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***Characterization and biological activities of
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application in the cosmetics industry***

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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*First and foremost, praise be to God,
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الملخص

التوصيف والأنشطة البيولوجية للمستخلصات النباتية بمنطقة الوادي وتطبيقها في صناعة مستحضرات التجميل

ركزت دراستنا على البحث عن اكتشاف النشاط المضادة للأكسدة والمضاد للالتهاب، وتقييم نشاط الحماية الضوئية للمستخلصات المائية لنبات بذور الجزر (*Daucus carota L*) وجذور عرق السوس (*glycyrrhiza glabra L*) المستخرجة باستخدام طريقة النقع، واستعمالهما في تركيب منتجات للعناية بالبشرة (مزيل المكياج في هذه الدراسة).

بعد إجراء الاختبارات الكيميائية النباتية ثبت أن كلتا النباتين المدروستين غنية بالمستقلبات الثانوية بما في ذلك البوليفينول، الفلافونويد، التربينويدات، الجليكوسيدات، الصابونوزيدات، القلويدات، التانينات، بينما ثبت غياب الصابونوزيدات في مستخلص بذور الجزر، أما بخصوص المردود فبلغ مردود المستخلص الخام نسبة 13.2 % بالنسبة لمستخلص بذور الجزر و10% لمستخلص جذور عرق السوس.

كما تم تقدير المحتوى الكلي للبوليفينول حيث أظهرت النتائج أن مستخلص جذور عرق السوس يحتوي على نسبة عالية من مادة البوليفينول تقدر بـ (108.42 ± 0.89 مغ مكافئ من حمض الغاليك/غ من المادة الجافة) مقارنة بمستخلص بذور الجزر الذي يقدر بـ (55.78 ± 0.35 مغ مكافئ من حمض الغاليك/غ من المادة الجافة). بينما تم تقدير الفلافونويدات، وأظهرت النتائج أن مستخلص جذور عرق السوس يحتوي على نسبة عالية من الفلافونويد تقدر بـ (76.28 ± 0.37 مغ مكافئ من الكرسيتين/غ من المادة الجافة) مقارنة بمستخلص بذور الجزر المقدرة عند (37.73 ± 0.34 مغ مكافئ من الكرسيتين/غ من المادة الجافة)، من ناحية أخرى، فإن محتوى التانينات المكثفة في مستخلص جذور عرق السوس (13.99 ± 0.63 مغ مكافئ من الكاتيشين/غ من المادة الجافة) أقل من محتوى مستخلص بذور الجزر (12.33 ± 0.48 مغ مكافئ من الكاتيشين/غ من المادة الجافة).

أظهرت نتائج النشاط المضاد للأكسدة الذي تم إجراؤه عن طريق اختبارين (قوة إحتجاز الجذر الحر DPPH والقدرة الإرجاعية للحديد FRAP) حيث بينت النتائج أن المستخلصين لهما تقريبا نفس القدرة المضادة للأكسدة. كما تم تقييم النشاط المضاد للالتهابات، وأظهرت النتائج أن مستخلص جذور عرق السوس أكثر نشاطاً من مستخلص بذور الجزر بقيمة تقدر بـ ($IC_{50} = 29.07 \pm 3.49 \mu g/ml$).

تم إجراء تقييم لنشاط الحماية الضوئية للمستخلصين حيث أظهرت النتائج أن مستخلصي جذور عرق السوس وبذور الجزر يتمتعان بقدرة جيدة على الحماية الضوئية.

في الأخير وبعد تحضير المنتج (مزيل المكياج) بخلصة النباتات المدروسة ذات الفعاليات المدروسة، تم تجريب المنتج على عينة تقدر بـ 100 امرأة متطوعة من نساء الوادي، وأظهرت نتائج الاستبيان إلى أن مزيل المكياج بخلصة بذور الجزر وخلصه جذور عرق السوس فعالة وتحظى بإقبال كبير من قبل النساء حيث كانا فعالان في تنظيف البشرة ولم تعاني معظم المتطوعات من تهيج أو حساسية، كما أشارت النتائج إلى أن معظمهن سيوصين بإستعمال المنتج. ولهذا نحن نشجع التقدم في هذا المجال لأننا نؤمن بأن له إمكانات واعدة للمستقبل.

الكلمات المفتاحية: *Glycyrrhiza glabra L*, *Daucus carota L*، المركبات الفينولية، النشاط المضاد للأكسدة، النشاط المضاد للالتهابات، نشاط الحماية الضوئية، مزيل مكياج.

Résumé

Notre étude s'est concentrée sur l'investigation de l'activité antioxydante et anti-inflammatoire, et évaluant l'activité photoprotectrice d'extraits aqueux de graines de carotte (*Daucus carota* L) et de racines de réglisse (*Glycyrrhiza glabra* L) extraites par macération, et en les utilisant dans des formulations de produits de soins de la peau (démaquillant dans cette étude).

Après avoir effectué des tests phytochimiques, il a été prouvé que les deux plantes étudiées sont riches en métabolites secondaires, notamment des polyphénols, des flavonoïdes, des terpénoïdes, des glycosides, des saponosides, des alcaloïdes et des tanins, tandis qu'il a été prouvé que les saponosides étaient absents dans l'extrait de graines de carotte. Le rendement de l'extrait brut atteint 13,2% pour l'extrait de graines de carotte et 10% pour l'extrait de racine de réglisse.

La teneur totale en polyphénols a également été estimée, et les résultats ont montré que l'extrait de racine de réglisse contient un pourcentage élevé de polyphénols, estimé par ($108,42 \pm 0,89$ mg EAG/gE) par rapport à l'extrait de graines de carotte, qui est estimé par ($55,78 \pm 0,35$ mg EAG/gE). Bien que les flavonoïdes aient été estimés, les résultats ont montré que l'extrait de racine de réglisse contient un pourcentage élevé de flavonoïdes estimé par ($76,28 \pm 0,37$ mg EQ/gE) par rapport à l'extrait de graines de carotte estimé par ($37,73 \pm 0,34$ mg EQ/gE). En revanche, la teneur en tanins condensés de l'extrait de racine de réglisse ($13,99 \pm 0,63$ mg EC/gE) est inférieure à la teneur en extrait de graines de carotte ($12,33 \pm 0,48$ mg EC/gE).

Les résultats ont montré l'activité antioxydante réalisée à travers deux tests (pouvoir de rétention des radicaux libres DPPH et capacité de récupération du fer FRAP). Les résultats ont montré que les deux extraits ont presque la même capacité antioxydante. L'activité anti-inflammatoire a également été évaluée et les résultats ont montré que l'extrait de racine de réglisse est plus actif que l'extrait de graines de carotte avec une valeur estimée de ($IC_{50}=29,07 \pm 3,49$ µg/ml).

Une évaluation de l'activité photoprotectrice des deux extraits a été réalisée et les résultats ont montré que les extraits de racine de réglisse et de graines de carotte ont une bonne pouvoire photoprotectrice.

Enfin, après avoir préparé le produit (démaquillant) à base des extraits des plantes avec les propriétés étudiées, le produit a été testé sur un échantillon de 100 femmes volontaires d'El-Oued, et les résultats du questionnaire ont montré que les démaquillants à base d'extrait de graines de carotte et d'extrait de racine de réglisse est efficace et très demandé par les femmes et étaient efficaces pour nettoyer la peau et la plupart des volontaires ne souffraient pas d'irritation ou de sensibilité. Les femmes recommanderaient d'utiliser le produit, c'est pourquoi nous encourageons les progrès dans ce domaine car nous pensons qu'il présente un potentiel prometteur pour l'avenir.

Mots clés : *Daucus carota* L, *Glycyrrhiza glabra* L, composés phénoliques, activité antioxydante, activité anti-inflammatoire, activité photoprotectrice, démaquillant.

Abstract

Our study focused on investigating the antioxidant and anti-inflammatory activity and evaluating the photoprotective activity of aqueous extracts of carrot seeds (*Daucus carota* L) and licorice roots (*Glycyrrhiza glabra* L) extracts. by maceration and using them in skin care product formulations (make-up remover in this study).

After carrying out phytochemical tests, it was proven that both plants studied are rich in secondary metabolites, including polyphenols, flavinoids, terpenoids, glycosides, saponosides, alkaloids and tannins, while it was proved that saponosides were absent in carrot seed extract. The crude extract yield reached 13.2% for carrot seed extract and 10% for licorice root extract.

The total polyphenol content was also estimated, and the results showed that licorice root extract contains a high percentage of polyphenols, estimated by (108.42 ± 0.89 mg EAG/gE) compared to the carrot seed extract, which is estimated by (55.78 ± 0.35 mg EAG/gE). Although flavonoids were estimated, the results showed that licorice root extract contains a high percentage of flavonoids estimated by (76.28 ± 0.37 mg EQ/gE) compared to licorice seed extract. carrot estimated by (37.73 ± 0.34 mg EQ/gE). On the other hand, the condensed tannin content of licorice root extract (13.99 ± 0.63 mg EC/gE) is lower than the content of carrot seed extract (12.33 ± 0.48 mg EC /gE).

The results showed the antioxidant activity achieved through two tests (DPPH free radical retention power and FRAP iron scavenging capacity). The results showed that both extracts have almost the same antioxidant capacity. The anti-inflammatory activity was also evaluated, and the results showed that licorice root extract is more active than carrot seed extract with an estimated value of ($IC_{50}=29.07 \pm 3.49$ μ g /ml).

An evaluation of the photoprotective activity of the two extracts was carried out and the results showed that licorice root and carrot seed extracts have good photoprotective power.

Finally, after having prepared the product (make-up remover) based on plant extracts with the properties studied, the product was tested on a sample of 100 female volunteers from El-Oued, and the results of the questionnaire showed that the make-up removers with base of carrot seed extract and licorice root extract is effective and highly demanded by women and were effective in cleansing the skin and most of the volunteers did not suffer from irritation or sensitivity. Women would recommend using the product, so we encourage advancements in this area as we believe it has promising potential for the future.

Key words: *Daucus carota* L, *Glycyrrhiza glabra* L, phenolic compounds, antioxidant activity, anti-inflammatory activity, photoprotective activity, make-up remover.

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Abbreviation List

A	
Abs	Absorbance
ARP	Anti-Radical Power
As	The absorbance of the sample
B	
BSA	Bovine serum albumin
BSTI	Baumann Skin Type Indicator
C	
CAT	Catalase
CF	Correction Factor
D	
DPPH	Diphenyl Picryl
E	
EAA	Equivalent of Ascorbic Acid
EAG	Gallic Acid Equivalent
ECT	Catechin Equivalent
EE	Erythema Effect
EQ	Quercetin Equivalent
FRAP	Ferric Ion Reducing Antioxidant Power
FeCl₃	Iron Chloride
I	
IC₅₀	Inhibitory Concentration of 50%
M	
Mg	Milligram
ml	Milliliter
N	
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
O	
O.D.	Optical Density
OTC	Over the counter Medicines
P	
Pcs	Phenolic Compounds
PH	Potential Hydrogen
PUFA	polyunsaturated fatty acids
R	
R•	Free Radical
ROS	Reactive Oxygen Species
S	
SLS	Sodium Lauryl Sulfate
SPF	Sun Protection Factor
T	
TCA	Trichloroacetic Acid
U	
USA	United States of America
UV	Ultraviolet
V	
VIP	Vasoactive Intestinal Peptide

Introduction

The human skin epidermis is a plate epithelium whose cells undergo a complex transition that converts live corneocytes from the basal layer to "horny" stratum corneum cells. This horny layer protects against unpleasant chemicals and physical agents and is essential in preventing transepidermal water loss **(Schrader & Rohr, 1996)**. The Baumann Skin Type Indicator (BSTI) now includes four main dichotomies or characteristics to better characterize skin types. By evaluating skin according to these parameters - dry or oily, sensitive or resistant, pigmented or nonpigmented, and wrinkled or unwrinkled- and thus differentiating among the 16 possible skin types, consumers can more easily identify the most suitable topical treatments for their skin **(Baumann, 2008)**

People are exposed to a wide range of chemicals on a daily basis, the majority of which exist naturally in the environment, but others are created from human activity and can be found in foods, water, and other ordinary products. Because our skin is the greatest surface of the body interacting with the outside environment, it is both involuntarily exposed to abiotic and biotic influences, and voluntarily **(Panico et al., 2019)**. Dirty skin is caused not only by the accumulation of soot and dust on the skin's surface, but also by natural skin by-products [decomposition products from cornification (cellular debris and lipids), sebaceous secretions, microorganisms and their by-products], as well as products intentionally or unintentionally applied to the skin (e.g. moisturizers, sunscreens, makeup, grease). This combination of natural and environmental greases and oils attracts and retains environmental dirt and/or particulate pollutants (soot and dust) on the skin's surface and within its pores, creating a material that is extremely difficult to remove with superficial cleansing/hygiene regimes **(Peterson et al., 2017)**

Regular daily skin care is crucial. Cleaning is a key component of skin care because it helps the skin absorb topically administered medications by clearing away dead surface cells and undesired debris, grime, and bacteria **(Subramanyan, 2004)**.

It is generally accepted that the first step in the skincare cosmetics routine is removing makeup. An impurity makeup is nice to remove entirely to optimize the benefits of skincare cosmetics products applied later **(Watanabe et al., 2005)**

Cosmetics, which are substances that are frequently applied straight to the human skin, mucous membranes, hair, and nails, ought to be safe for health, but lately, worries about this have grown. Sadly, there are situations where employing these goods might lead to

unfavorable outcomes from the purposeful or unintentional presence of chemicals, such as hazardous metals. Metals found in makeup can either be retained and act directly on the skin, or they can enter the bloodstream through the skin, build up in the body, and have harmful effects on different organs. **(Borowska & Brzóśka, 2015)**

However, some cosmetics have components with medicinal qualities in their formula that have positive local effects and guard against degenerative skin conditions. It enhances beauty by giving skin the vital nutrients it needs to stay healthy. They can lessen wrinkles and enhance the tone, texture, and brightness of the skin. The fastest-growing segment of the natural personal care market is cosmetics. Natural components are increasingly showing up in modern formulas, despite being utilized for centuries for skin care purposes. Anything or any element that is created by nature, found in nature, and taken straight from plants or animal products is referred to as "natural" **(Ribeiro et al., 2015)**.

People from worldwide have been using plant-based substances (Natural Products) to enhance the appearance since the existence of mankind **(Desam & Al-Rajab, 2021)**. Medicinal plants, with their wide variety of bioactive compounds, have long been a dependable source of therapy for human skin problems **(Sitarek et al., 2020)**.

Among these plants, we chose the following plants "*Glycyrrhiza glabra*" and "*Daucus carota L*" because of their benefits and use for alternative medical purposes in skin care. The main aim of this study was to discover the therapeutic properties and the effective substances contained in the plant species and their beneficial components, also to incorporate them in cosmetics products for the skin. We divided our research into two parts: a theoretical part and an experimental part.

The theoretical part includes three chapters: the first chapter about the skin, the second chapter about cosmetics products, and the third chapter about the plant species "*Glycyrrhiza glabra*" and "*Daucus carota L*" plants.

For the experimental part we opened this part with the chapter of material and methods, then the chapter of results and discussion. Finally, we concluded our research with a conclusion that summarize the results.

First part

Bibliographic

Part

Chapter one

Skin

I.1. Generalities

The skin is defined as the external covering organ of mammals (**Ferrag,2007**). It accounts for around 16% of an adult's total body weight (**McLafferty et al.,2012**). The skin is a complex organ whose functioning has two purposes: the first, ensuring communication between our own organism and the surrounding environment; the second, to protect our body from external attacks (**Dréno,2009**).

Its three-layered structure gives the skin unique mechanical qualities. It is a heterogeneous, anisotropic adhesive material with a nonlinear stress-strain relationship. Its mechanical behaviour is also quite complex. Understanding these properties is vital for cosmetic and clinical studies (**Boyer et al., 2009**).

I.2. Skin Physiology

The skin serves as a protective barrier between our Internal and external environments. It consists of three layers: the epidermis, dermis, and hypodermis. An adult human body weighs about 3 kg, has a surface area of 2 m², and varies in thickness from 1 to 5 mm based on location (**Baures et al.,2009**).

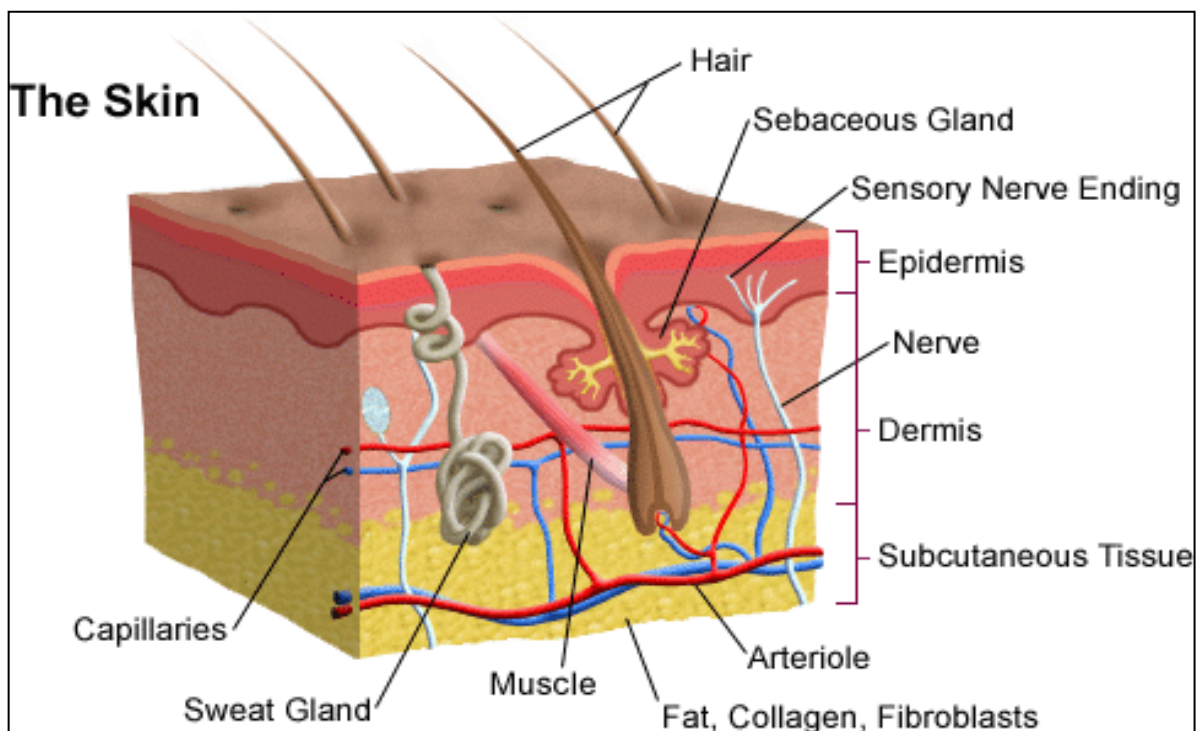


Figure 01: Skin structure (**Wazaefi, 2013**).

