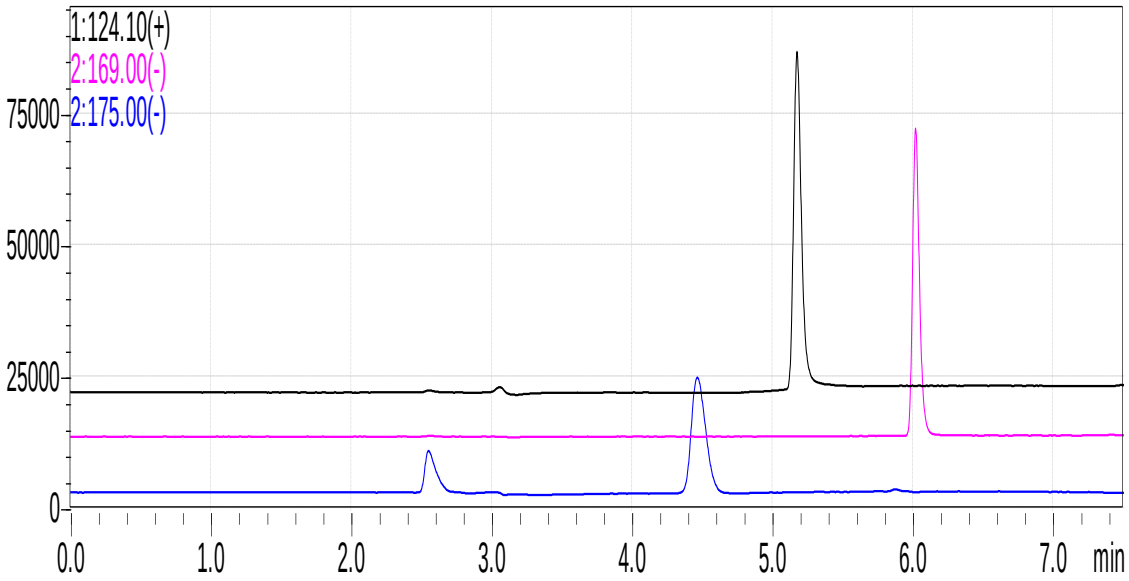


Figure 3.4: The HPLC chromatogram of hibiscus ,ginger and colves level 1of standards

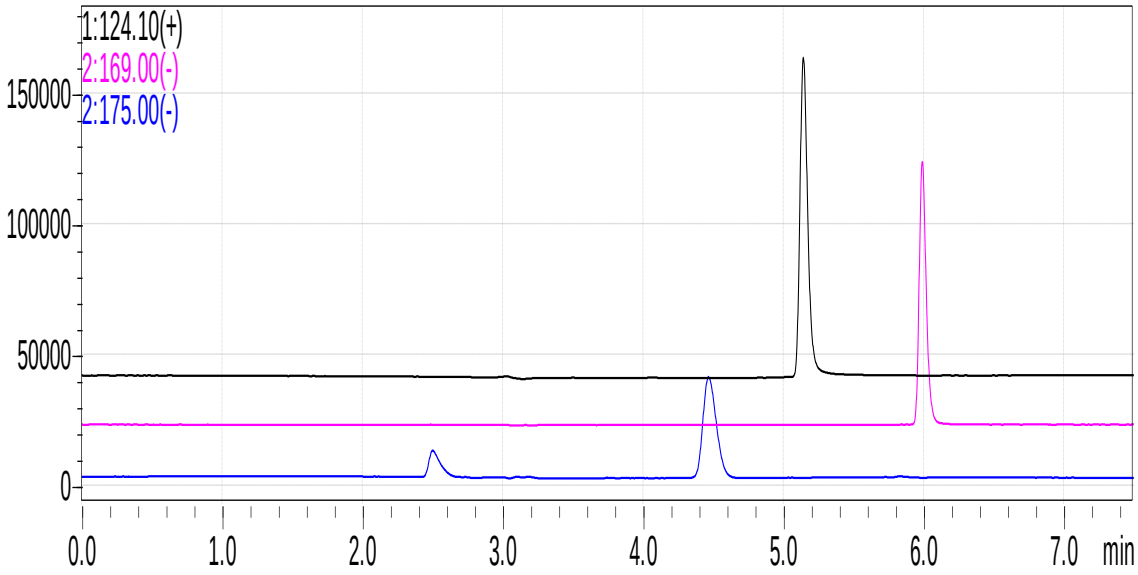


Figure 3.5: The HPLC chromatogram of hibiscus, ginger and colves level 2of standards

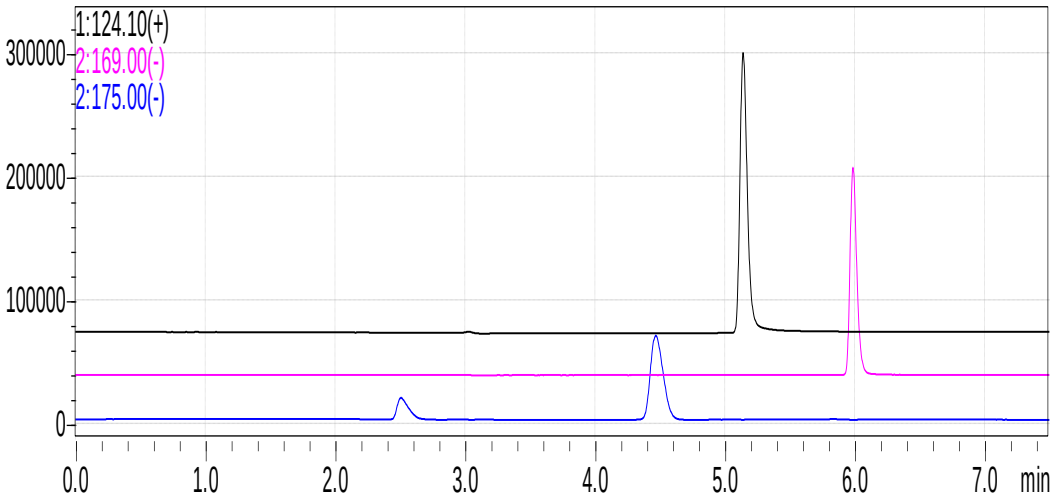


Figure 3.6: The HPLC chromatogram of hibiscus, ginger and colves level3of standards

HPLC chromatogram of ginger, colves and hibiscus are shown in figure 3.7, 3.8and 3.9, respectively.

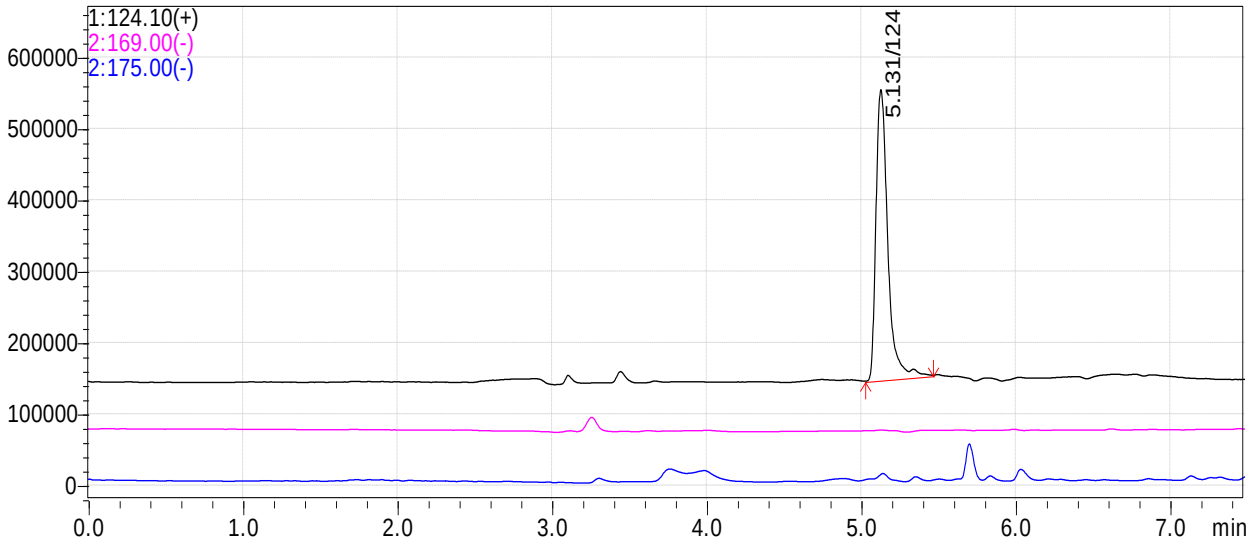


Figure 3.7: The HPLC chromatogram of ginger

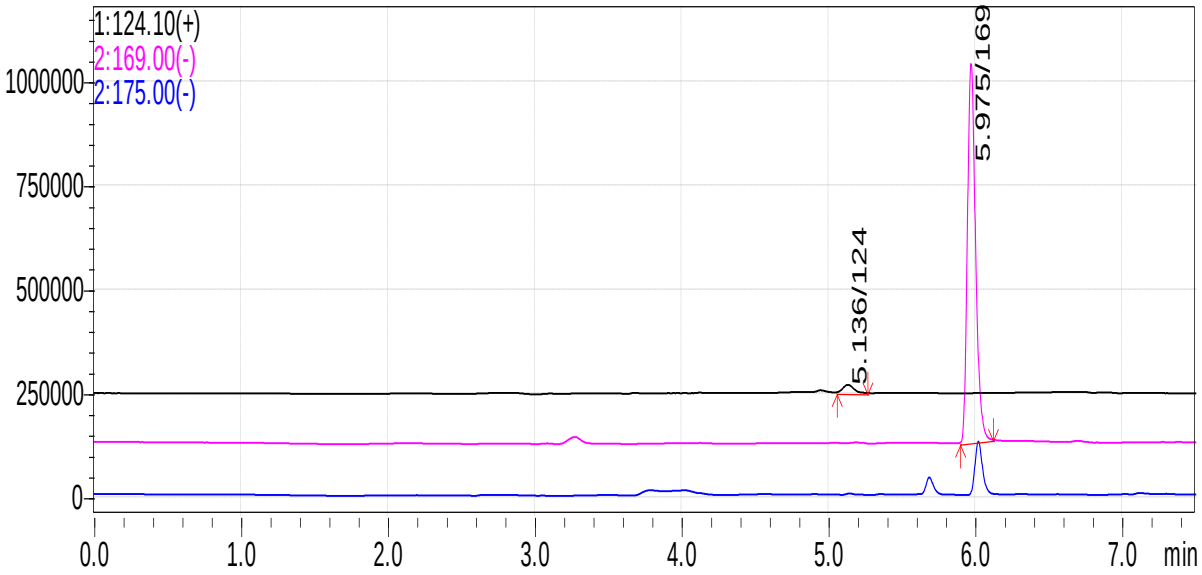


Figure 3.8: The HPLC chromatogram of colves

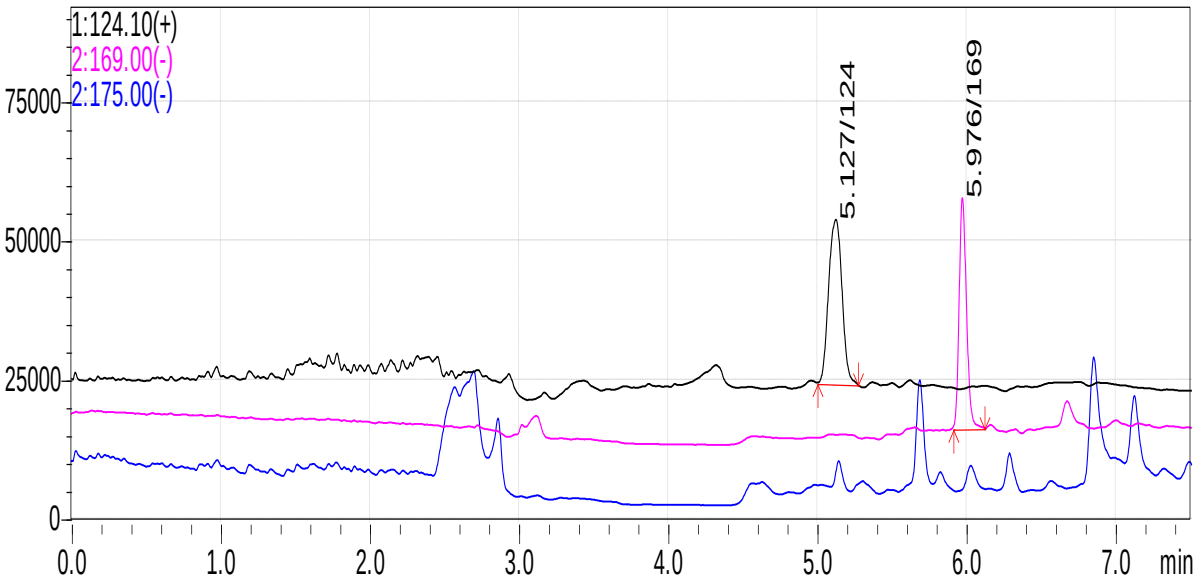


Figure 3.9: The HPLC chromatogram of hibiscus

Niacine, vitamin C and gallic acid inginger, colves and hibiscus were extracted with 30% of methanol and 70% distilled water, acidified to pH3 with phosphoric acid, and determined using high-

performance liquid chromatography/ mass spectroscopy (HPLC/MS) . The results obtained are shown in Table 3.5.