I.2.1. Epidermis

Epidermis is the outermost layer. Because the epidermis lacks blood arteries, nutrients are delivered to it via the dermis. The epidermis is made up of cells known as keratinocytes and melanocytes. Keratinocytes (80%) stack on top of each other, providing thickness, resistance to stretching, and protection (**Baures *et al.*,2009**).

I.2.1.1. Basal (germinative) layer

It is made up of a single layer of cells, approximately 50% of which are in mitosis and produce the cells of the layer immediately above, the stratum spinosum (**Martini,2011**).

I.2.1.2. Stratum spinosum

The stratum spinosum (or Malpighian mucous body) is made up of polyhedral cells firmly attached to each other by desmosomes, which are proteinaceous in nature. Their cytoplasm contains tonofilaments, also proteinaceous in nature, which are grouped into bundles, the tonofibrils. As they move upwards, they differentiate biochemically and become more and more active in the syntheses (**Martini,2011**).

I.2.1.3. Stratum granulosum (granular layer)

At its level, there is the development of fundamental molecules responsible for the physicochemical characteristics of the upper layers with the formation of keratohyaline grains and the appearance of Odland bodies.

Keratohyalin grains consist either of a protein rich in cystine which contributes to the formation of interfibrillary cement, or of a phosphoprotein rich in histidine, profilaggrin. The profilaggrin is itself the precursor of filaggrin (filament aggregating protein), a protein which aggregates the cells together in the stratum corneum. In the stratum granulosum, there are still living cells with nucleus. During keratinization, they will flatten and lose their nucleus to turn into corneocytes which constitute the stratum corneum (**Martini,2011**).

I.2.1.4. Stratum corneum (corneal layer)

It is made up of three layers:

Stratum lucidum (present only in the palm of the hands and the despieds plant);

Stratum compactum, which represents the stratum corneum proper;

Stratum disjonctum (couche la plus externe, desquamante).

The set of these three layers has a thickness of about 10 μm . The stratum corneum has completely different characteristics and biochemical composition than the epidermis's underlying layers. Corneocytes are the cells responsible for the ultimate step of keratinization. These cells, which are sometimes considered dead, lack a nucleus and are virtually entirely made up of keratin. They do, however, include several enzymes that are involved in the metabolization process. Furthermore, they contain a variety of more or less hygroscopic chemicals that help to fix water (**Aminata,2010**).

Corneocytes are structured schematically, much like a brick wall. The cement that holds them together is lipid-based and composed of polyunsaturated fatty acids (PUFA), cholesterol, and ceramides. Desmosomes are then changed into corneosomes or corneodesmosomes. Desquamation is primarily governed by corneocyte cohesion, which is maintained by the lipid cement and the presence of corneodesmosomes. Corneodesmosomes are connected by desmosomal cadherins and a particular protein called corneodesmosin. Corneocyte desquamation is linked to corneodesmosin breakdown, which is regulated by the proteolytic enzymes' trypsin and chymotrypsin-like, both of which cause desquamation. These enzymes can be blocked by cholesterol sulfate, a process that becomes more prevalent with age and leads to the formation of corneocytes (**Martini,2011**).

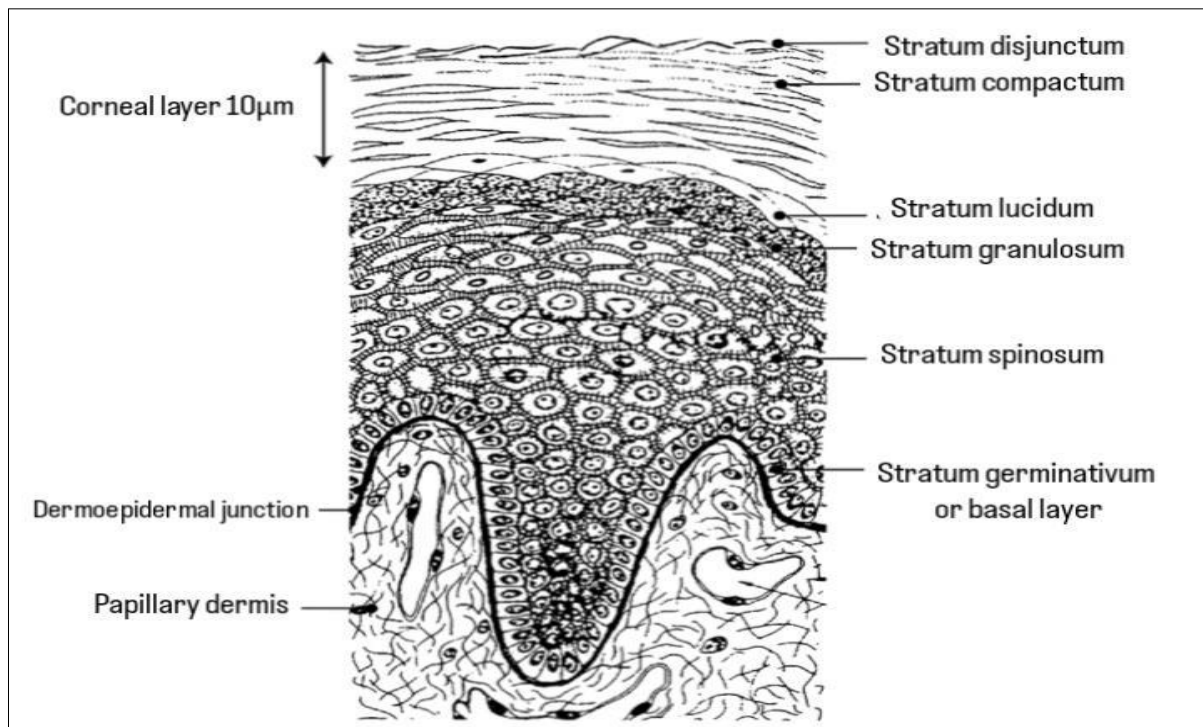


Figure02: Schematic structure of the epidermis (**Martini,2011**).

I.2.1.5. Other cells of the epidermis

I.2.1.5.1. Keratinocytes: are the most abundant cells, approximately 80% of the cell population of the epidermis. Their main characteristic is their ability to differentiate by producing keratin in a process called keratinization. Keratin is a structural protein whose fibrous nature gives the epidermis its protective function (**Eckert& Rorke,1989**).

I.2.1.5.2. Melanocytes: are cells capable of synthesizing melanin located in granules called melanosomes. Melanin has the property of absorbing UV rays from the sun which have not been reflected by the surface of the skin. It absorbs radiation from 200 to 2000 nm, and thus protects cells and their vital organelles (**wickett& Visscher,2006**).

I.2.1.5.3. Langerhans cells: located in the epidermis, belong to the immune system. These cells resemble the dendritic cell family and fulfill the role of sentinel in the epidermis. They play a role in non-specific immunity by presentation of phagocytosed antigen to T lymphocytes in the lymph nodes (**Banchereau et al.,2000**).

I.2.1.5.4. Merkel cells: are neuroendocrine cells. They are involved in touch and produce neuroactive substances such as vasoactive intestinal peptide (VIP), substance P, somatostatin and serotonin. These molecules probably allow the exchange of information with adjacent neurons (**Tachibana&Nawa,2005**).

I.2.2. Dermis

provides nutrition, support, flexibility, strength, and hydration to the skin. It is thicker. Collagen, elastic fibers, and hyaluronic acid are all present. It has many blood vessels (**Baures et al., 2009**).

I.2.3. Hypodermis

is the innermost layer. It is primarily composed of lipids and blood vessels and serves, among other things, to soften shocks (**Baures et al.,2009**).

I.3. Functions of the skin

It is a barrier against water evaporation. It maintains normal body temperature; therefore, it is a thermoregulatory organ which allows energy to be assimilated to synthesize vitamin D (**Travkine, 2012**). Each cell, each skin layer and all the elements constituting the skin will have a different function and metabolism, in close relation to their architecture and their surrounding environment. Thus, different phenomena will be encountered within the skin:

skin pigmentation as well as phenomena contributing to the barrier effect of the skin against the penetration of external substances **(Derras&bechlagem,2017)**.

It plays an essential role as an interface with the external environment; it is a means of protection against the environment and subsequently against external aggression: ultraviolet rays, dehydration, temperature variations, microorganisms and various physical, mechanical and chemical attacks **(Travkine, 2012)**.

It has metabolic activity in the cells of the dermis and those of the epidermis, for example: the synthesis of vitamin D in particular, prostaglandin, mucopolysacharides, keratins, melanins and their precursors **(Hé, 2006)**.

This activity allows the storage of triglycerides as an energy reserve. Finally, the skin and its various ancillary glands produce numerous secretions such as pheromones, milk, sebum, sweat, etc. which fulfill important and varied roles **(Hé, 2006)**.

I.3.1. Maintaining Body Temperature

Sweating helps to control body temperature since it increases with temperature and cools the body through evaporation from the surface. It lowers when the temperature drops (**Dréno,2009**).

I.3.2. Protective barrier against the external environment

The skin serves as a physical barrier, shielding tissues and organs from external attack. It provides a good barrier against microorganisms.

It also prevents body fluid loss and functions as a semi-permeable membrane that faces the external liquid.the skin also shields the body from mechanical stress, chemical poisons, UV radiation, and infectious agents like bacteria and fungi.it protects against the sun's rays, particularly due to its pigmentation **(Dréno,2009)**.

I.4. Mechanisms of skin absorption

I.4.1. Transcutaneous routes

There are several pathways crossing the stratum corneum barrier. Two main pathways stand out: the intercellular pathway and the transcellular pathway are the most used pathways. A third minority route is the route using the cutaneous annexes **(Amadine, 2008)**.

I.4.1.1. Transcellular passage

Transcellular passage is mainly used by small molecules. Substances must be able to integrate into the double layer of phospholipids constituting cell membranes to penetrate them. This pathway could therefore be followed by amphiphilic or lipophilic molecules. The speed of penetration by this route is very slow but it is compensated by the fact that the surface area is very large (**Amadine, 2008**).

I.4.1.2. Intercellular passage

takes the tortuous path of lipid cement. This would be the most used by all amphiphilic or lipophilic molecules, uncharged and of low molecular weight (**Amadine, 2008**).

I.4.1.3. Passage through the skin appendages

I.4.1.3.1. Transfollicular passage

The molecules diffuse through the lipophilic secretion of hair follicles and sebaceous pores. Molecules whose diffusion through the Stratum Corneum is very weak, due to their tendency to form chains with keratin, use the transfollicular route, which reduces their transport speed. Large molecules with a polar group, such as steroids, are those that use this route the most. However, the latter constitutes less than 1% of the body surface area (**Scheuplein&Blank, 1973**).

I.4.1.3.2. Sweat glands passage

Some hydrophilic compounds of low molecular weight, as well as electrolytes, diffuse within the aqueous liquid contained in the sweat ducts to reach the base of the gland which is in contact with numerous blood vessels in the dermis. However, this route of penetration represents only 0.1% of the body surface area (**Pinnagoda et al., 1990**).

I.4.2. Kinetics of transcutaneous passage

Transcutaneous absorption is a passive diffusion phenomenon which occurs at each layer of the skin. The molecules must first cross the skin barrier of a lipid nature, then diffuse into the different layers of the totally hydrophilic epidermis (**Martini, 2011**).

I.5. Mechanical properties of the skin

The skin is an organ in the form of a flexible membrane enveloping the entire body surface. Each skin layer, by the physical nature of its components and their organization, contributes

to the great complexity of the mechanical properties of the skin. The mechanical resistance of the skin is closely linked to its firmness and flexibility, reflecting the properties of the connective tissue of the dermis. With age, this network becomes disorganized, its firmness and flexibility decrease. The baby's skin, for its part, is much denser **(Lefrançois, 2015)**.

The mechanical protection of the skin is provided by all its layers, but especially by the stratum corneum, the outermost. On the one hand, the protein part of the protein and lipid envelope of corneocytes gives it its mechanical resistance. On the other hand, corneal desmosomes, ensuring connections between corneocytes, play an important role with respect to the mechanical constraints to which the skin is subjected. Extensibility, allowing it to resist stretching and friction, is essentially due to the presence of keratin in the stratum corneum. The epidermis, waterproof, protects the dermis which is mechanically resistant but hydrophilic **(Lefrançois,2015)**.

The dermis, 10 to 40 times thicker than the epidermis, has a complex structure. It is very durable, but also stretchy and elastic. Collagen fibers give it resistance to tension and traction, while elastin fibers give it its elastic properties. The hypodermis is a very loose tissue, which has a thermal and mechanical insulating role. It is a sort of protective cushion, which separates the skin from the fibrous membranes surrounding the muscles and bones **(Lefrançois,2015)**.

I.6. Skin toxicity

Skin toxicity is most often linked to the interaction of the surfactant with several charged molecules, proteins and lipid bilayers; these surfactants will penetrate these molecules more easily **(Lamery,2015)**.

Cosmetic products composed of chemical molecules, their toxic physicochemical properties, trigger a danger signal responsible for activating the innate response **(Lamery,2015)**.

Haptens are responsible for allergic contact eczema, the presence of erythema, edema, dry skin, cracks, scaling, itching and pain are signs of irritant contact dermatitis, but also of contact allergy **(Lamery,2015)**.

The irritant power of a substance is linked to its ability to penetrate the skin barrier and to its toxicity leading to the disruption of skin cell membranes **(Lamery,2015)**.

The effect of irritants on cells is characterized by a disruption in cell functioning. Solvents and detergents disrupt cell membranes and their fluidity through their actions on lipids, such as sodium lauryl sulfate (SLS) often used in cosmetics **(Lamery,2015)**.

These modifications induce the death of cells by apoptosis or necrosis depending on the nature and dose of the irritant, these cells will thus release pro-inflammatory mediators (histamines, prostaglandins, cytokines, etc.) which will be responsible for the activation of the system. Innate cutaneous immune system inducing inflammation **(Lamery,2015)**.

Chapter Two

Cosmetics

II.1. Generalities

The use of cosmetics has been a significant part of human life for thousands of years. They made it possible to utilize scent, protect the skin, take care of teeth, enhance appearance, and paint the skin for religious and cultural reasons. All cosmetics were created with natural materials in the past, primarily from plants, minerals, and anima (**McMullen & Dell'Acqua, 2023**).

The oldest traces of cosmetic uses were found in Egypt about 4000 Befor Christ, while the precise date and timing of cosmetology's inception remain uncertain. A concoction of lead, mercury, ashes, and other materials was utilized by the ancient Egyptians. The use of cosmetics and other beautifying substances dates to the beginning of human history. In the past, people utilized animal fat as a moisturizer to keep their skin smooth. Archaeological evidence suggests that cosmetics were utilized in ancient Greece and Egypt. Ancient Egyptians utilized castor oil, beeswax, olive oil, honey, and rose water to treat their skin. The Greeks employed cosmetics as well; they moisturised their faces at night with roghan-e-zaitoon (olive oil) and used bread and milk as a nightly anti-aging ritual (**Latif, 2021**).

II.2. Cosmetics

Cosmetics preparations consisting of natural or synthetic materials that are applied externally to the various body parts, including the skin, capillary system, nails, lips, genitalia, teeth, and mucous membranes of the mouth, with the sole or primary goal of cleaning, scenting, changing the appearance, and/or addressing body odors, as well as protecting or keeping them in good condition (**Barros *et al.*, 2020**). The cosmetic products protect the skin from drying, making it soft, supple and offer comfort and beauty (**Stankovic *et al.* 2014**).

II.3. Types of cosmetics

II.3.1. Natural cosmetics

As implied by the name, herbal cosmetics are free of all dangerous synthetic ingredients that could otherwise be hazardous to the skin. These products, including aloe vera gel and coconut oil, use various plant materials and plant extracts in place of conventional synthetic ones. They also include organic minerals such as vitamin E (**Bijauliya *et al.*,2017**).

II.3.2. Chemicals cosmetics

chemicals used as preservatives, UV filters, color additives, etc., can cause cancer, mutation, developmental and reproductive toxicity, endocrine disruption, etc., Most of the cosmetic

products are applied on the skin, which results in the toxic/harmful chemicals penetrating the stratum corneum barrier and reaching the dermis of the human skin **(Kaushik et al., 2023)**.