Table3.5.Results of (HPLC/MS) determination of niacine, vitamin Cand gallic acid in ginger , hibiscus and colves

Samples	Conc. Of niacine ppm	Conc. of gallic acid ppm	Conc. of vitamin C ppm
Hibiscus	1.04	12.05	*N.D.
Colves	0.525	2. 265	N.D.
Ginger	11.48	56	N.D.

*not detected

The HPLC method mentioned here represented an excellent technique for simultaneous determination of niacine and gallic acid in aqueous extract of ginger , hibiscus and colves plants. The method gives a good resolution among niacine and gallic acid with a gradient elution. The results obtained showed the presence of niacine and gallic acid, and the absence of vitamin C,in all herbal samples. Ginger gave high concentration of niacine (11.48 ppm), then hibiscus (1.04 ppm) and colves (0.525ppm) .Gingergave a high concentration of gallic acid (56ppm), then hibiscus (12.05ppm) and colves (2. 265ppm). However,The concentration of gallic acid is not detected in Ginger.

Vitamin C was not detected in the three herbal samples because the method might not be suitable for its extraction.

3.5. Results of development and validation of spectrophotometric determination for niacin content in ginger

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular

summation of the characteristics applicable to identification, control of impurities and assay procedures is included.

The plant selected for the present study is ginger. It contains many minerals, vitamins and oils as well as antioxidants and so it has many medical and aesthetic benefits. Also, it makes hair more manageable, softer and shinier. In the present study, efforts will be made to develope and validate a simple, specific and economic UV spectrophotometric method using water as solvent to determine niacin content in ginger water extracts according to the ICH guidelines (2005).

The λ-max of niacin in distilled water was found to be 262 nm. The absorbance maximum of the drug was recorded by taking scan of the niacin sample solution in the UV region (200-400 nm)(Figur3.10).

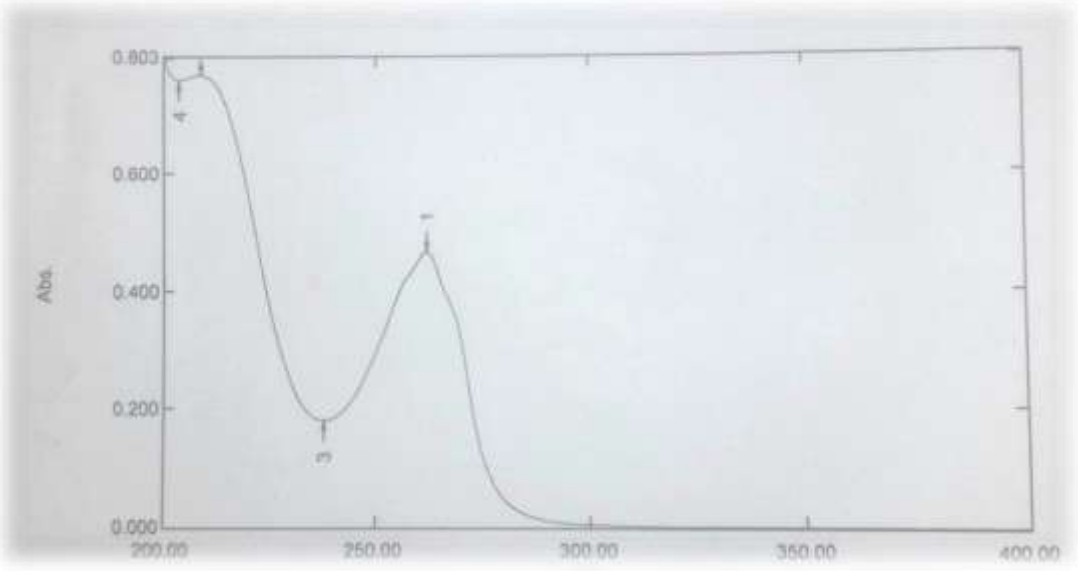


Figure 3.10: UV Spectrum of standard Niacin

3.5.1 Validation parameters of developed analytical method

The method was validated following ICH guidelines (Q2 (R1)).

3.5.1.1 Linearity and range

Good linear correlation was observed between absorbance and concentration in the selected concentration range of 5-30 μg/cm^3.

The regression equation was recorded to be y = 0.034x - 0.057. The correlation coefficient (R^2) of the standard curve was found to be 0.9955,(Figure 4.2). The results are tabulated in Table 3.6.

Table 3.6.: Spectrophotometric data for calibration curve of niacin at 262 nm.

Concentration (µg/ cm^3)	*Absorbance S.D.(nm)
5	0.133
10	0.256
15	0.465
20	0.603
25	0.818
30	0.961

*Each value is the average of three determinations

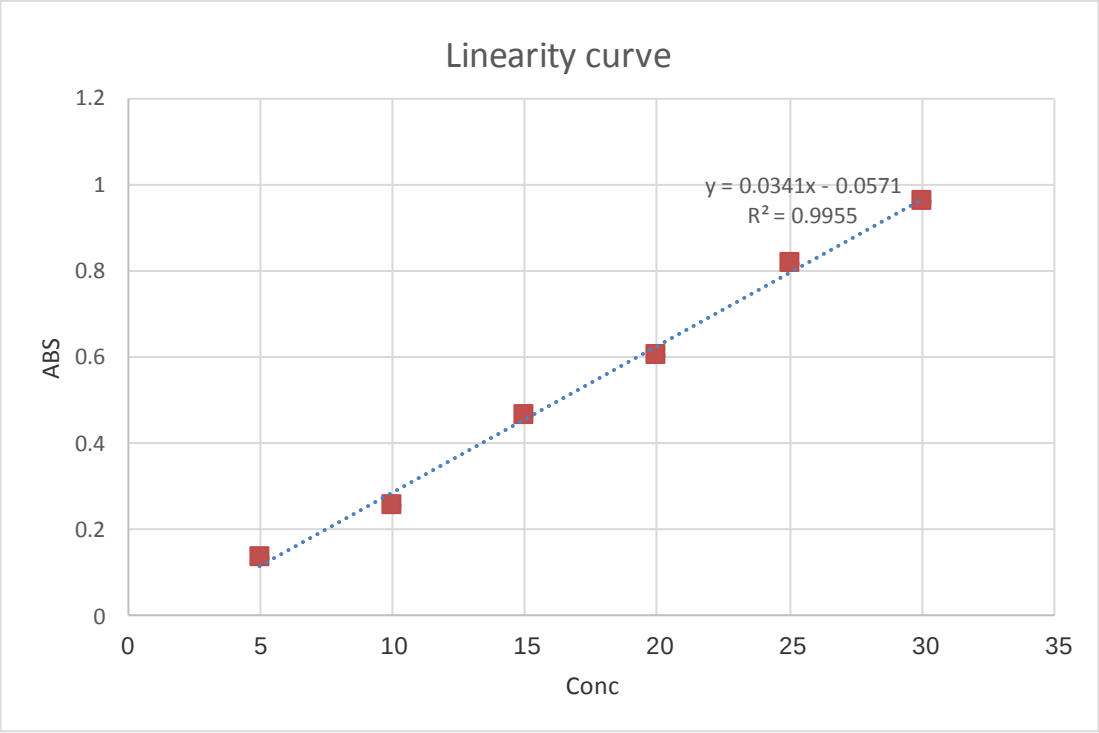


Figure 3.11: Linearity curve of niacin at 262 nm.