II.4. Composition of cosmetic products

The manufacture of a cosmetic product requires numerous ingredients subtly chosen and dosed to obtain a harmonious mixture and, above all, an effective preparation. This mixture is made up of 3 main families of components:

- The active ingredient to which the effectiveness of the cosmetic product is attributed.
- The excipient which constitutes the vehicle.
- Adjuvants which bring together all the components added to improve certain properties of the product **(Tec & Doc, 2006)**.

II.5. Constituents of cosmetics

Water, oil, silicones, surfactants, polymers, poly-hydric alcohols, saccharides, organic solvents, acid and alkali salts, powders, both inorganic and organic, pigment colors, proteins, amino acids, vitamins, plant extracts, UV absorbers, chelating agents, preservatives, antioxidants, oxidizing and reducing agents, and aromatic essential oils are some of the ingredients present in cosmetics. It is convenient to group the ingredients into those that give the product its form, stabilize it, affect on the users' senses, and have efficacy and effects. Cosmetic ingredients are blended to provide the desired results and to be suitable for the intended use, body area to be treated, and application technique **(Iwata et al., 2012)**.

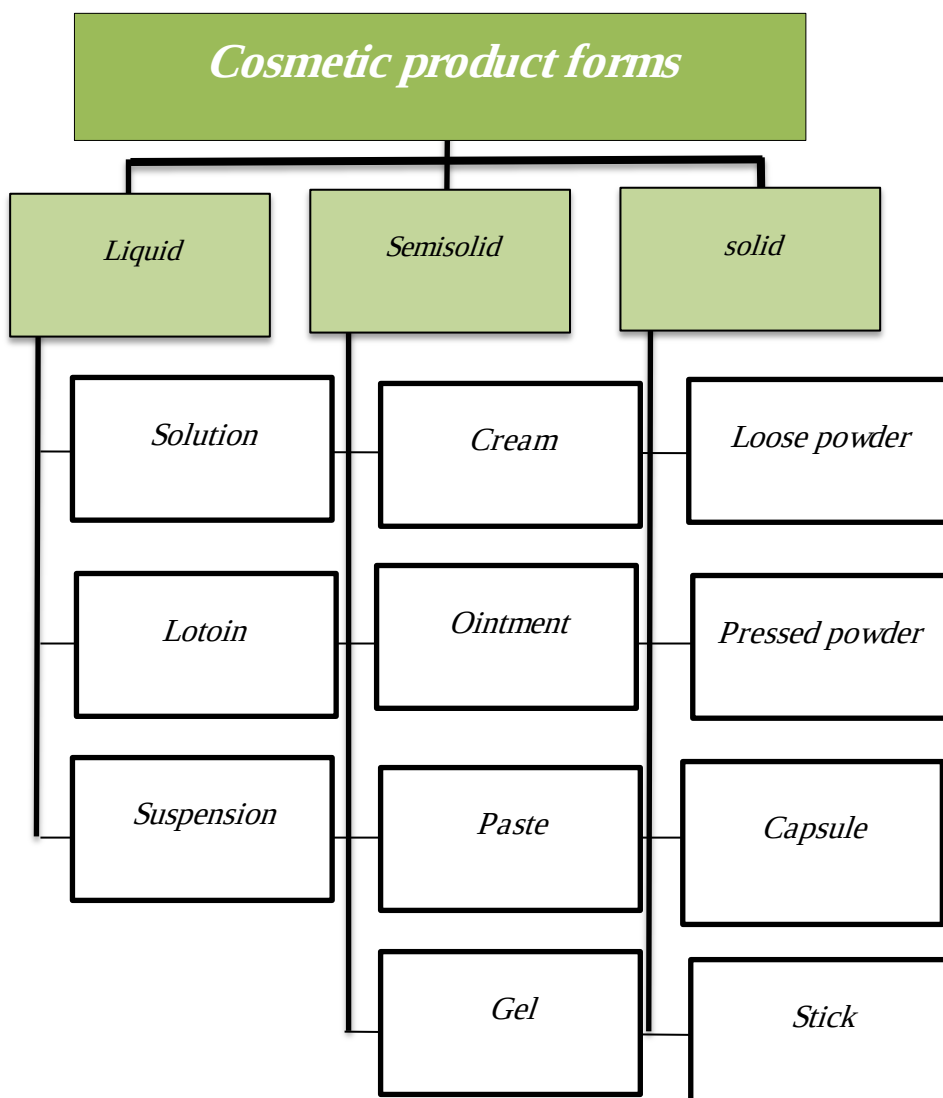
Table1: Basic formulations of soaps and cleansers (Iwata *et al.*, 2012)

Toilet soap Fatty acids or oils Glycerin Sodium hydroxide Chelating agent (EDTA-4Na)	60-70% 0-8% 18-22% of the fatty acids or oils	Beef tallow: 80%, coconut oil: 20% Also add sucrose or other saccharides as necessary (EDTA-4Na)
Liquid soap Fatty acids or oils mainly consisting of lauric acid Glycerin Chelating agent (EDTA-4Na) Potassium hydroxide Anionic surfactants Amphoteric surfactants Nonionic alkanol amide surfactants Preservative	5-25% 1-6% 20-23% of the fatty acids or oils	Emulsifier, touch improver Stabilizer, viscosity adjuster, moisturizer (EDTA-4Na, sodium citrate) Foaming agent Stimulus reducer Thickener, foam stabilizer
Cream cleanser Fatty acids Glycerin Sodium hydroxide Anionic surfactants Amphoteric surfactants Nonionic surfactants Chelating agent Preservative Purified water	5-20% 1-6% 20-23% of the fatty acids or oils 0-20% 0-12% 3-6%	Detergent Moisturizer, stabilizer Detergent, foaming agent Touch improvement, hair protection, and stimulus reduction Thickener, foam stabilizer

Table 2: Basic formulations of cosmetics mainly consisting of oil (Iwata *et al.*, 2012)

<i>Solid type</i>		
<i>Liquid oils</i>	20-100%	<i>Emulsifier</i>
<i>Waxes</i>	0-30%	
<i>Nonionic surfactants</i>	0-25%	
<i>Hair oil</i>		
<i>Silicone</i>	90-100%	
<i>Liquid oils</i>	Minute to small amount	

II.6. Cosmetic product formes

**Figure 03:** Main product forms for cosmetic and OTC drug-cosmetic products (Baki, 2022)

II.7. Classification of cosmetics products

The following classification is more suitable: hygiene products (shampoos, deodorants, soaps); Second, skin and hair care products (toothpaste, topical items); Third, decorative products (lip colors, perfumes); Fourth, preventive products (sunscreen and anti-wrinkle products); Fifth, corrective products (hair dyes, face masks); Sixth, upkeep products (moisturizers, shaving creams); and Seventh, active products (antiseptics, fluoride toothpaste)(Liu, 2022).

Table3: Eau de colognes are used less compared to the USA and Europe. This may be because of differences in lifestyle body construction.

<i>Classification</i>		<i>Usage</i>	<i>Main Products</i>
For Skin	Skin care cosmetics	Cleansers	Face Cleansing Creams and Foams
		Conditioners	Lotions, Packs, Massage Creams
		Protectors	Milky Lotions, Moisture Creams
	Makeup cosmetics	Base makeups	Foundations, Face Powders
		Point Makeups	Lipstick, Blushers, Eye Shadow, Eye Liners
		Nail Care	Nail Enamels, Nail Polish Removers
	Body cosmetics	Bath	Sunscreen Creams, Sun Oils
		Suncare and Suntans	Deodorant Sprays
		Antiperspirants & Deodorants	Bleaching Creams, Depilatory Creams
		Bleaching, Depilatory	Insect Repellent Lotions and Sprays
		Insect Repellents	Shampoos
For Hair & Scalp	Hair care Cosmetics	Cleansing	Rinses, Hair Treatments
		Treatments	Hair Mousses, Hair Liquids, Pomades
		Hair Styling	Permanent Wave Lotions (Agent N ^o 1, N ^o 2)
		Permanent Waves	Hair Colors, Hair Bleaches, Color Rinses
		Hair Colors and Bleaches	Hair Growth Promoters, Hair Tonics
	Scalp care Cosmetics	Hair Growth Promoters	Scalp Treatments
		Treatments	Toothpastes
For Oral	Oral care Cosmetics	Toothpastes	Mouthwashes
		Mouthwashes	
	Fragrances	Fragrances	Perfumes, Eau de Colognes

II.8. Important conditions regarding substances in cosmetics

- Not cause skin irritation or toxicity.
- Not interfere with the skin's natural processes.
- Remain steady and resist changing in hue or scent.
- Not break apart, not coagulate, not precipitate, and not experience property changes.
- Not significantly alter in viscosity with time or temperature changes Stop the growth of microorganisms and secondary pollutants **(Iwata & Shimada, 2012)**.

II.9. Microbiological Safety of Cosmetic Products

In general, microbiological contamination can occur in any product, including cosmetics, that contains water and organic or inorganic substances under the right physicochemical circumstances. This explains why these products need to be adequately and effectively protected against the spread of microorganisms. **(Halla *et al.*, 2018)**.

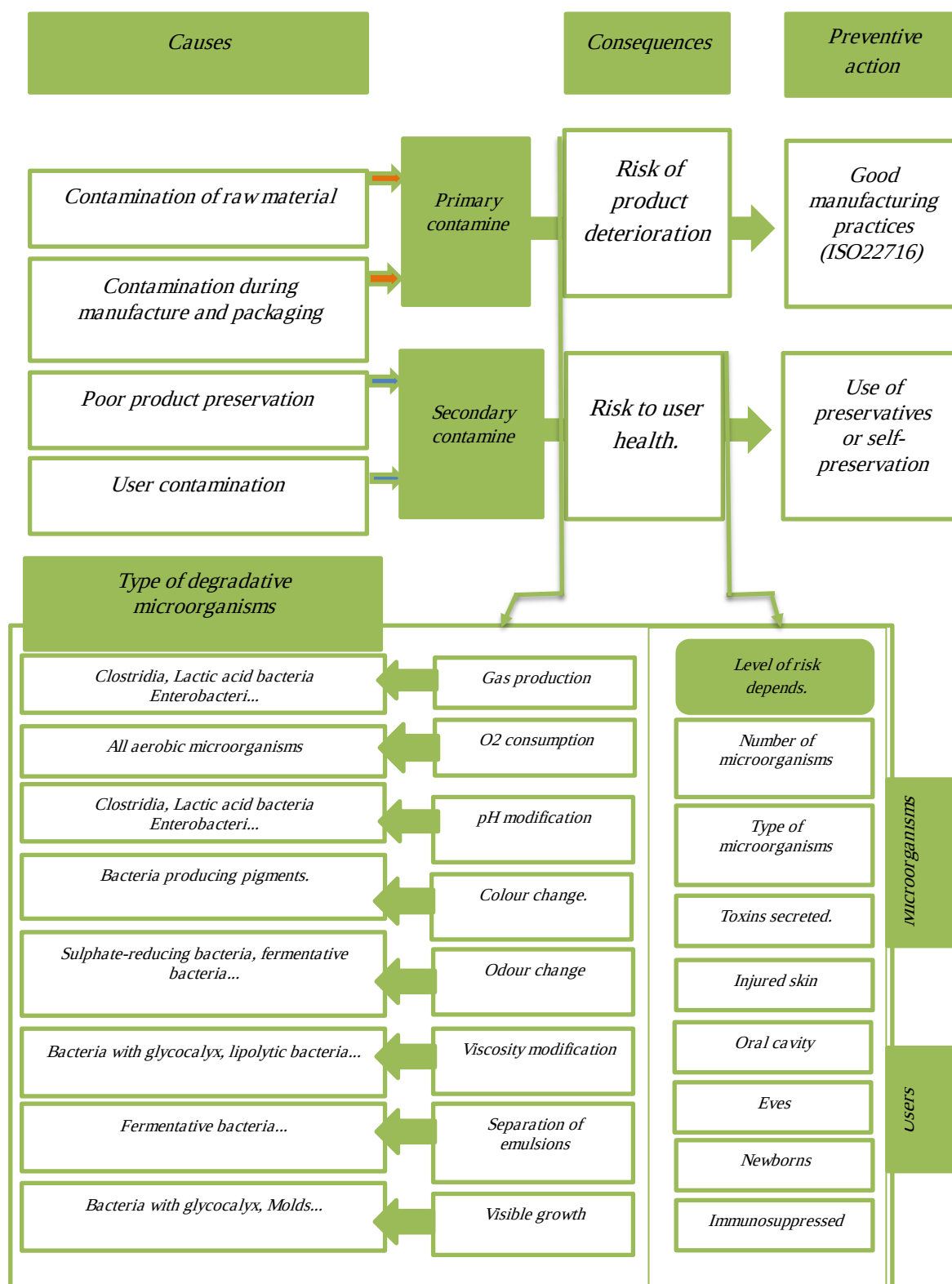


Figure 04: Causes, consequences, and ways of preventing cosmetics contamination (Halla et al., 2018)

Chaptre three

Plants

III.1. Generalities

Plants are one of the two main groups of organisms that are considered essential. It is considered an entity for the function of the biosphere. Plants can be found all over the known Earth in all shapes and sizes (**Fernando et al.,2012**). Additionally, some plants are considered an important source of nutrition and therefore these plants are recommended for their therapeutic action. (**Rasool Hassan,2012**). Plants have evolved to be able to synthesize an extremely diverse range of chemical compounds. Known as secondary metabolites. These have various roles. Its obvious role in the growth and development processes of primary plants, it is therefore unique to plants of a single species and increases during periods of high stress, e.g. Such as dehydration, temperature and many bacterial infections. These compounds have antimicrobial and antioxidant properties. Finally, the beneficial properties to which these activities can be attributed. The presence of a variety of phytochemical components, which can be divided into three main chemically distinct groups: terpenes, phenols, and nitrogen compounds (alkaloids). (**Ghorbanpour et al.,2017**).

These characteristics have led to the usage of plants in numerous disciplines. Since the beginning of time and throughout all civilizations, people have used cosmetics for body hygiene, beauty, and well-being. These products range from the most basic (soap, shampoo, and shower gel) to the most advanced (anti-aging serum, hair masks, makeup, etc.) (**Pereki, 2009**).

Most of the plants used in cosmetics are called aromatic plants, a group of plants that have various culinary properties. Suitable for cosmetics and hair treatments. These plants constitute the category of aromatic plants and its crops are used for flowers, trees, bulbs, roots, grains, flowers, bark, etc. (**Verdrager,1978**).

III.2. Family of *Apiaceae*

Daucus carota L are a biennial herbaceous plant belonging to the family *Apiaceae* (**Que, 2019**) includes cumin, caraway, fennel, coriander, anise, dill, and parsley, which are widely cultivated. Globally, they cover over 1.2 million ha and produce approximately 25 million tons per year. (**Sayed-Ahmad, 2017**). Plants in this family have a distinct pungent or aromatic scent. The presence of essential oil or oleoresin (**Singh & Jain, 2007**). they are introduced and cultivated worldwide due to their usage in foodstuff, pharmaceutical, perfume and cosmetic productions (**Hunault et al., 1989**). *Apiaceae* family is significant in Algerian

flora, with 56 genera, 130 species (24 endemics), and 26 subspecies recognized. (Pujadas Salvà *et al.*, 2003).

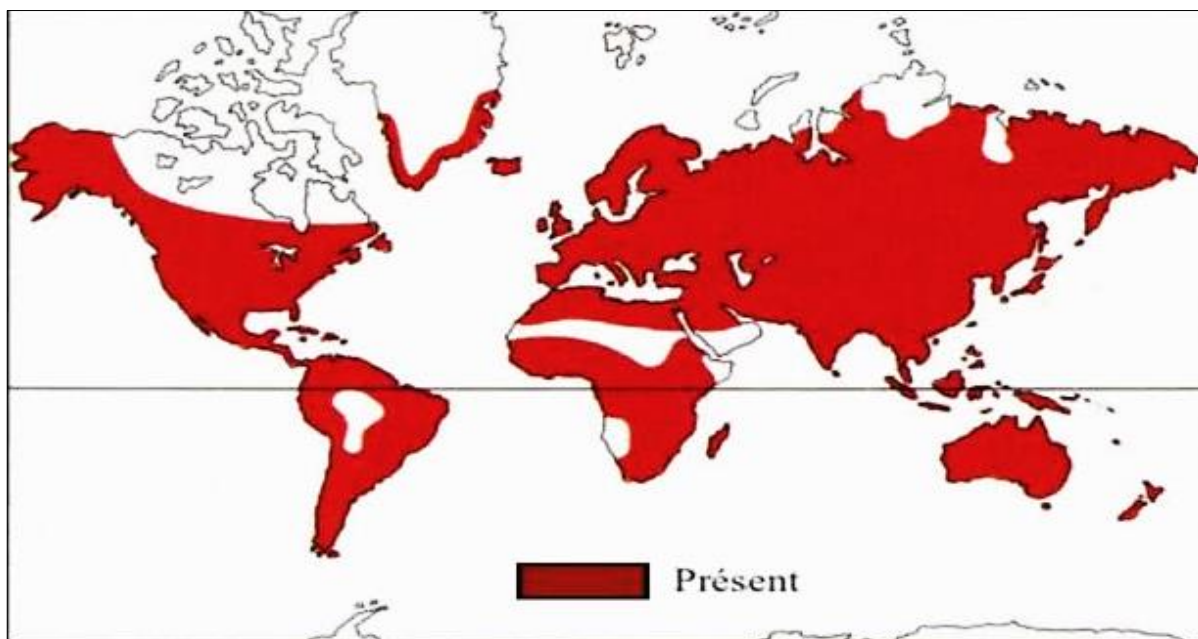


Figure 05: Geographic distribution of *Apiaceae* worldwide (Pimenov *et al.*, 1993).

III.3. *Daucus carota* L

The carrot (*Daucus carota* L., *Apiaceae*) is one of the most widely consumed vegetables in the world (Özcan, 2007). Among the top ten most important vegetable crops in the world in terms of economic value are carrots. (Koley *et al.*, 2014). It is a vegetable that can be used in a variety of ways. hues, forms, and dimensions (John & Jules, 2011). It is an aromatic, flowering, celery/parsley-like plant, including edible (Ismail *et al.*, 2023).

III.3.1. Botanical description

Biennial with spreading, upright branches that often jut out to a height of one meter. Ultimately divided lower leaves that are more than one millimeter broad. extended results of 2-4 mm, usually with spines that are shorter than the diameter (Miara, 2013).

III.3.2. Nomenclature of *Daucus carota* L

Table 04: The names of *Daucus carota* plant (chaabane & latreche, 2020)

Scientific name	<i>Daucus carota</i> L
English	Carrot
French	Carrot or Queen Anne's Lace
Arab	الجزر

III.3.3. Chemical composition

Table 05: Different compounds of *Daucus carota* L (Adjabi & Chabana, 2020)

Constituent name	Average	content name	Average conten
weight (g)	100	Sodium (mg)	< 40
Water (g)	89	Ca (mg)	30
Calories (kcal)	33	K (mg)	300
Proteins (g)	0.8	P (mg)	25
Lipids (g)	0.3	Fe (mg)	0.3
Carbohydrates(g)	6.7	Fiber(g)	3
Vitamin C (mg)	10	β-carotene (mg)	7

III.3.4. Morphology of *Daucus carota* L

Daucus carota L consist of leaves, flowers, tubers, and roots. *Daucus carota* L leaves are double or triple pinnate compound, and leaflets are lanceolate (stripes). It has 5-7 petiole, stiff and thick petiole with a smooth surface. At the same time, the leaves are limp and thin.



Figure 06: Morphology of *Daucus carota* L (Engla *et al.*,2021)

The stems of the carrot plant are so short they are barely visible. The trunk is also round, not woody, a little stiff, and small in diameter, dark green. It has no branches but is overgrown by

longleaf stalks so that it looks branched. *Daucus carota* L plants have taproots. The taproot will change its form and function to become a storage area for food reserves. Roots will turn out to be large and round lengthwise, up to 6 cm in diameter and up to 30 cm long, depending on the variety. *Daucus carota* L plant flowers grow on the tip of the plant and are white. Flowers have short, thick stalks. The flowers lie in the same plane. *Daucus carota* L flowers that have been pollinated will produce fruit and seeds that are small and hairy. *Daucus carota* tubers have a reddish yellow, white, yellow, and purple color (Lesmana *et al.*,2015).

III.3.4.1. Seeds

It means fruits with an oval shape, containing secondary metabolites such as flavonoids, polyphenols and essential oils including asarone, carotol, Pinene, limonene, sesquiterpene, beta-bisabolene (Tirilly *et al.* ,1999).



Figure 07: Real photo of *Daucus carota* L seeds (Original photo)

III.4. Uses and benefits

Due to their numerous advantages, carrot seeds have been utilized in a variety of fields in the past. For application in the pharmaceutical, *daucus carota* L is used to extract carotene and oleoresins (Doré & Varoquaux, 2006). They can supply several vitamins and minerals, function as a diuretic, and aid in cleansing the digestive system. Alkaline components in the

material cleanse and invigorate the blood. It has vital antioxidant components for protecting against coronary heart disease and maintaining health (**Koch et al.,2005**).

Carrot (*Daucus carrota* L) is one of the organic sources of cosmetics (**Anderiani et al., 2009**) These cosmetic emulsions have enough potential to be employed as possible precursors for skin rejuvenation (**Singh et al.,2019**) Given its current found effectiveness in causing structural alterations in the skin, seeds and seed oils are frequently used in commercial cosmetic and therapeutic procedures. Beta carotene, which is found in *Daucus carrota*L, protects the skin from photosensitivity and the damaging effects of sun exposure. (**Machewad et al.,2003**). Finally, eating them greatly enhances eye health and provides better eyesight protection. All things considered, *Daucus carrota* L seem to be a flexible and powerful dietary addition, providing a range of health advantages from skin protection to immunological support (**Kamiloglu et al.,2016**).

III.5. Family of *Fabacées*

Fabaceae family of flowering plants is one of the largest, with over 730 genera and 19,400 species that are found in both tropical and temperate regions. The herbaceous forms are more common in hotter regions, while the arborescent forms temperate areas. Nonetheless, this family of plants' preference for dry or semi-arid environments. (**Bourgaud et al.,2001**). The nitrogen-dependent metabolism of desert animals is thought to be an adaptation to climatic and erratic habitat fluctuations. (**Bourgaud et al.,2001**) .

III.6. *Glycyrrhiza* genus

The *Glycyrrhiza* genus, which is extensively dispersed globally, is a member of the Leguminosae family, which is usually referred to as the Fabaceae. It comprises around thirty species. "*Glycyrrhiza*" comes from the Greek words "glykys" and "rhiza," which respectively mean "sweet" and "root" (**Batiha et al.,2020**), Other names for it include sweet wood, *glycyrrhiza*, liquorice, licorice, and *Liquiritiae radix* (**Pastorino et al.,2018**).

III.6.1. Botanical Description

Herbaceous plants in the temperate and subtropical zones are called *Glycyrrhiza spp.* Generally found in sandy, fertile soil, the plants can grow up to 2 meters in height, and their underground stems can extend up to 2 meters horizontally.(**Husain et al., 2021**).

Plants have slender flowers that range in hue from lavender to violet, as well as pinnae. Three to eight brown reniform seeds are included in the oblong, legume-like fruit. The brown roots have a good degree of development. The roots have a characteristic scent and taste, and they shatter with a fibrous fracture.(**Karkanis et al.,2018**).



Figure 08: *Glycyrrhiza glabra* L plant (**Murray, 2020**)

III.6.2. Nomenclature of *Glycyrrhiza glabra*

Table 06: *Glycyrrhiza glabra* L nomenclature (**Rahmouni and Reghis., 2016**)

Scientific name	<i>Glycyrrhiza glabra</i> L
In French	Réglisse
English name	liquorice root
In Arabic	عرق السوس

III.6.3. Chemical composition

Table 07: Chemical compounds found in *Glycyrrhiza glabra* L (**Wright, 2004**)

<i>Glycyrrhizic acid</i>	Phytosterins
coumarin, maltol (0.03% of the extract)	Alkaloids
Flavonoids	Saponins
Liquiritoside	Phytoestrogens
Mannite	Asparagine
Isoliquiritoside	ACTH-like steroids

III.6.4. Morphology of *Glycyrrhiza glabra*

- a. **Flowers;** Typically blue in hue, the flowers might be more or less violaceous. These are rather little, ranging in length from 10 to 13 mm, and grouped in large numbers (20 to 30 flowers), in elongated grappes (Cael, 2009).
- b. **Leaves;** Several pairs of oval, obtuse, and petaloid leaves make up alternates (Chouitah, 2012) .
- c. **Stem;** Nearly linear annuities that can reach a height of one meter, well-groomed, stiff, and crumbly (Cael, 2009).
- d. **Fruits;** Being a member of the legume family, reglisse has gousse as its fruit. The presence of tiny brownish grains in the reptile's gousse makes it aplatie and bosselée. There are around five grains in each *Glycyrrhiza glabra* berry. The grains have a diameter of 2 to 4 millimeters and a brown color (Chouitah, 2012).
- e. **The roots;** *Glycyrrhiza Glabra* roots are vibrant, average 1-2 meters high, and have a sweet, pleasant flavour. Brown on the outside and yellow on the inside..(Bouriquat, 2020).



Figure 09: Morphology Of *Glycyrrhiza Glabra* L (Dilekh &Messaoudi, 2020)

III.6.4.1. Roots

The stolons are cylindrical, and the root has few branches. There are longitudinal streaks in the gray-brown outer bark, which also displays evidence of lateral roots. It is not present in the original worlded. The stolons and roots are fibrous when broken. The suber is thin, the center cylinder is yellow (isoliquiritoside present), and the inner bark is thick and radially striated (**Bouriquat, 2020**).



Figure 10: Roots of *Glycyrrhiza glabra* L (original photo)

III.7. Uses and benefits

Glycyrrhiza glabra L has long been used for both culinary purposes used to flavor candies and sweetener medical remedy licorice (**Fintelmann, 2004**). They have been used to treat a variety of conditions relating to the digestive system, the respiratory tract, epilepsy, fever, sexual debilitation, paralysis, rheumatism, leucorrhoea, psoriasis, prostate cancer, malaria, hemorrhagic infections, and jaundice, either by themselves or in combination with other herbs (**Batiha et al.,2020**). are utilized in cosmetic preparations because of their anti-inflammatory, anti-sensitizing, and skin-whitening qualities (**Vanitha et al.,2017**) Their excellent whitening effect led to their widespread use in commercial items, particularly in cosmetics. There are several products on the market that include *Glycyrrhiza glabra* extracts.

are mostly used on a regular basis with Sun Protection Factor (SPF) products that contain extract from the roots of *Glycyrrhiza glabra* L. Claimed to have anti-aging properties, the extract is added to the internal aqueous phase of water/oleum emulsion in cream and serum compositions. It also has benefits on wrinkles, hyperpigmentation, and skin whitening. *Glycyrrhiza glabra* L extracts are utilized to achieve these effects in sunscreen formulations as well as in personal care products like toners, shampoos, cleansers for the face, and makeup removers. *Glycyrrhiza glabra* L extract is also an ingredient in makeup products like BB creams, lipsticks, primers, concealers, and around-the-eye creams. (Wang *et al.*, 2020).

Second part

Experimental

Part

Chapter one

*Material &
Methods*

I.1. Material

I.1.1. Plant material

a. *Daucus carota*.L

The plant material is carrot seeds (*Daucus carota* .L) obtained in September in the Taghzout area (El-Oued). This plant was collected and dried away from light and moisture at room temperature for two to three weeks. After drying, it was carefully stored in a closed glass bottle in a dry place for later use (Figure 11A).

b. *Glycyrrhiza glabra* .L

The plant material is licorice roots (*Glycyrrhiza glabra*.L), which was harvested from the Taghazout region (El-Oued). This plant was dried away from light and moisture at room temperature for two to three weeks. After drying, it was crushed and turned into crumbs, then carefully stored in a closed glass bottle in a dry place for later use (Figure 11B).



Figure11: A: *Daucus carota*.L and B: *Glycyrrhiza glabra*.L, respectively (original photo,2024).

I.1.2. Chemicals and biochemical product

Table08: The list of chemicals and biochemical products used in our study.

Product	Form	Chemical formula
-2,2diphenyl-1-picrylhydrazyl (DPPH)	Powder	C ₁₈ H ₁₂ N ₅ O ₆
Aluminum trichloride	Powder	AlCl ₃
Chloroform	Liquid	CHCl ₃
Ethanol	Liquid	C ₂ H ₅ OH
Folin-Ciocalteu	Liquid	/
Hydrochloric acid	Liquid	HCl
Iron trichloride	Liquid	FeCl ₃
Magnesium chips	/	Mg
Mayer reagent	Liquid	/
Potassium ferricyanide	Liquid	K ₃ Fe(CN) ₆
Sodium carbonate	Liquid	Na ₂ CO ₃
Sulfuric acid	Liquid	H ₂ SO ₄
Trichloroacetic acid (TCA)	Liquid	C ₂ HCl ₃ O ₂
Vanillin	Powder	C ₈ H ₈ O ₃
Bovine serum albumin (BSA)	Powder	/
Distilled water	Liquid	/
Urea	Liquid	CH ₄ N ₂ O
Citric acid	Powder	C ₆ H ₈ O ₇
Vitamin E	Liquid	/
Glycerin	Liquid	C ₃ H ₈ O ₃
Tween 80	Liquid	C ₆₄ H ₁₂₄ O ₂₆

I.2. Methods

The study focuses on extracting the active compounds from *Daucus carota*.L and *Glycyrrhiza glabra*.L plants by maceration method. Therefore, the most important studies investigate the biological properties, including antioxidant, anti-inflammatory, and photoprotective activities of carrot and licorice seeds.

I.2.1. Phytochemical tests

The solution used for the chemical group detection tests was conducted on the aqueous extracts of the vegetable powders obtained by decoction, according to **Kaneria et al., (2012)**, 5 g of dried powder of *Daucus carota*.L and *Glycyrrhiza glabra*.L was boiled, separately with 100 ml of distilled water for 30 min in a water bath. Then the extracts are filtered using Whatman N°01 paper. This preparation has been preferred to be as close as possible to the

conditions of use of *Daucus carota*.L and *Glycyrrhiza glabra*.L in traditional medicine. We used the analytical techniques described in the work.

a. Polyphenols

Polyphenols The polyphenols were characterized using a reaction with iron trichloride (FeCl₃). To 2 ml of aqueous extract, we added a drop of 2% iron trichloride alcoholic solution. The presence of polyphenols was indicated by the emergence of a blue-blackish or greenish tint that was more or less dark (**N'Guessan *et al.*, 2009**).

b. Tannins

The presence of tannins is demonstrated by adding to 1 ml of the aqueous extract, 1 ml of water and 1 to 2 drops of FeCl₃ solution (2%). The appearance of a dark green or blue-green color indicates the presence of tannins (**Lock *et al.*, 2006**).

c. Flavonoids (Shibata reaction)

When 5 ml of the aqueous extract, 5 ml of hydrochloric alcohol (4 ml EtOH and 1 ml of concentrated HCl) and approximately 0.5 g of magnesium shavings are added, the mixture turns pink, orange, or purplish red when flavonoids (flavonols, flavones, flavonones) are present (**Lock *et al.*, 2006**).

d. Alkaloids

To acidify the media, 5 ml of 1% HCl is mixed with 1 ml of the aqueous extract. The mixture is cooked in a water bath and then separated into two equal portions. The reagents for treating the two volumes separately are:

- In the first tube, add a few drops of Mayer reagent (yellowish color).
- In the second tube, add a few drops of Dragendorff reagent (orange color) (**Tlili, 2015; Fettah, 2019**).

e. Terpenoids

To 5 mL of extract, add 2 mL of chloroform and 3 mL of sulfuric acid (H₂SO₄). a positive test indicates the presence of two phases and a brown tint at the interface (**Tlili, 2015; Fettah, 2019**).

f. Saponins

In a test tube, add 10 mL of the extract to be examined, shake for 15 seconds, and allow settle minutes. The emergence of persistent moss implies the presence of saponosides (**Fettah, 2019**).

g. Glycosides

Two ml of chloroform are added to 1 ml of the extract, the appearance of a reddish brown color after the addition of H₂SO₄ indicates the presence of cardiac glycosides (**Tlili, 2015; Fettah, 2019**).

I.2.2. Maceration

Maceration is also known as cold extraction. The maceration technique was used to separate the numerous components and elements of the plants into a crude extract (**Irshad et al., 2012**) It consists of contacting the vegetable mat with the solvent sans or with agitation. This procedure, due to long periods of extraction and use of a large amount of solvent, is relative to the effect. Moreover, this product is at low temperatures, which is very positive for maintaining the integrity of polyphenols (**Fettah, 2019**). The aqueous extract of the plant are carrot seeds (*Daucus carota*.L) & Licorice roots (*Glycyrrhiza glabra*.L) were prepared according to the method described by **Coulibaly et al., (2011)**. 50 g of the two plants material were macerated it in 500 ml of distilled water with magnetic stirring and at room temperature. This maceration is repeated 3 times successively with renewal of the solvent every 24 hours. The aqueous macerate obtained is subjected to double filtration on Whatman No. 1 filter paper (**Coulibaly et al.,2011**).

The filtrates obtained are concentrated the rotary evaporator (Rotavapor BUCHI Haeting bath R-210) at a temperature of 45°C and 50°C respectively. After drying in an oven (45°C) for 24 hours, the raw extracts were stored in sterile bottles labeled then stored in the freezer at 4°C until used for phytochemical and biological tests (**Coulibaly et al.,2011**).

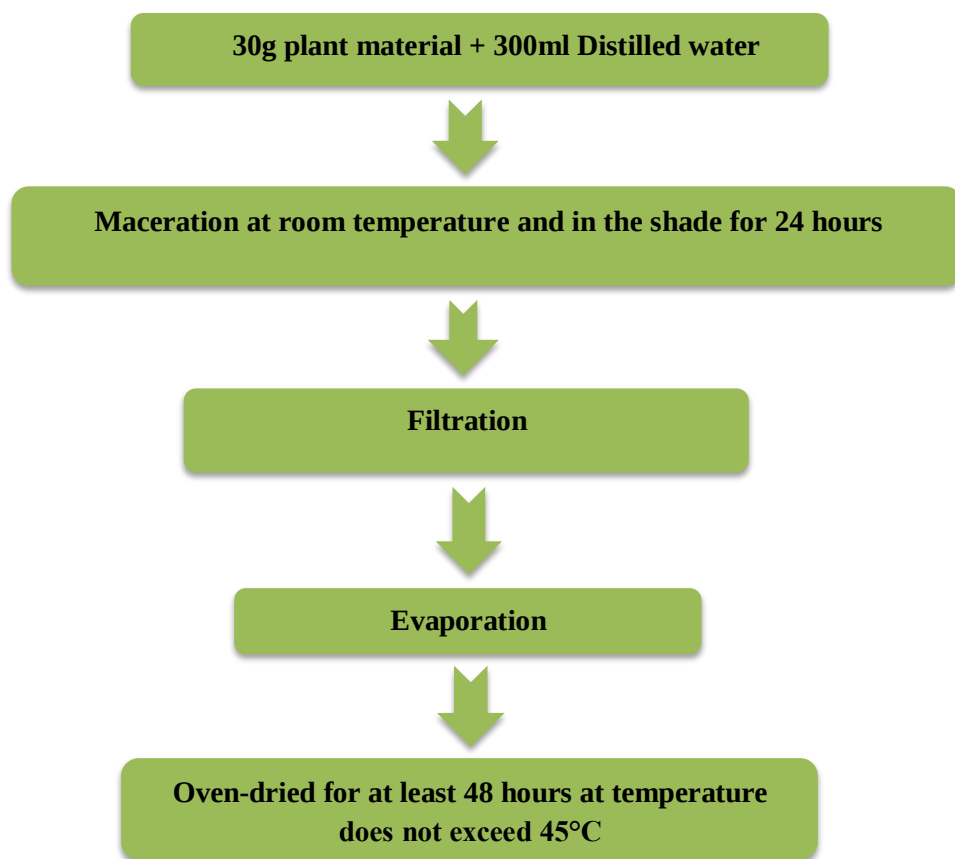


Figure12: Maceration protocol of *Daucus carota.L* and *Glycyrrhiza glabra.L*.