3.5.1.2 Sensitivity

Calculations of LOD and LOQ of method are based on the standard deviation of y-intercept of regression line (σ) and the slope (s) of the calibration curve at levels approximating the LOD and LOQ. LOD and LOQ were calculated according to the formulae: LOD = 3.3 σ/S = 2.996 μg/cm^3 LOQ = 10 σ/S = 9.08 μg/cm^3.

3.5.1.3 Accuracy/recovery

Results of recovery study were within the range of 99.15- 99.66 % indicating that the developed method is an accurate method for determination of niacin.

3.5.1.4 Precision

The samples were estimated similarly daily, for three consecutive days. The developed method was found to be precise as the average % RSD values for intraday and inter-day precision study was found to be 0.378% , 0.355%and 0.343% respectively . The results obtained from intra-day and inter- day precision are shown in Table 3.7.

Table 3.7.: Results of intraday and inter day precision

S. no.	Conc. mg/ cm^3	Abs Day 1	AbsDay 2	Abs Day 3
1	0.8	**0.188**	**0.198**	**0.207**
2	0.8	**0.187**	**0.201**	**0.202**
3	0.8	**0.186**	**0.199**	**0.208**
Mean		**0.187**	**0.199**	**0.206**
SD		**0.0007**	**0.0007**	**0.0007**
% RSD		**0.378**	**0.355**	**0.343**

3.5.1.5 Robustness

Robustness studies assume that the obtained results are insignificantly affected by small variations in any of the variables

(Table 3.8) they ensured the reliability of the proposed method during routine analysis.

Table 3.8: Results of robustness studies

Sample NO	Conc. ($\mu g/cm^3$)	Abs at 261nm	Abs at 262 nm	Abs at 263nm	Abs at 264nm
1	**20**	**0.561**	**0.565**	**0.552**	**0.528**
2	**20**	**0.567**	**0.566**	**0.562**	**0.526**
3	**20**	**0.563**	**0.564**	**0.562**	**0.531**
Mean		**0.564**	**0.565**	**0.557**	**0.528**
SD		**0.0007**	**0.0007**	**0.0007**	**0.0007**
RSD%		**0.1254**	**0.125**	**0.1265**	**0.1339**

3.5.1.6 Repeatability

The repeatability of the instrument was validated by taking the absorbance of six samples of the same concentration (0.8mg/ml). The mean absorbance was computed to be 0.253. The results are tabulated in Table 3.9.

Table 3.9: **Results of repeatability studies of niacin at 262 nm.**

Concentration (mg/ml)	Absorbance S.D.	Statistical analysis
0.8	0.257	Mean = 0.253
0.8	0.251	SD = 0.00089
0.8	0.251	%RSD = 0.3535
0.8	0.252	
0.8	0.254	
0.8	0.255	

3.9 **Results for determination of heavy metals in some natural hair Cosmetics**

Inductive coupled plasma(ICP) spectroscopy is commonly used today as a good element analysis technique and it has got the capacity to measure considerable amount of elements at the same time. This study is to determine heavy metals content in natural hair cosmetics using inductive coupled plasma optical emission spectrometry(ICP-OES) .

Table 3. 10:The concentration levels of the metals with ppm unit in the sample

The(ICP) study measured the concentration levels of the various

Sample Elements	Hibiscus	Cloves	Ginger	Wadak	Camel hump	Alsia butter
Al	57.02	49.50	216.6	3.210	3.540	9.450
As	<0.002547	<0.002547	<0.002547	<0.002547	<0.002547	<0.002547
B	49.38	13.55	1.300	<0.00035	0.9000	<0.00035
Ba	17.25	16.20	32.15	<0.00057	<0.00057	<0.00057
Be	<0.00030	<0.00030	<0.00030	<0.00030	<0.00030	<0.00030
Ca	16881	8118	1615	84.90	<0.001685	42.50
Cd	<0.000198	<0.000198	<0.000198	<0.000198	<0.000198	<0.000198
Co	<0.000198	<0.000198	<0.000198	<0.000198	<0.000198	<0.000198
Cr	6.960	0.3200	0.4300	<0.000583	<0.000583	<0.000583
Cu	5.470	5.200	6.210	1.7500	<0.000298	0.9800
Fe	196.21	105.71	437.91	10.07	21.33	32.43
K	19647	14497	20847	87.60	14.30	21.30
Li	<0.001279	<0.001279	<0.001279	<0.001279	<0.001279	<0.001279
Mg	4185	3353	2813	3.000	<0.000217	11.53
Mn	139.4	446.5	267.6	<0.000075	<0.000075	<0.000075
Mo	<0.000277	<0.000277	<0.000277	<0.000277	<0.000277	<0.000277
Na	<0.1421	76.40	<0.1421	<0.1421	<0.1421	<0.1421
Ni	0.4200	0.8600	43.05	2.050	<0.0000674	68.54
P	1740	970.3	1838	46.20	<0.004732	26.00
Pb	<0.004727	<0.004727	<0.004727	<0.004727	<0.004727	<0.004727
Sb	<0.006078	<0.006078	<0.006078	<0.006078	<0.006078	<0.006078
Se	<0.004993	<0.004993	<0.004993	<0.004993	<0.004993	<0.004993
Si	95.87	43.25	71.97	6.030	13.92	12.68
Sn	<0.009724	0.1100	0.0400	<0.009724	<0.009724	0.3600
Sr-	58.41	14.52	<0.000147	<0.000147	<0.000147	<0.000147
Ti	<0.000147	<0.000147	0.9900	<0.000147	<0.000147	<0.000147
V	4.750	2.980	2.890	<0.000099	<0.000099	<0.000099
Zn	34.24	4.970	15.72	<0.000112	<0.000112	<0.000112

metals in six different samples of Sudanese hair cosmetic products. Bestminerals that are necessary to keep hair healthy, strong and shiny and prevent hair loss are zinc, magnesium, silica, sulphur , selenium and iron .