I.2.3. Calculation of dry extract yields

To calculate the yield of dry extracts from *Daucus carota.L* and *Glycyrrhiza glabra.L*, we can use the following ratio:

$$\text{Yield (\%)} = P1/P2 \times 100$$

P1: weight of the balloon after evaporation.

P2: weight of the starting plant material

I.2.4. Quantitative Estimation

I.2.4.1. Estimation of total polyphenols contents

The polyphenols were measured by spectrophotometry using the method of Folin Cioclateu (Singleton *et al.*, 1999). This yellow-colored reagent is composed of phosphotungstic acid and phosphomolybdic acid. When polyphenols are oxidized, they convert the Folin-Cioclateu reagent to a blue complex containing tungsten oxide and molybdenum. The color

intensity is proportional to the amounts of oxidized phenolic chemicals (**Boizot & Charpentir, 2006**).

The assay methodology is performed as follows: Mix 200 µl of each extract of extracts of *Daucus carota*.L and *Glycyrrhiza glabra*.L with 1 ml of Folin Ciocalteu reagent (diluted 10 times in distilled water). After 4 minutes of incubation at room temperature, 800 µl of 7.5% Na₂CO₃ dissolved in distilled water is added to the mixture. The previously shaken mixture is incubated in the dark for 2 hours. A UV/visible spectrophotometer is then used to read the absorbance at 765 nm.

I.2.4.2. Estimation of Flavonoid contents

The quantitative estimation of the flavonoids contained in the extracts of *Daucus carota*.L and *Glycyrrhiza glabra*.L is carried out following a colorimetric method of aluminum trichloride AlCl₃ with slight modification, 1 ml of extract (prepared in methanol) is added to 1 ml of 2% AlCl₃; then the mixture is vigorously shaken, the whole is incubated in the shade at room temperature for 30 minutes, the absorbance is read at 430 nm. The quantification of flavonoids is done according to a calibration curve produced by a standard flavonoid: Quercetin. The flavonoid content is expressed in milligrams of quercetin equivalent per gram of dry matter (**Kosalek et al., 2004**).

I.2.4.3. Estimation of condensed tannins contents

The condensed tannins in extracts *Daucus carota*.L and *Glycyrrhiza glabra*.L are determined using the **Schofield et al. (2001)** technique .

This assay involves attaching the vanillin aldehyde moiety to the catechin A-ring carbon 6 to generate a red chromophore complex that absorbs light at 500 nm. To each 400 µl sample or standard, add 3 ml of vanillin (4% in methanol) and 1.5 ml of strong hydrochloric acid. The mixture is incubated for 15 minutes, and the absorbance is measured at 500 nm. The concentrations of condensed tannins are determined using the calibration ranges established with catechin (0-0.5 mg/ml) and are expressed in milligrams of catechin equivalent per gram of extract (Mg EC/gE).

I.2.5. Evaluation of biological activities

I.2.5.1. Antioxidant activity

I.2.5.1.1. DPPH radical trapping test

The antioxidant activity of the different extracts is measured by the method described by **Brand-williams *et al.* (1995)**. The test involved combining 50 µl of extract or standard with 1.95 ml of DPPH dissolved in methanol (4%). After shaking, the reaction was kept away from light for 30 minutes, and the absorbance was measured at 517 nm. Inhibition of free radical DPPH in percent (I%) was computed as follows :

$$I \% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100;$$

Where A_{blank} is the absorbance of the control reaction (with all reagents except the samples) and A_{sample} is the absorbance of the tested samples. The extracts concentration that provided 50% inhibition (IC_{50}) was obtained using a graph showing inhibition percentage against extracts concentration. The tests were conducted in duplicate (**Saffidine *et al.*, 2015**).

I.2.5.1.2. Ferric reducing antioxidant power (FRAP)

The reducing power of *Daucus carota L* and *Glycyrrhiza glabra L* extracts was tested using the (**Do *et al.* 2014**) technique. This method assessed the reduction of Fe^{3+} to Fe^{2+} by measuring the absorbance of the Perl's Prussian blue complex. This approach relies on reducing (Fe^{3+}) ferricyanide in a stoichiometric excess relative to antioxidants.

For this purpose, 1mg of each extract was diluted with 1ml of distilled water. 0.5 ml of diluted extract was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution in a test tube, followed by incubating in a water bath at 50 °C for 30 minutes. At the end of the incubation, 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% iron trichloride freshly prepared was added, absorbances of these mixtures were measured at 700 nm using a UV spectrophotometer.

A calibration curve with different concentrations (2.5 µg / ml to 500 µg / ml) is carried out using ascorbic acid with the same experimental procedure. The results are expressed in milligrams (mg) equivalent of ascorbic acid per gram of extract (mg EAA / g E).

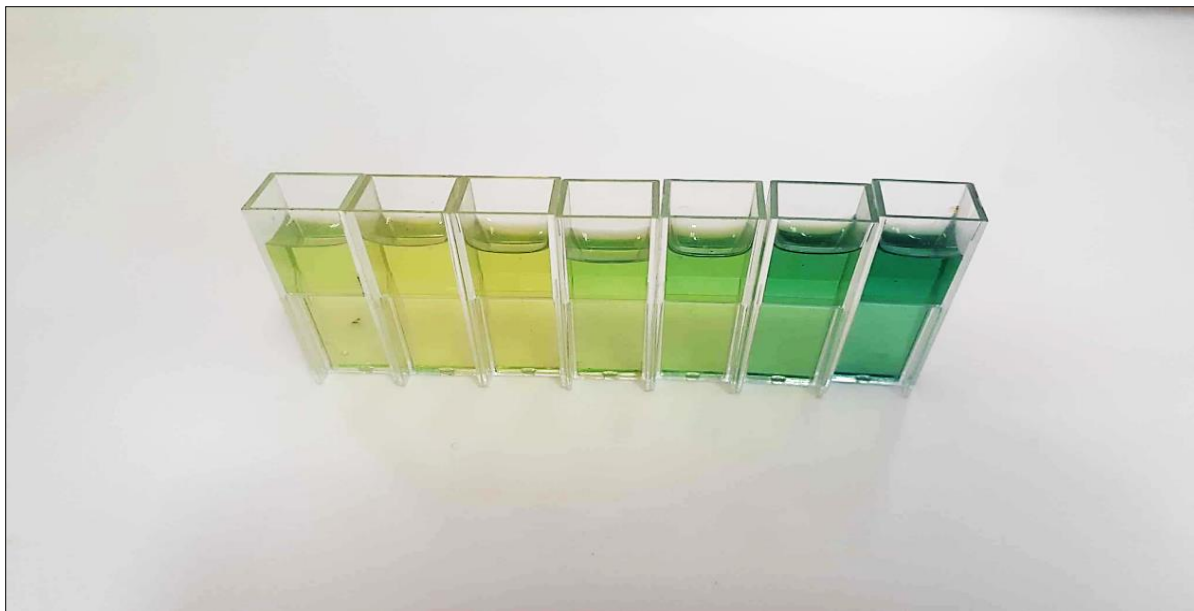


Figure13 : The calibration of Ascorbic acid for the measure of reducing power of *Daucus carota*.L and *Glycyrrhiza glabra*.L extracts (**Original photo, 2024**).

I.2.6. Photoprotective activity

The effectiveness of *Daucus carota*.L and *Glycyrrhiza glabra*.L extracts against UV rays was assessed in vitro using UV-Visible spectrophotometry to determine the sun protection factor (SPF). The SPF was calculated, by measuring the difference between the spectroscopic readings of the aqueous extracts (500 µg/mL) in the range of 290 nm-320 nm according to the method described by (**Benine et al. 2023**).

$$\text{SPF}_{\text{Spectrophotometric}} = \text{CF} \sum_{\lambda=290}^{320} \text{EE}(\lambda) \times (\lambda) \times \text{Abs}(\lambda)$$

Where CF: correction factor (=10); EE: erythemal effect spectrum; I: solar intensity spectrum; Abs: absorbance of the sample. The results obtained were compared with the categories of sunscreens mentioned in Table 09.

Table 09. Categories of sunscreens based on the value of the SPF.

Protection Level	SPF Value
Maximum	>50
High 30-50	30-50
Medium	15-30
Low	02-15

2.7.I. Anti-inflammatory activity

To study the potential anti-inflammatory effects of *Daucus carota*.L and *Glycyrrhiza glabra*.L extracts, the albumin denaturation assay was utilized with Aspirin serving as a control as described by (Benine *et al.* 2023). To perform this assay, a reaction mixture of 500 µl of various concentrations of *Glycyrrhiza glabra*.L and *Daucus carota*.L extracts or the standard (ranging from 62.5 to 1000 µg/mL) and 700 µl of phosphate-buffered saline (pH 6.4) was combined with 500 µl of BSA (Bovine serum albumin). The reaction mixture was then incubated for 15 min at 27 ± 1 °C. Denaturation of albumin was induced by heating the mixture in a hot bath at 70 °C for 10 min. After cooling, the absorbance at 660 nm was measured using distilled water as a blank. The experiment was carried out in triplicate. The inhibition of albumin denaturation was calculated using the following formula:

$$\text{Albumin Inhibition (\%)} = [(A_{\text{Sample}} - A_{\text{Control}}) / A_{\text{Control}}] \times 100$$

Where A_{control} is the absorbance of the control reaction, and A_{sample} is the absorbance of the test extract/standard. The extract concentration that provided 50% inhibition (IC_{50}) was obtained using a graph showing inhibition percentage against extracts concentration. The tests were conducted in duplicate.

I.3. Preparation of the Bioproduct (make-up remover)

Two makeup remover Bioproducts were prepared, the first based on *Daucus carota*.L extract and the second on *Glycyrrhiza glabra*.L extract.

I.3.1. Makeup remover with *Daucus carota*.L and *Glycyrrhiza glabra*.L extract

a. Ingredients

***Daucus carota*.L extract:** The powerful antioxidants in *Daucus carota*.L extract neutralize free radicals to prevent collagen breakdown and protect the skin's ability to repair itself.

Glycyrrhiza glabra.L extract: it contains antioxidants, antimicrobial chemicals that produce skin inflammation, and whitening agents that help erase pigmentation and dark spots, making it a popular ingredient in many skin products.

Almond oil: is an effective anti-inflammatory that helps reduce swelling of the skin. With its whitening properties, it can also help lighten dark spots and dark circles.

Glycerin: is a natural product that is. a special cleanser and moisturizer for the skin, extracted from vegetable oil and does not easily oxidize during storage, it tones the skin, therefore it is considered a good alternative to many products that contain harmful chemical compounds.

Citric acid: is a natural preservative extracted from citrus fruits such as lemon and orange that promotes cell regeneration and helps remove dead cells, so skin feels smooth and clean.

Vitamin E: soothes the skin, helps soothe irritation and inflammation and is also considered a skin nutrient.

Urea: an exfoliating active ingredient for the skin; from 10% concentration, urea (or carbamide) changes properties and becomes exfoliating. It performs a keratolytic action on the skin by eliminating dead skin on the surface of the stratum corneum.**Distilled water.**



Figure 14: Make-up remover preparations (Original photo,2024).

Table 10: Ingredients used in Makeup remover with *Daucus carota*.L extract.

Ingredients	Volume (%)
Distilled water	88%
Urea	5%
Citric acid	1%
Vitamin E	1%
Glycerin	2%
Tween 80	1%
Almond oil	1%
<i>Daucus carota</i> L extract	0.8%

Table 11: Ingredients used in Makeup remover with *Glycyrrhiza glabra*.L extract.

Ingredients	Volume (%)
Distilled water	88%
Urea	5%
Citric acid	1%
Vitamin E	1%
Glycerin	2%
Tween 80	1%
Almond oil	1%
<i>Glycyrrhiza glabra</i> L extract	0.8%

I.4. Investigation relating to the application of make-up remover

I.4.1. Questionnaire formulation

The questionnaire (Annexe 01) formed according to the questions took from these references (Sedrati *et al.*,2021; Sehib *et al.*,2020). This is to evaluate the effectiveness of the makeup remover with licorice root extract and the effectiveness of the makeup remover with carrot seed extract and choose the best between them.

a. Inclusion criteria

- Women over 20 and under 50.
- Women who use makeup.
- Women who do not have any skin diseases.

b. Exclusion criteria

- Girls under 20 years old.
- Women who do not use make-up.
- Women who have skin diseases.

Chapter two

Results & Discussion

II.1. Results

II.1.1. phytochemical screening:

Through phytochemical screening, we were able to determine which secondary metabolites were found in plant tissues and to gather enough data about their medicinal and cosmetic qualities.

Table 12: Results of phytochemical tests of *Daucus carota*.L and *Glycyrrhiza glabra*.L

Test of	<i>Daucus carota</i> L	<i>Glycyrrhiza glabra</i> L
polyphenols	+	+
Flavonoïdes	+	+
Alkaloids	+	+
Terpenoids	+	+
Glycosides	+	+
Saponosides	—	+
Tanins	+	+

(+): Presence, (-): Absence

The phytochemical analysis revealed that *Daucus carota*.L plant seeds included both polyphenols, flavonoids, alkaloids, terpenoids, glycosides, and tannins, but not saponosides. Polyphenols, Alkaloids, Terpenoids, Glycosides, Tannins, and Saponosides are all present in the *Glycyrrhiza glabra*.L plant, but the flavonoid metabolite is absent.

II.1.2. Yields of dry extracts

The dry weight obtained was calculated by weighing the raw extracts from *Daucus carota*.L and *Glycyrrhiza glabra*.L that were recovered after evaporation. The outcomes were given as a percentage.

Table13: Yields, aspects and colors of extracts of *Glycyrrhiza glabra*.L and *Daucus carota*.L

Extraction	Yield (%)	Aspects	Colors
Aqueous extract <i>Glycyrrhiza glabra</i> L	13.2%	powder	Brown
Aqueous extract <i>Daucus carota</i> L	10%	powder	Brown

The extraction yields obtained depend on the plant studied and the extraction process road. The results obtained indicate that there is a slight difference between the yield of two different types of plants, which are respectively 13.2% and 10%.

II.1.3. Quantitative Estimation

II.1.3.1. Estimation of total polyphenols contents

The aqueous extracts were analyzed quantitatively by spectrophotometer. Their total polyphenol contents were determined using the Folin-Ciocalteu method. Results obtained It is expressed as mg gallic acid equivalent per gram of extract (mgEAG/gE).

This content is calculated using a linear regression equation for the calibration curve plotted with gallic acid (**Figure 15**)

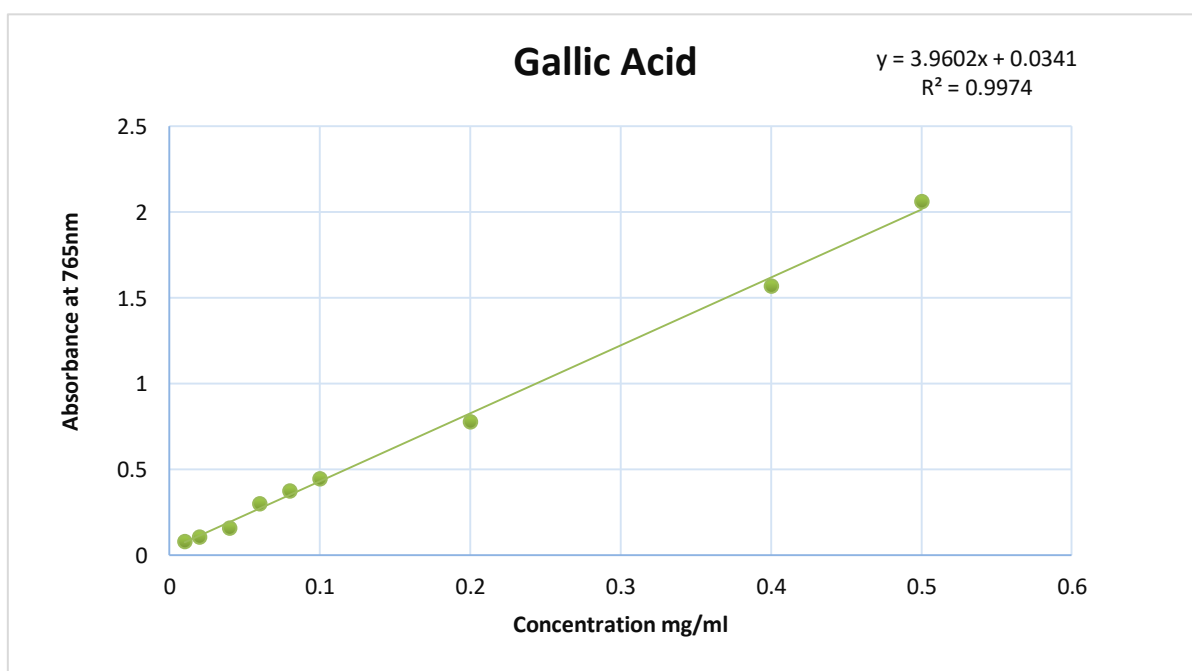


Figure 15: Gallic acid Calibration curve for the determination of total phenols.