The results obtained showed that the concentration of zinc,(from 4.970 ppm to 34.24ppm), magnesium(from 2813 ppm to 4185ppm), silicon(from43.25 ppm to95.87ppm)and iron(from196.21 ppm to437.91ppm)in plant extracts samples was higher than animal fats samples (from0.000112 ppm to32.43ppm) .The selenium concentration found to be the same in all samples (0.004993 ppm).

Industrial hair cosmetics includes hair cleansing cosmetics, hair growth promoters, and hair grooming cosmetics , containing limited concentration of heavy metals .For example selenium shampoo that is used as anti-dandruff was measured to have2% selenium . However, natural hair cosmetics contained variable concentration of elements but lower than that of the industrial hair cosmetics. Consequently, the use of industrial hair cosmetics could give better and more rapid effect than that given by natural hair cosmetics .

3.10 Resultsof visible spectrophotometer determination of antioxidants and proteins in some natural hair cosmetics in Sudan

The samples to be examined are: beeswax, lamb grease, sesame oil, bone marrow and ethanolic extracts of ginger , hibiscus and colves. All these samples are used as a hair grooming product to treats many problems of hair and scalp.

The percentageof antioxidants in some Sudanese hair cosmetic products are summarized in Table3.11.

Table 3.11: the percentage of antioxidants in some Sudanese hair cosmetic products

No.	Sample	Antioxidants content %
1	Sesame Oil	48.1
2	Beeswax	26.4
3	Bone marrow fats	3.1
4	Camel hump fats	14.3
5	Lamb grease	4
6	Animal fats	2.02
7	Colves	90.4
8	Ginger	85.7
9	Hibiscus	20.5

Since the colves, ginger and hibiscus herbal plants had a higher content of phenolic compounds and polyphenols, it had a stronger Antioxidant activities. The antioxidant properties of phenolic compounds originate from their properties of proton loss, chelate formation, and dismutation of radicals. Their structure–activity relationships are examined for this purpose. Phenols are compounds that have the ability to destroy radicals because they contain hydroxyl groups. These important plant components give up hydrogen atoms from their hydroxyl groups to radicals and form stable phenoxyl radicals; hence, they play an important role in antioxidant activity. Therefore, determination of the quantity of phenolic compounds is very important in order to determine the antioxidant capacity of plant extracts (De Gaulejac 1999).

In addition, antioxidants are added to cosmetic to prevent deterioration in their taste, smell, and colour (Kulisic 2004). The uses of these herbal plants extracts give a good effects to hair.

As a result, owing to its antioxidative properties, sesame oil is thought to be a natural source of antioxidants. It was observed to be the strongest compared with other animal fats and beeswax. It was observed that the radical scavenging effect of Sesame Oil was(48.1%),beeswax was(26.4%),bone marrow fats was(3.1),camel hump fats was(14.3%),lamb grease was(4%).

The ethanolic extracts of colves, ginger and hibiscus hada radical scavenging effect as shown intable 6.1 were:90.4%, 85.7%, 20.5% respectively.

Table 3.12: The results of moisture content, FFA content and protein content in some Sudanese hair cosmetic products

No	Sample / Test	Moisture content	FFA content	Protein content
1	Sesame oil	0.3036	0.2022	_
2	Ground nut oil	0.21	0.266	_
3	Beeswax	0.8337	0.2017	3.3667
4	Animal Ghee	0.864	0.8133	_
5	Camel hump fats	0.8333	0.2567	1.3633
6	Bone marrow fats	0.4162	6.4533	0.6967
7	Lamb grease	0.9575	7.9	2.46

Protein treatments are a popular option these days for addressing a number of hair problems, especially hair loss. They generally work by coating the outside of hair follicles and strands with proteins to harden them and protect them from breakage and further damage (Arshiya 2018).The results obtained as in(Table 3.12) showed the FFA content , protein content of moisture content in sesame oil, ground nut oil, bees wax, bone marrow fats, animal Ghee, camel hump fats and lamb grease Sudanese hair cosmetic products. The highest moisture content and FFA content in this samples were found in Lamb grease (0.9575%),(7.9%) respectively. The highest Protein content in this samples was found in Beeswax (3.3667%).

General conclusions

The objectives of this study were extraction of natural active ingredients, determination of heavy metals levels and determination of antioxidants and proteins, in natural herbal plants and animal fats used as hair cosmetic products in Sudan. In the current study, 12 samples of Sudanese hair cosmetic products (3 were plant extracts and 9 were animal fats samples).

The plant samples were subjected to preliminary phytochemical screening to identify the chemical constituents. In addition to total flavonoids and tannins, phytochemical analysis gave positive results for tannins and flavonoid swhich are applied as dye for hair to give hair excellent color strength and smooth surface morphology.The extraction of active ingredients from Sudanese medicinal plants, using 80% methanol has been successfully made. The extraction was made by shaking the samples in the respective solvent for 48 hours at room temperature. Results showed that the hibiscus gaven higher content of total tannins(189. 05 mg/dm^3) than that give by colves (48.03mg/dm^3). The latter , however, colves gave higher content of flavonoids (311.86mg/dm^3)than that given by hibiscus(54. 86 mg/dm^3). A visible spectrophotometric determination of vitamin C in these plants extracts of hair cosmetics was done. Vitamin C absorbance of the three different solution plant extracts was measured at 760nm .It was found that the highest content of vitamin c was determined in colves (1.155% w/v) ,the lowest in ginger (0.349% w/v) and the middle in hibiscus (0.855% w/v) .

A simple high performance liquid chromatographic method has been performed to estimte niacine, ascorbic acid and gallic acid in ginger, hibiscus and colves samples using aqueous extract. The ginger gave high concentration of niacine (11.48 ppm), then hibiscus (1.04 ppm) and Colves (0.525ppm) . Ginger gave a high

concentration of gallic acid(56 ppm), then hibiscus (12.05 ppm) and colves (2. 265 ppm). However, The concentration of vitamin C was not detected in the three herbal samples because method might not suitable for its extraction.
The developed and validated spectrophotometric determinationmethod for niacin content in ginger was found to be simple, specific, economic, precise and rapid for the determination of niacin in ginger.

Inductive coupled plasma optical emission spectrometry(ICP-OES) was applied to determine the concentration levels of the various metals in six different samples of Sudanese hair cosmetic products. To keep hair healthy, strong and shiny and to prevent hair loss, zinc, magnesium, silica, sulphur , selenium and iron are the active metals. The results obtained showed that the concentration of zinc,(from 4.970 ppm to 34.24 ppm), magnesium (from 2813 ppm to 4185 ppm), silicon (from 43.25 ppm to 95.87 ppm) and iron (from 196.21 ppm to 437.91 ppm)in plant extracts samples was higher than that of animal fats samples (from 0.000112 ppm to 32.43 ppm) .The selenium concentration was found to be equal but in trace amount in all samples (0.004993 ppm). Industrial hair cosmetics should include hair cleaners, hair growth promoters, and hair groomer singredient and limited amounts of heavy metals . For example selenium shampoo that is used as anti-dandruff was measured to have as much as 2% selenium . However, natural hair cosmetics contained variable concentration of elements but lower than that of the industrial hair cosmetics. Consequently, the use of industrial hair cosmetics could give better and more rapid effect than given by natural hair cosmetics .

Since the colves, ginger and hibiscus herbal plants showed a high content of phenolic compounds and polyphenols, it had strong antioxidant activities. As a result, owing to its antioxidative properties, sesame oil is thought to be a natural source of

antioxidants. It was observed to be the strongest source compared with other animal fats and beeswax source. It was found that the radical scavenging effect of Sesame Oil was 48.1%,beeswaxwas 26.4%,bone marrow fats was 3.1%,camel hump fatswas14.3%,lamb greasewas4%.Theethanolic extract of colves, ginger and hibiscus was found to have a radical scavenging effectof90.4%, 85.7%, 20.5% respectively.

The results obtained showed the FFA content , protein content of moisture content in Sudanese hair cosmetic products. The highest moisture content and FFA content in this samples were found in Lamb grease 0.9575%, 7.9% respectively. The highest Protein content in this samples was found in Beeswax3.3667%.

General recommendations

Suggestions for further research work include :

- Effects of heavy metals deficiency, malnutrition and anaemia on hair health.
- Identifition and determination of tannins, flavonoids, antioxidants and heavy metals in other natural Sudanese hair cosmetics.
- Extraction and isolation of pigments from natural colorants from Sudanese herbal plants, animals and even in organic source for possible potential use as hair colouring dyes.

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Appendixes

criterions, respectively, where σ is the standard deviation of the y- intercept of the regression line and s is the slope of the calibration curve.

Accuracy

Accuracy is the percentage of analyte recovered by assay from known added amount. Solutions were prepared at levels 75%, 100% and 150% of 20 µg/cm^3 test concentration of the sample solution using standard working solution as per the test method and absorbance was noted down. The whole procedure was done in triplicate (Sethuraman 2013).

Precision

Precision of an analytical method is the degree of repeatability under the normal operation conditions. The precision was determined with standard quality control samples prepared in triplicate at same concentration covering the entire linearity range. The precision of assay was determined by intra-day and intermediate, i.e. inter-day, precision (comparing the assay conducted on 3 different days) and were recorded as % RSD for a statistically significant number of replicate measurements (Bhavar 2015) [1].

Repeatability

Repeatability analysis was performed by analyzing samples of same concentrations (six times) of standard niacin (0.8 µg/cm^3). From the resulting absorbance, SD (standard deviation) and RSD (relative standard deviation) were calculated.

Robustness

The robustness of any analytical method is the measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Robustness is an indicative of reliability of a method during normal usage. Robustness was tested by varying detection wavelength (±1 nm) of optimized conditions from the standard detection wavelength (262 nm) (Desai P 2013) [2].

Results and discussion

The λ-max of niacin in distilled water was found to be 262 nm. The absorbance maximum of the drug was recorded by taking scan of the niacin sample solution in the UV region (200-400 nm) (Figur.1).

Fig 1: UV Spectrum of standard Niacin

Niacin was found to be linear within the concentration range 5-30 µg/ml and exhibited correlation coefficient of 0.995. The result of regression analysis is given in Table 1.

Validation parameters of developed analytical method

Linearity and range

Good linear correlation was observed between absorbance and concentration in the selected concentration range of 5-30 µg/cm^3. The regression equation was recorded to be y = 0.034x - 0.057. The correlation coefficient (R^2) of the standard curve was found to be 0.9955, (Figure 2). The results are tabulated in Table 1.

Table 1: Spectrophotometric data for calibration curve of niacin at 262 nm

Concentration (µg/ cm^3)	*Absorbance S.D. (nm)
5	0.133
10	0.256
15	0.465
20	0.603
25	0.818
30	0.961

*Each value is the average of three determinations

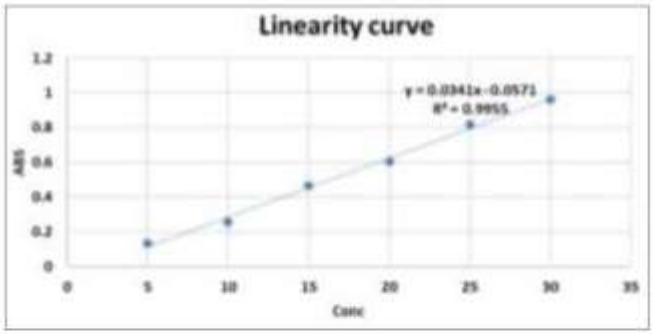

Fig 2: Linearity curve of niacin at 262 nm.

Sensitivity

Calculations of LOD and LOQ of method are based on the standard deviation of y-intercept of regression line (σ) and the slope (s) of the calibration curve at levels approximating the LOD and LOQ. LOD and LOQ were calculated according to the formulae: LOD = 3.3 σ/S = 2.996 µg/cm^3 LOQ = 10 σ/S = 9.08 µg/cm^3.

Accuracy/Recovery

Results of recovery study were within the range of 99.15-99.66 % indicating that the developed method is an accurate method for determination of niacin.

Precision

The samples were estimated similarly daily, for three consecutive days. The developed method was found to be precise as the average % RSD values for intraday and inter-day precision study was found to be 0.378%, 0.355%and 0.343% respectively. The results obtained from intra-day and inter- day precision are shown in Table 2.

Table 2: Results of intraday and inter day precision

S. no.	Conc. mg/ cm3	Abs Day 1	Abs Day 2	Abs Day 3
1	0.8	0.188	0.198	0.207
2	0.8	0.187	0.201	0.202
3	0.8	0.186	0.199	0.208
Mean		0.187	0.199	0.206
SD		0.0007	0.0007	0.0007
% RSD		0.378	0.355	0.343

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Development and validation of spectrophotometric determination for niacin content in ginger

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Abstract
A novel, simple, specific and economic UV spectrophotometric method was developed using water as solvent to estimate niacin content in ginger. The λ-max of niacin was found to be 262 nm. Linearity in the concentration range of 5-30 µg/cm^3 was found to be exhibiting good correlation coefficient (R2=0.9955). The developed method was validated statistically to demonstrate linearity, accuracy, precision, LOD and LO. The results of the study proved the applicability of the present method in routine analysis of niacin in ginger.