The findings demonstrated that there are substantial differences in the amounts of phenolic components in each plant extract (**Figure 16**). We observe that the following are present in the extract we made from *Glycyrrhiza glabra L* extract 108.42 ± 0.89 mg EAG/gE is a high content of total polyphenols when compared to the extract we obtained from *Daucus carota L* seeds, which has contents of 55.78 ± 0.35 mg EAG/gE.

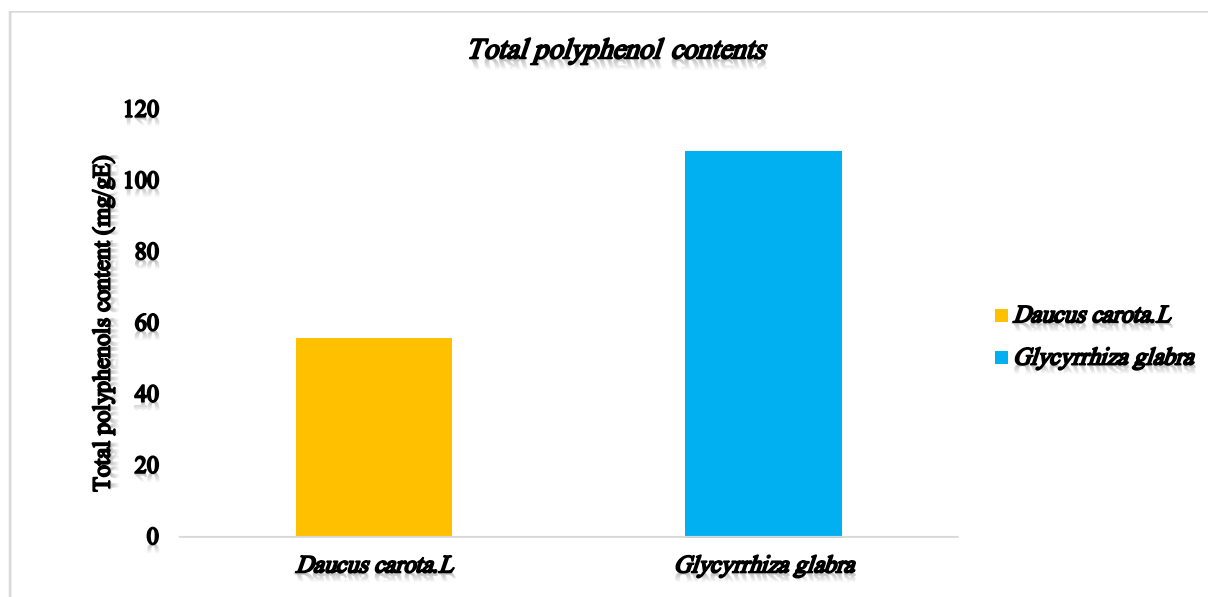


Figure16: Evaluation of the total polyphenols of the two extracts.

II.1.3.2. Estimation of Flavonoid contents

The aluminum chloride (AlCl_3) colorimetric method was used to measure the flavonoids. The results are reported using the equation of the linear regression of the quercetin standard curve (**Figure 17**) and are given in mg of Quercetin equivalent per gram of the extract (mg EQ /g E).

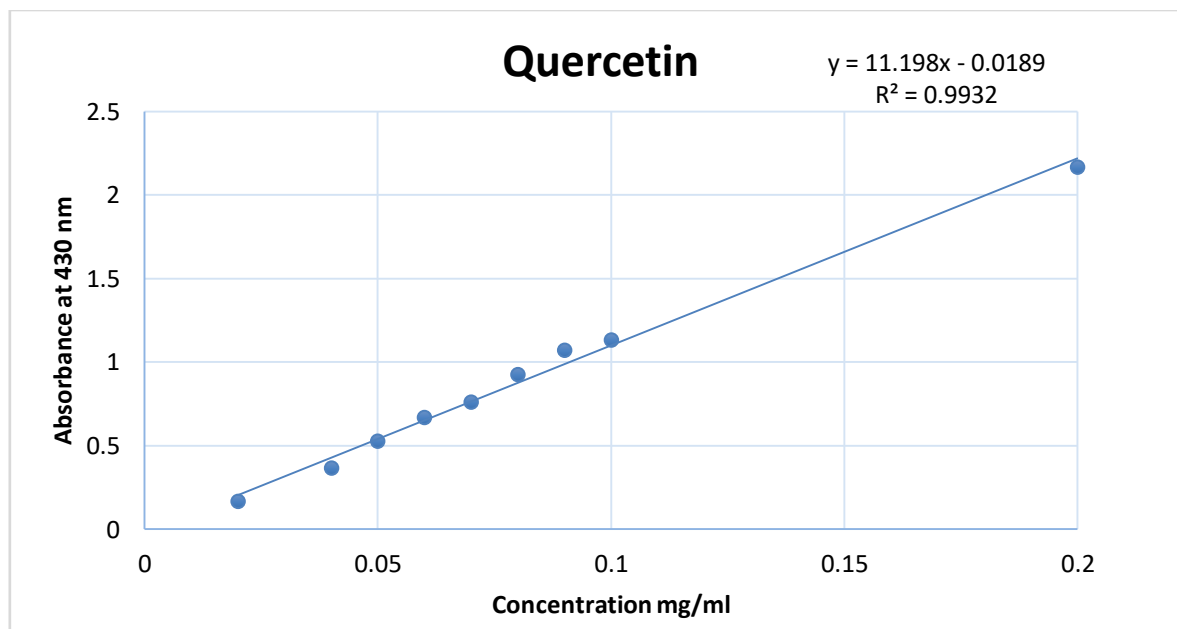


Figure 17: Quercetin calibration curve for the determination of flavonoids

According to the findings, it contains flavonoids and differs significantly between the two plants (**Figure 18**). The results observe that *Glycyrrhiza glabra* L extract has high total flavonoid content (76.28 ± 0.37 mg EQ/gE) in comparison to *Daucus carota* L extract it was extracted from fresh *Daucus carota* L seeds, whose concentrations range from 37.73 ± 0.34 mg EQ/gE.

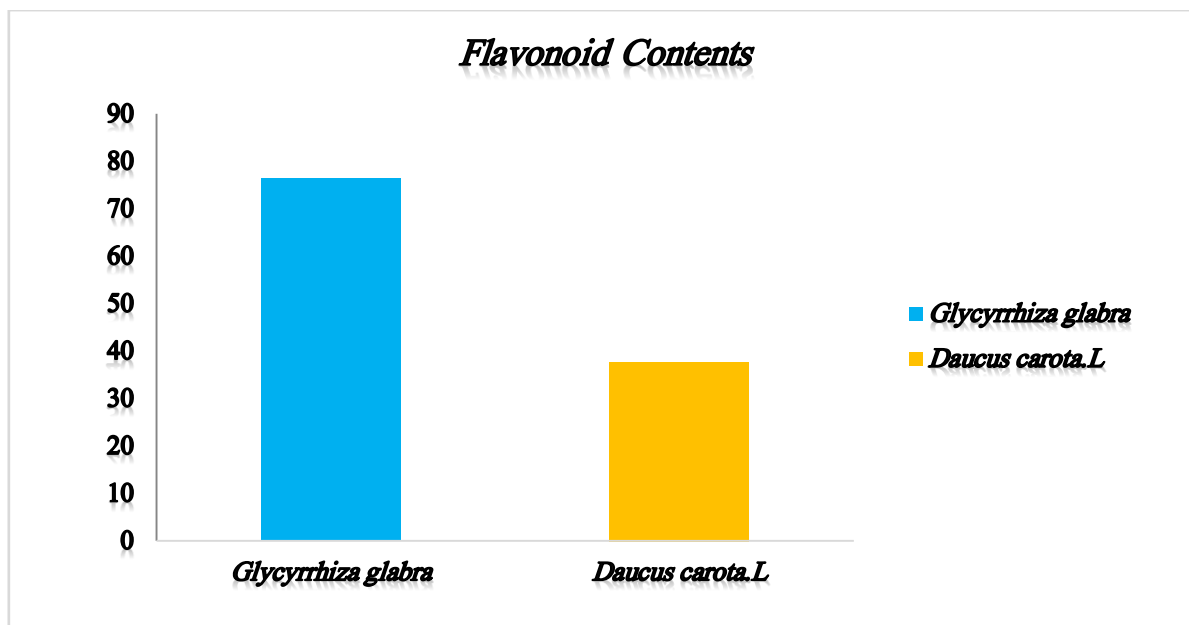


Figure 18: Evaluation of the Flavonoid contents of the two extracts.

II.1.3.3. Estimation of condensed tannins contents

The linear regression equation of the calibration curve produced for catechin (**Figure 19**) is used to express the results obtained for the determination of condensed tannins in milligrams of catechin equivalent per gram of the extract (mg EC/g E).

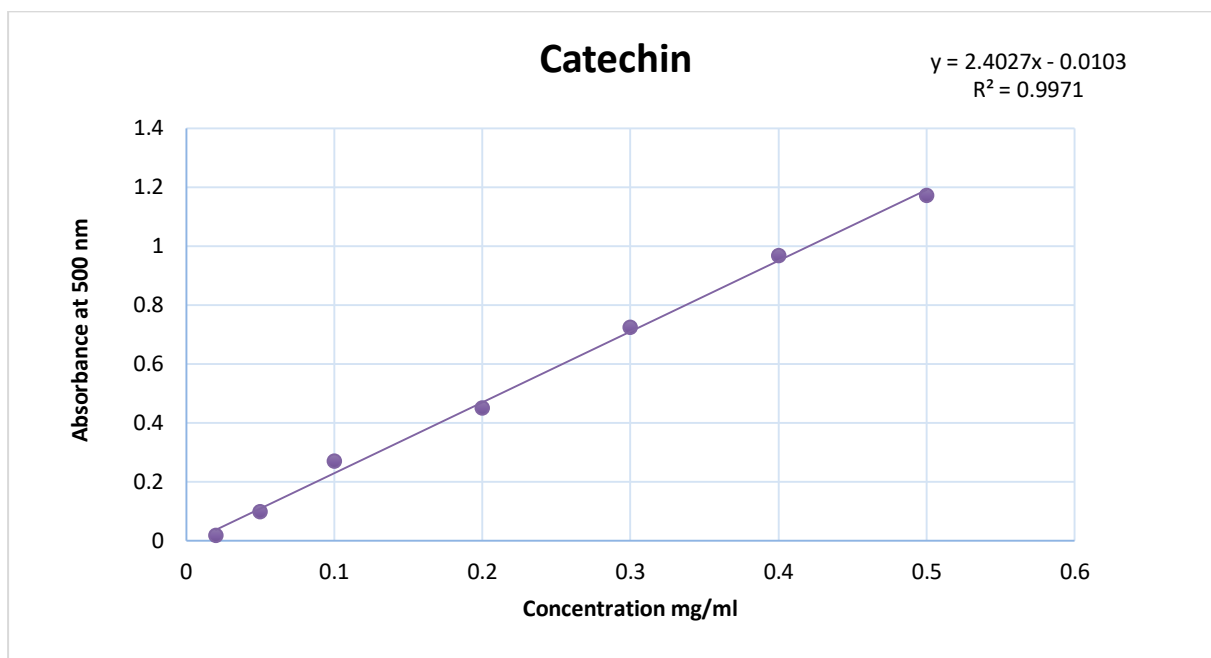


Figure19: Catechin calibration curve for the determination of condensed tannins.

In contrast to flavonoids and polyphenols, the results presented in **(Figure 20)**. *Glycyrrhiza glabra* extract has a tannin content of 13.99 ± 0.63 mg EC/gE, which is close to *Daucus carota* seed extract which is 12.33 ± 0.48 mg EC/gE.

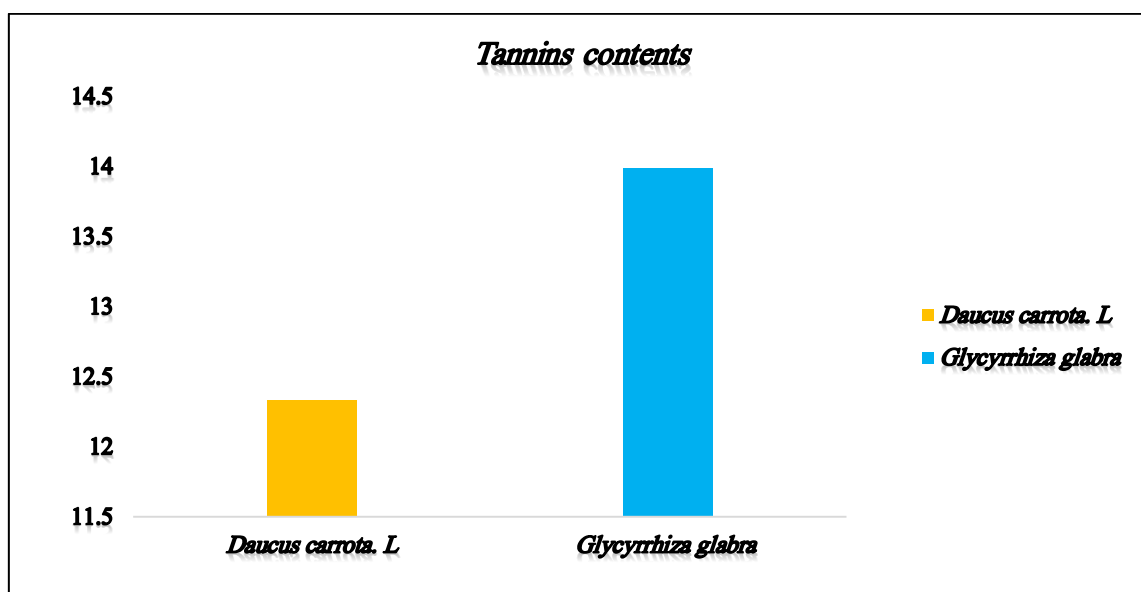


Figure 20: Evaluation of the Condensed Tannins contents of the two extracts.

II.1.4. Evaluation of biological activities

II.1.4.1 .Antioxidant activity

II.1.4.1.1 .DPPH radical trapping test

Because of the radical's stability and the ease of its analysis, the DPPH radical is often one of the most frequently employed substances for the quick and accurate assessment of antioxidant activity (Mensor *et al.*,2001). Ascorbic and gallic acid are used as reference molecules, and the results are represented as a percentage of 50% as was calculated from the first parameter noted: "ARP" (anti-radical) power, equal to $(1 / IC_{50})$. More these values move away from zero, more the antioxidant power increases. expression of the free radical DPPH (Singh, S *et al.*,2021).

Table14: Results of the antioxidant activity evaluated by the DPPH test.

Sample	IC ₅₀ (µg / mL)	ARP (1/IC ₅₀)
<i>Daucus carota L</i>	19.35 ± 1.44	0.06
<i>Glycyrrhiza glabra L</i>	22.09± 0.29	0.07
Ascorbic Acid	0.08 ± 0.006	12.5

For comparative purposes, two standard antioxidants are used ascorbic acid (**Figure 21**), they showed a powerful anti-free radical activity with IC₅₀ 0.08 ± 0.006 µg / mL and ARPs of around 12.5 respectively. The lower the IC₅₀ value, the more the extract is considered a powerful antioxidant.

For our extracts, it represents *Daucus carota L* extract most active extract, With IC₅₀ in the range of 19.35±1.44 mg/ml and ARP: 0.06 followed by *Glycyrrhiza glabra L* extract With IC₅₀ 22.09±0.29 µg/ml and ARP: 0.07.