Keywords: Niacin, UV Spectrophotometry, Method development, validation, ICH guideline

Introduction
Ginger is one of the medicinal plants known for its therapeutic benefits since ancient times, and is used in several forms fresh, dried, powder or oil. Ginger contains many minerals, vitamins and oils and antioxidants and so it has many medical and aesthetic benefits (Shady 2013) [10].
Niacin, or vitamin B-3, or nicotinic acid, is chemically pyridine-3-carboxylic acid, official in IP (Indian Pharmacopoeia 2007) which is a colorless, water- soluble solid. It has high stability towards light and heat. Niacin helps to maintain the structure of the blood cells and improves blood circulation. That's why niacin brings more blood flow to the scalp, bringing more oxygen and nutrients to the hair follicles. Literature survey has revealed various analytical methods for determination of niacin in pharmaceutical formulations in combination with other drugs (Narayankar 2015). In the present study, efforts will be made to develope and validate a simple, specific and economic UV spectrophotometric method using water as solvent to determine niacin content in ginger water extracts according to the ICH guidelines (2005).

Materials and methods
Materials
Niacin standard, distilled water, ginger powder, some plants extract, whattman filter paper no. 41.

Instrument
Spectrophotometric analysis was done using UV spectrophotometer. (Shimadzo, 1650PC, Japan) with 1cm matched quartz cells.

Methods
Determination of wavelength of maximum absorbance(λ-max)
A standard stock solution of niacin (100 µg/cm^3) was prepared using distilled water as solvent and 0.2cm^3 was diluted to 10cm^3 with the same solvent to obtain 2 µg/cm3 standard solution. The standard solution was scanned in the wavelength region of 200- 400 nm.

Assay of content of niacin in ginger
A method for the determination of niacin was developed and applied to analyze it in ginger and some plants extract. 0.5 g of ginger was powdered and dissolved into 25 cm3water by shaking for one hour for complete extraction of niacin. The solution was then filtered through Whattman filter paper no. 41. This filtrate was diluted suitably with distilled water. The absorbance of this solution was measured and the amount of niacin was read from the calibration curve.

Method validation
Validation of the developed method was done following the guidelines laid down in International Conference on Harmonization (ICH) guidelines (2005).The following parameters were evaluated:

Linearity and range
Linearity of any analytical method is its ability, within a given range, to get test results that are directly, or through a mathematical transformation, proportional to concentration of analyte. Six different concentrations (5- 30µg/cm^3) of niacin were scanned on UV spectrophotometer in UV-range (i.e., 200-400 nm). The spectrum was recorded. Least square regression analysis was done by constructing the calibration plot between concentration and absorbance (Jain 2011) [5].

Sensitivity
Sensitivity of the developed method was determined with respect to limit of detection (LOD) and limit of quantification (LOQ). A series of varying drug concentrations (5-30µg/ml) were analyzed to find LOD and LOQ. LOD is the lowest detectable amount of an analyte in a given sample that may or may not be quantified, under the stated experimental conditions, whereas LOQ is the lowest quantifiable amount of analyte in any sample. LOD and LOQ were computed by using standard deviation (σ) and slope value (s) obtained from calibration curve (Revathi 2014) [9].
Equations: LOD = 3.3 σ/s LOQ = 10 σ/s The LOD and LOQ were calculated according to the 3.3 σ/S and 10σ/S

Robustness

Robustness studies assume that the obtained results are insignificantly affected by small variations in any of the variables (Table 3), they ensured the reliability of the proposed method during routine analysis.

Table 3: Results of robustness studies

Sample NO	Conc. (µg/cm3)	Abs at 261nm	Abs at 262 nm	Abs at 263nm	Abs at 264nm
1	20	0.561	0.565	0.552	0.528
2	20	0.567	0.566	0.562	0.526
3	20	0.563	0.564	0.562	0.531
Mean		0.564	0.565	0.557	0.528
SD		0.0007	0.0007	0.0007	0.0007
RSD%		0.1254	0.125	0.1265	0.1339

Repeatability

The repeatability of the instrument was validated by taking the absorbance of six samples of the same concentration (0.8mg/ml). The mean absorbance was computed to be 0.253. The results are tabulated in Table 4

Table 4: Results of repeatability studi

Concentration (mg/ml)	Absorbance S
0.8	0.257
0.8	0.251
0.8	0.251
0.8	0.252
0.8	0.254
0.8	0.255

Conclusion

The method proposed in the above s simple, specific, economic, precis determination of niacin in ginger. Sa formulations were in good agreemen with their respective label claims without interference of excipients. Being economic and precise, the developed method could be preferred as an alternative method for the routine analysis of the niacin in ginger.

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High-performance liquid chromatographic analysis of the extracts of ginger, hibiscus and colves herbal plant

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Abstract
A high performance liquid chromatographic method was carred out to estimte niacine, ascorbic acid and gallic acid in ginger, hibiscus and colves samples using aqueous extract; a very simple method was performed. A C18 column was used with a gradient elution of mobile phase at a flow rate of 0.38 cm^3/min. The results obtained showed the presence of niacine and gallic acid, in all herbal samples. Ginger gave high concentration of niacine (11.48 ppm), then hibiscus (1.04 ppm) and colves (0.525ppm). Ginger gave a high concentration of gallic acid (56 ppm), then hibiscus (12.05 ppm) and colves (2. 265 ppm). However, The concentration of vitamin C was not detected in the three herbal samples because extraction method used might not be suitable for its extraction.

Keywords: HPLC /MS, ascorbic acid, gallic acid, niacin, aqueous extract

1. Introduction
Aqueous extract of ginger , hibiscus and colves plants, is rich in many chemical compounds, for example ascorbic acid, niacin and gallic acid. Vitamins A, C and E obtained from vegetables and fruits protect cells and tissues against damaging effect of free radicals (Mukherjee2009). On the other hand, some ingredients from natural products are incorporated in cosmetic preparations owing to their various therapeutic properties, e.g. antiaging, moisturizing, antioxidant, antiinflammatory and antimicrobial effects, hair stimulants, etc. (Aburjai2003) [4]. Hibiscus is a typical plant of tropical climates found in the regions of mangroves in significant quantities. Hibiscus plant have pharmaceutical and cosmetics benefits. Previous pharmacological investigations of the genus hibiscus plants indicated the presence of species with useful biological activities. The studies conducted to date have demonstrated that plants of the Hibiscus containing anthocyanins that use for hair dyeing (Rosa RM 2006) [6]. Ginger and clover are the medicinal plants known for their therapeutic benefits since ancient times, and their use in several forms either fresh or dried , powder , or oil. (Shady 2013, Widyarini 2001) [2, 5]. The main function of niacin is to help to maintain the structure of the blood cells and improves blood circulation (Eshe 2017) [3] Gallic acid is commonly used in the pharmaceutical industry (Fiuza2004).
Owing to the remarkable antioxidants properties of ascorbic acid, it is widely employed in pharmaceutical and cosmetic industry (Tapan 2016).