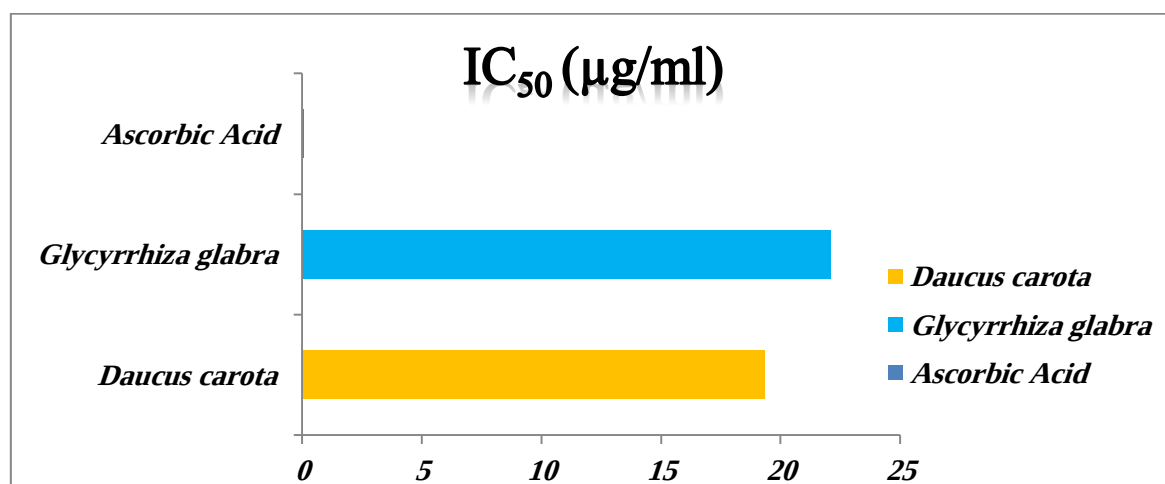
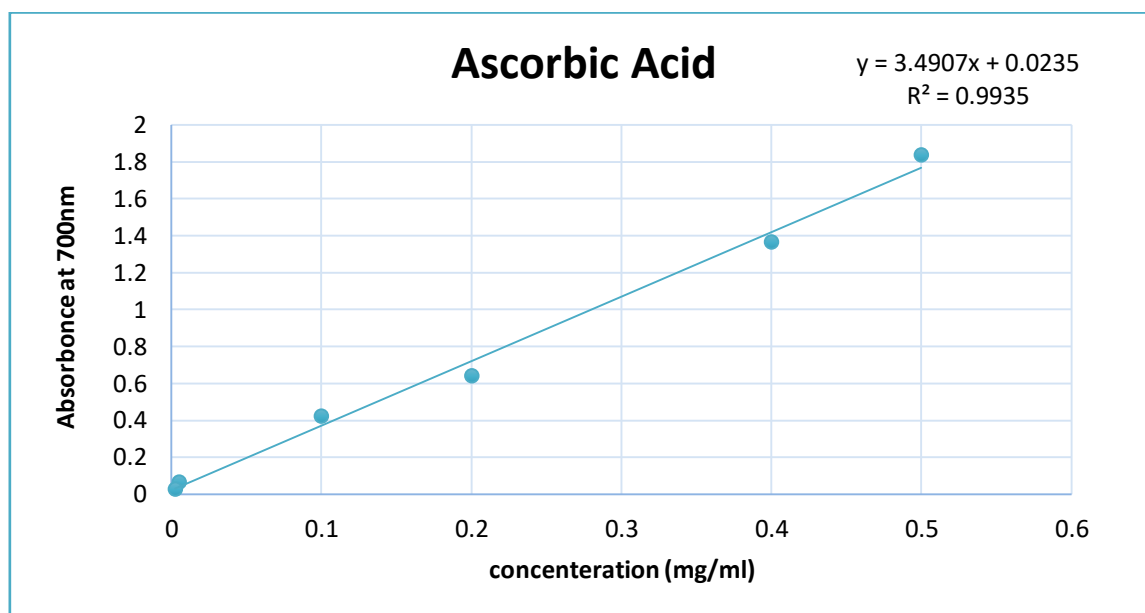


Figure 21: Histogram of the results of 50% inhibitory concentrations of DPPH.**II.1.4.1.2. Ferric reducing antioxidant power (FRAP)**

In this study, we chose to test different aqueous extracts (*Glycyrrhiza glabra L*, *Daucus carota L* seed). A calibration curve (**Figure 22**) is performed using ascorbic acid. The results are expressed in Milligrams (mg) ascorbic acid equivalent per gram of extract (mg EAA/g).

**Figure 22** Ascorbic acid calibration curve for the FRAP test.

The FRAP test results mentioned in Table 15 indicate that *Glycyrrhiza glabra* extract Antioxidant effect within 1.59 ± 0.045 mg EAA/g, *Daucus carota* extract with a value equal to 1.38 ± 0.201 mg EAA / g

Table 15: Results of the antioxidant activity evaluated by the FRAP test.

Extracts	Antioxidant activity (EC ₅₀ mg EAA/g)
<i>Glycyrrhiza glabra L</i>	1.59±0.054
<i>Daucus carota L</i>	1.38±0.201

II.1.5. Photoprotective activity

The SPF value is a mark that must be given cosmetics and personal products identified for their effectiveness in sun protection skin cooperation. The SPF value is determined by able to block, reflect or scatter solar rays (**Aida, B et al.,2022**).

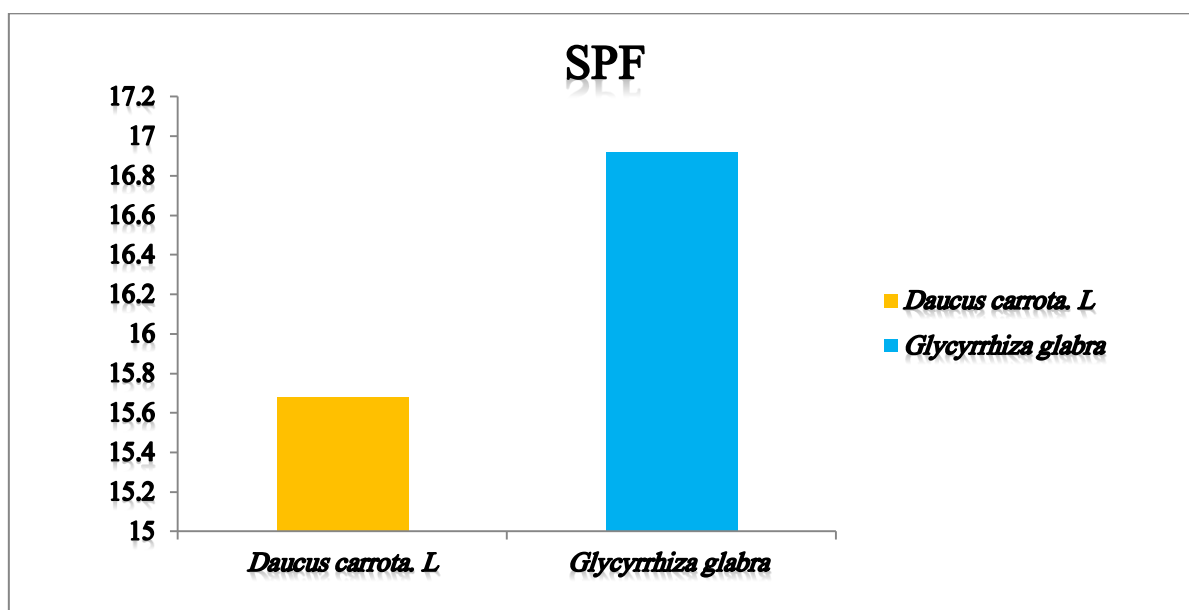


Figure 23: Histogram of the results of photoprotective of extracts

The results of the Photoprotective test for both extracts showed, respectively, *Daucus carota L* extract 15.68 ± 0.19 , and the results for *Glycyrrhiza glabra L* extract were higher, reaching 16.92 ± 0.54 .

II.1.6. Anti-inflammatory activity

The anti-inflammatory assay of extracts was tested by detecting the inhibition of heating induced denaturation of Albumin. The results show that the extract and standard drug have shown the dose-dependent ability for protein denaturation inhibition. demonstrates that the aqueous extract of extracts has an anti-inflammatory potential comparable to that of Aspirin[®].

Table16: IC₅₀ values for anti-inflammatory assay of aqueous extracts and aspirin standard

Sample	Inhibition of protein denaturation Assay (IC ₅₀ µg/mL)
<i>Glycyrrhiza glabra L</i>	329.07±3.49
<i>Daucus carota L</i>	113.50±4.46
Aspirin [®]	154.97 ± 0.87

The aqueous extract of *Glycyrrhiza glabra L* revealed a very effective activity in The inhibition rate is 50% of the protein denaturation (Figure 23) with IC₅₀=329.07±3.49 µg/ml, and for *Daucus carota L* its value was 113.50±4.46µg/ml Aspirin[®], It was used as a control, and showed IC₅₀ =154.97 µg/mL

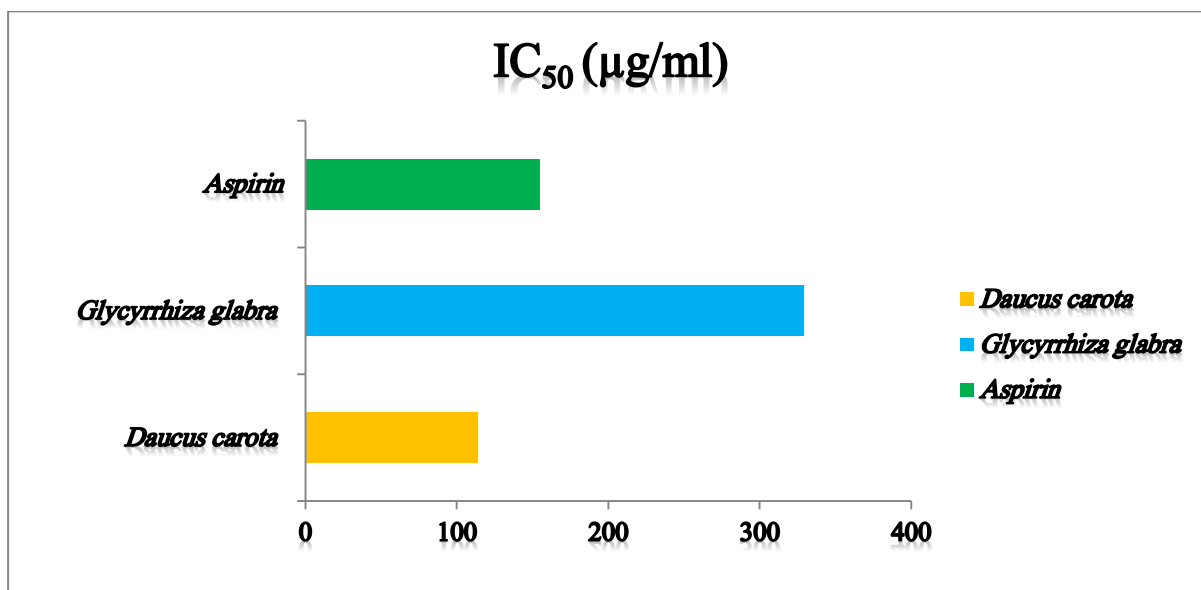


Figure 24: Inhibition of albumin denaturation at different concentrations of extracts and aspirin standard.

II.1.7. Preparation of Bioproduct

The product has been prepared in large quantities. The packaging has been prepared with a design for the skin care bioproduct. The final appearance of the make-up remover shown in Figure 25.



Figure 25: The final appearance of the products

II.1.7.1. Investigation relating to the application of make-up remover

a. Age group

According to the inquiry, the product testing rate for the 21-35 age group was assessed at 52%, the 36-50 age group at 23%, the under 20 age group at 17%, and the over 50 age group at 8%.

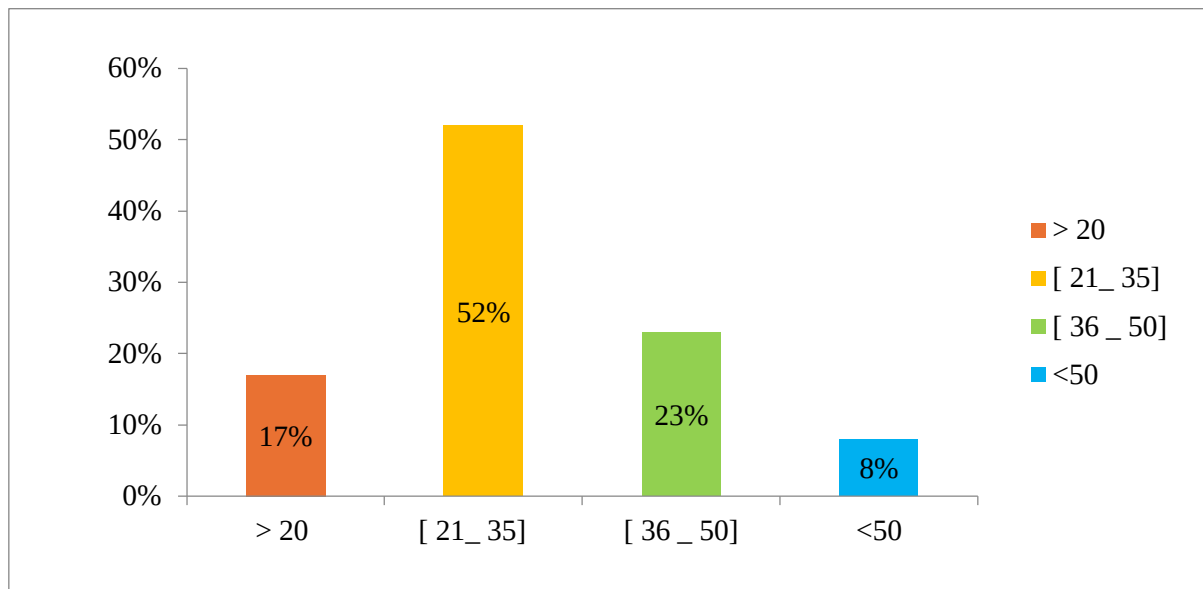


Figure 26: Age group

b. Educational level

According to our findings, the university level accounts for 47% of product experimentation, with the Uneducated group accounting for an average of 29% and the baccalaureate category accounting for only 24%.

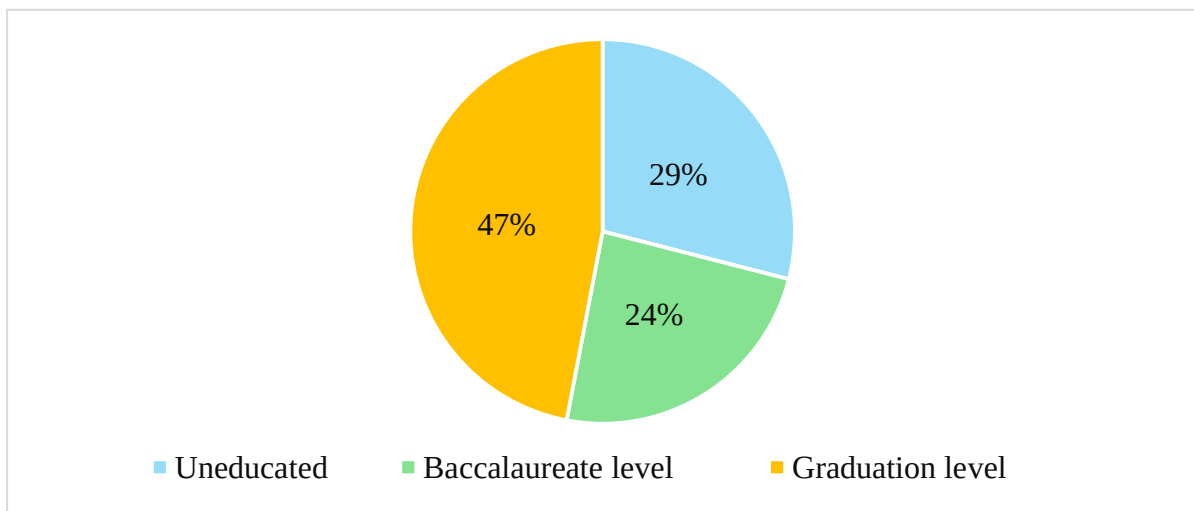


Figure27: Educational level

c. Professional category

According to our findings, the most frequent users of the product were women who stayed at home (54%), followed by Educated women (29%), and employees (17%).

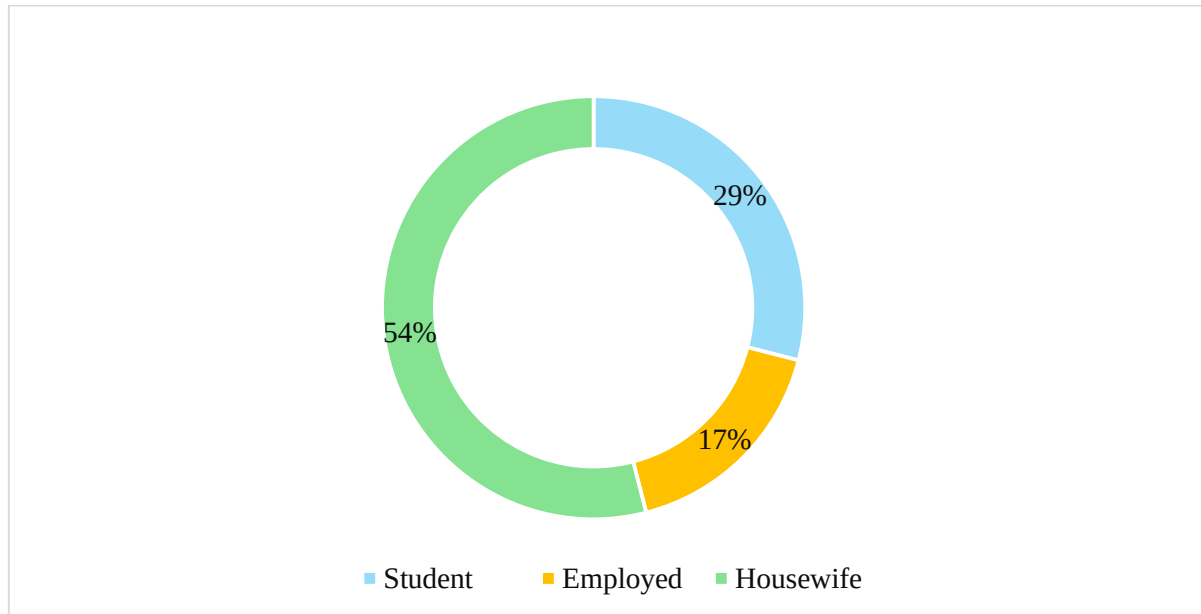


Figure28: Professional category

d. Skin types

According to our findings, combination skin received the highest use (47%), followed by oily skin (30%) and dry skin (24%).