2. Materials and Methods
Chemicals and reagents
- Standards chemicals like niacine , ascorbic acid and gallic acid.
- The HPLC-grade solvents such as, methanol, acetic acid from Merck (Germany).

Instrumentation
High- performance liquid chromatography (HPLC) equipped with an analytical column A C-18 LUNA (5 micron 25 cm×4.6 mm). prominence UFLC Shimadzu Corporation.

Methods
Preparation of aqueous extract
About 5 g of samples powder was weighed accurately, transferred to falcon tube, extracted with 30% of methanol and 70% distilled water and acidified to pH 3 with phosphoric acid, by shaking for 30 min .The samples then filtered with 0.45 µL, and subjected to determination of niacine, ascorbic acid and gallic acid in ginger , hibiscus and colves .

Preparation of mobile phase
The mobile phase was prepared by mixing methanol and formic acid (0.1% in water) for gradient elution system. Table 1 shows the chromatograph operating conditions.

Table 1: HPLC instrument Operation conditions

Time min	Methanol(B)	formic acid 0.1% in water(A)
0.01	5	95
5	90	10
9	5	95
10		

Flow rate : 0.38 cm^3/min
Column Temp : 40° C
The injection volume : 10µL

Mass spectroscopy (MS) Conditions
Instrument :MS 2020(Shimadzu corporation).
Ionization :ESI positive (DUIS PROBE)
Dl :400
Dry gas :5 L

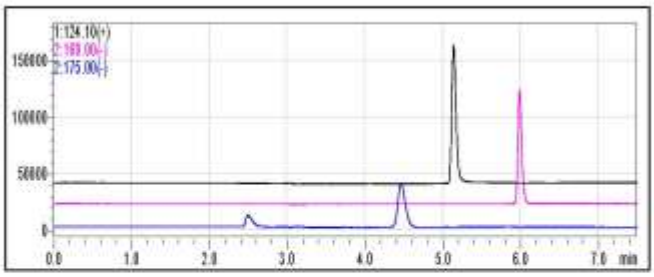

Fig 5: The HPLC chromatogram of hibiscus, ginger and colves of level 2 standards

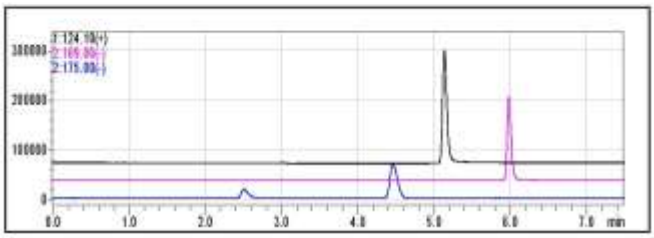

Fig 6 : The HPLC chromatogram of hibiscus, ginger and colves of level3 standards

HPLC chromatogram of ginger, colves and hibiscus are shown in figure 7, 8 and 9 respectively.

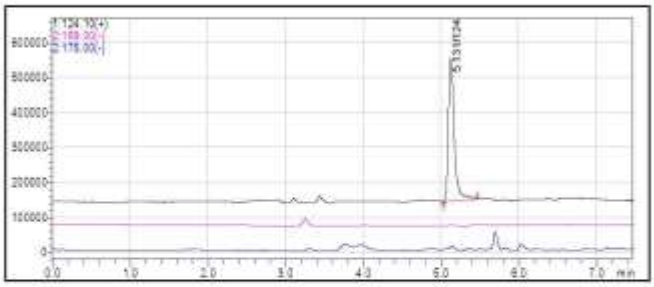

Fig 7: The HPLC chromatogram of ginger

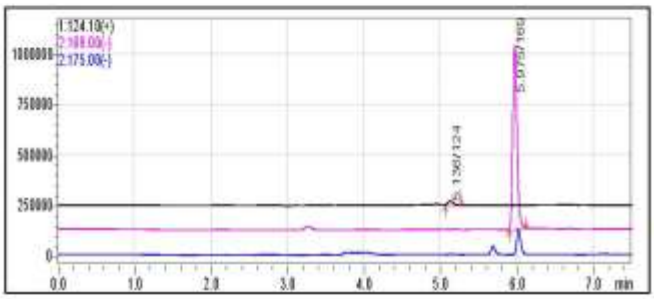

Fig 8: The HPLC chromatogram of colves

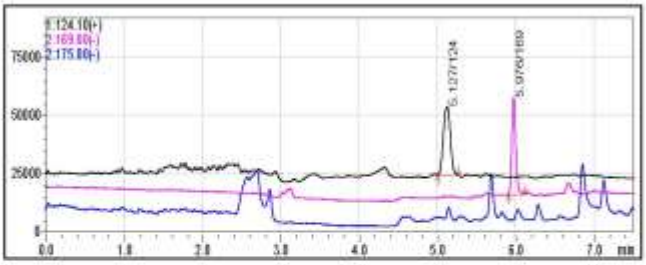

Fig 9: The HPLC chromatogram of hibiscus

Niacine, vitamin C and gallic acid in ginger, colves and hibiscus were extracted with 30% of methanol and 70% distilled water, acidified to pH 3 with phosphoric acid, and determined using high-performance liquid chromatography/ mass spectroscopy (HPLC/MS). The results obtained are shown in Table 2.

Table 2: Results of (HPLC/MS) determination of niacine, vitamin C and gallic acid in ginger, hibiscus and colves

Samples	Conc. Of Niacine ppm	Conc. of Gallic acid ppm	Conc. of vitamin C ppm
Hibiscus	1.04	12.05	*N.D.
Colves	0.525	2. 265	N.D.
Ginger	11.48	56	N.D.

*N.D.: not detected

The results obtained showed the presence of niacine and gallic acid, and the absence of vitamin C, in all herbal samples. Ginger gave high concentration of niacine (11.48 ppm), then hibiscus (1.04 ppm) and Colves (0.525ppm). Ginger gave a high concentration of gallic acid(56 ppm), then hibiscus (12.05 ppm) and colves (2. 265 ppm). However, The concentration of vitamin C was not detected in the three herbal samples because extraction method might not suitable for its extraction.

Conclusion

The applied HPLC method represented an excellent technique for simultaneous determination of niacine and gallic acid in aqueous extract of ginger, hibiscus and colves plants. The method gave a good resolution among niacine and gallic acid with gradient elution. The concentration of vitamin C was not detected in the three herbal samples because the extraction method used might not be suitable for its extraction.

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