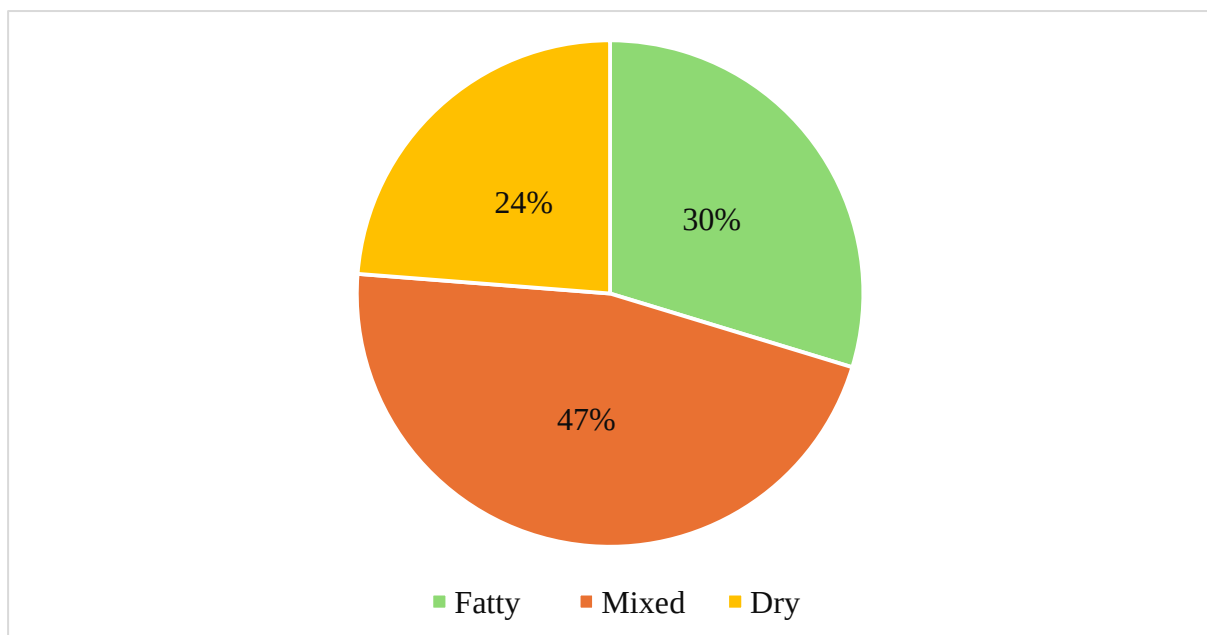


Figure 29: Skin types

e. Skin care and cosmetics uses

We see that the answer "sometimes" earned the largest percentage (49%), followed by "yes" (42%), and "no" (9%).

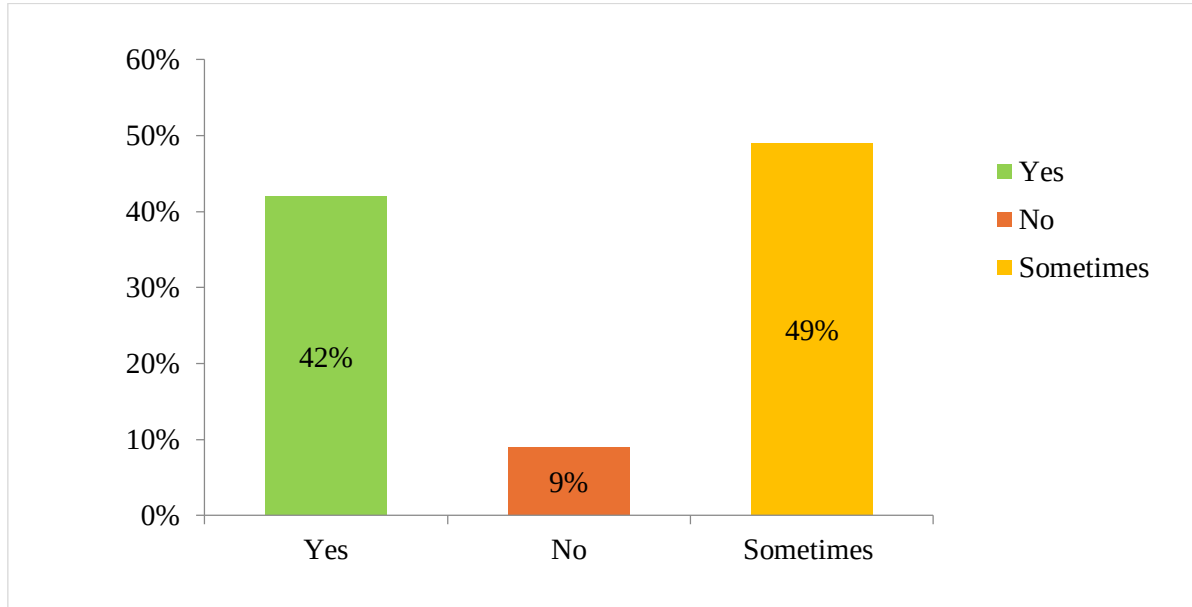


Figure 30: Skin care and cosmetics uses.

f. Read the list of ingredients on skin care products

We see that the answer "sometimes" earned the largest percentage (36%), followed by "daily" (32%) and "never" (32%).

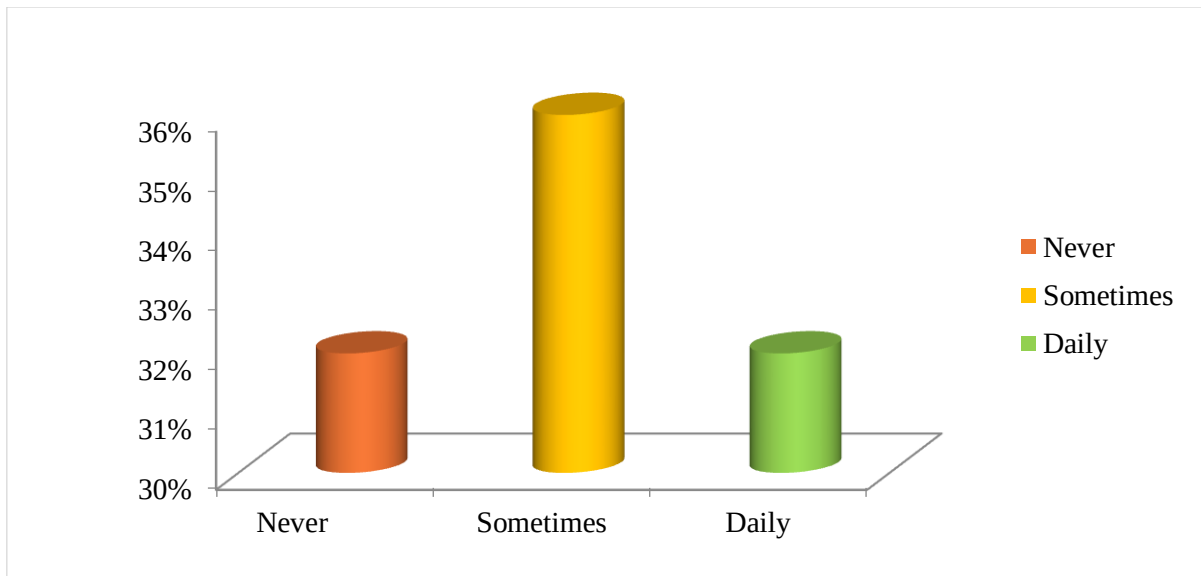


Figure 31: Read the list of ingredients on skin care products.

g. Support the idea of using skin care products

We see that the answer "yes" earned the largest percentage (53%), followed by "sometimes" (40%), and "no" (7%).

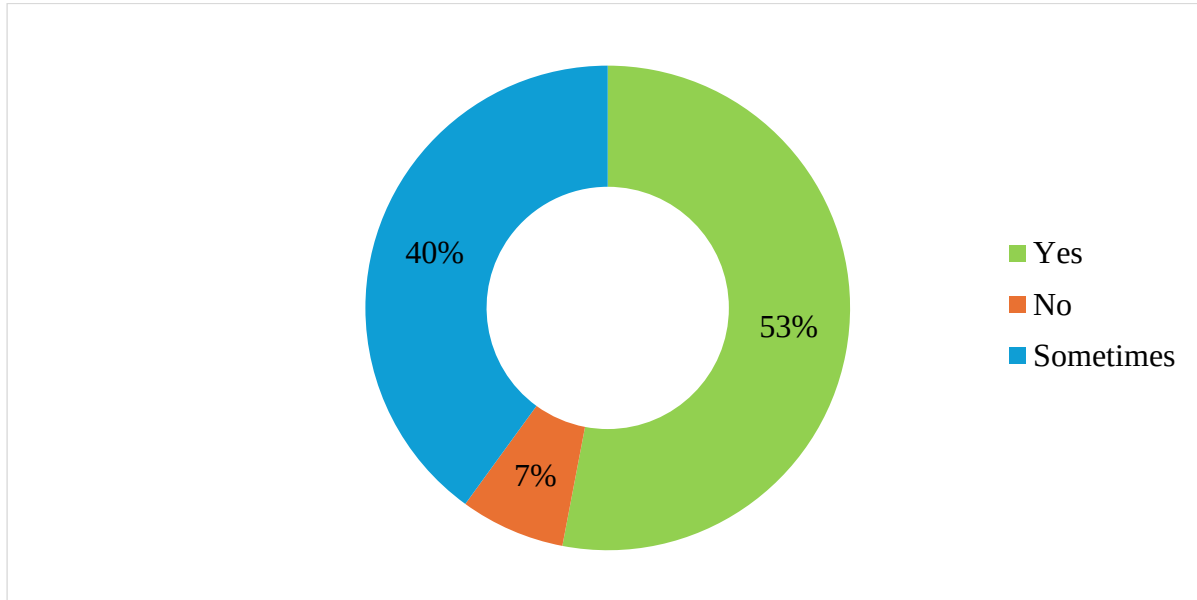


Figure32: Support the idea of using skin care products.

h. Like makeup remover

We see that the answer "yes" earned the largest percentage (55%), followed by "sometimes" (41%), and "no" (5%).

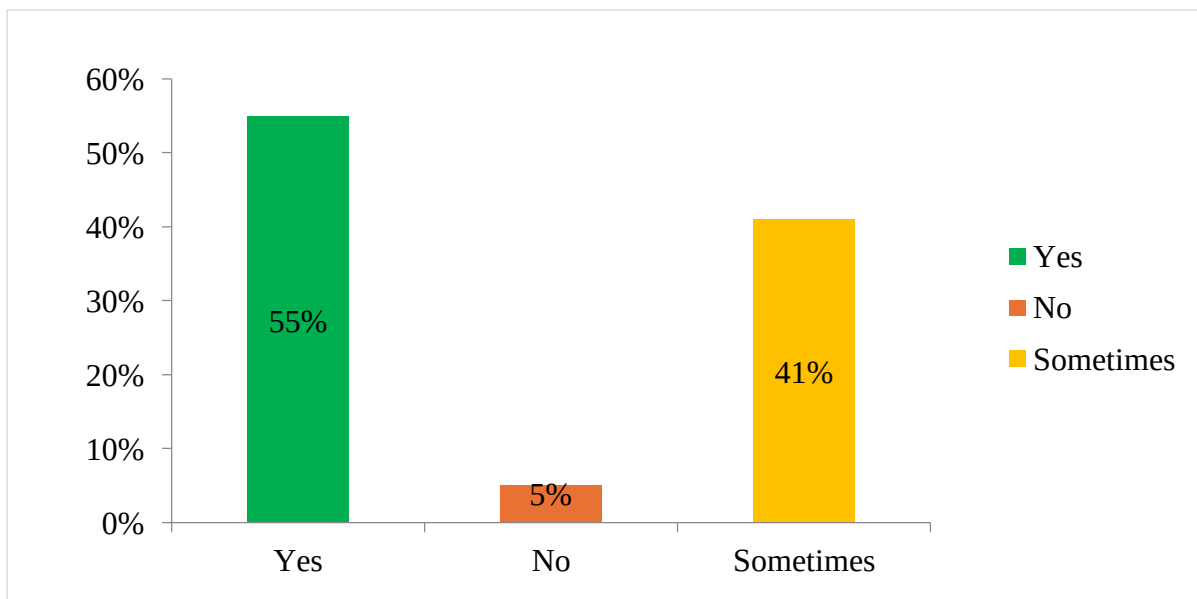


Figure 33: Like makeup remover

i. The number of times use makeup removers per week

67% use makeup remover once to three times a week, 19% use it four to six times, and 14% use it more than six times every week.

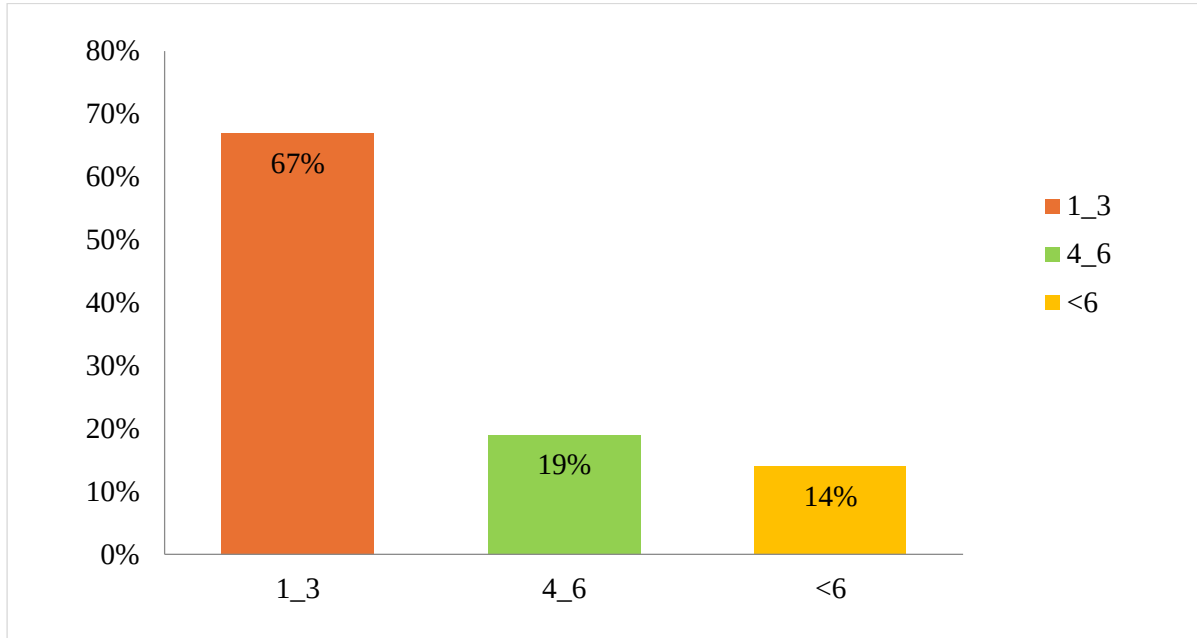


Figure 34: The number of times use makeup removers per week.

j. Type of makeup removers used

43% tried makeup remover with licorice extract, 43% tried makeup remover with carrot extract, and 44% tried both.

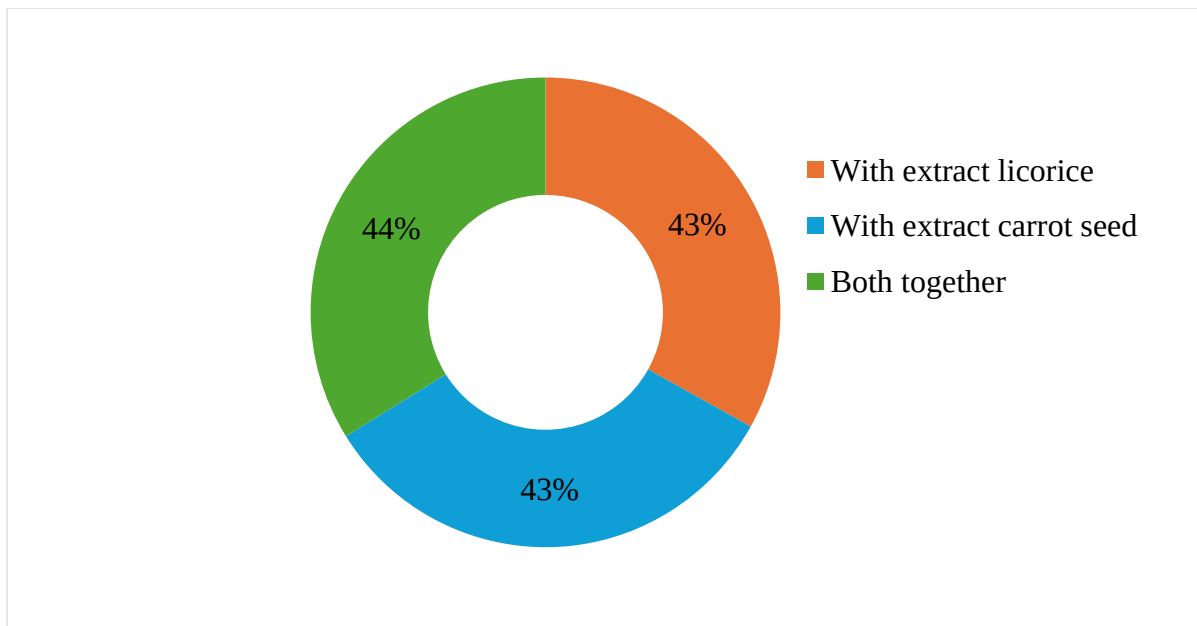


Figure 35: Type of makeup removers used.

k. Evaluation of makeup removers

We see that the answers in carrot seed are "very good" (30%), "good" (32%), "acceptable" (33%), and "unacceptable" (5%). But in with licorice the answers are "very good" (16%), "good" (51%), "acceptable" (31%), and "unacceptable" (3%).

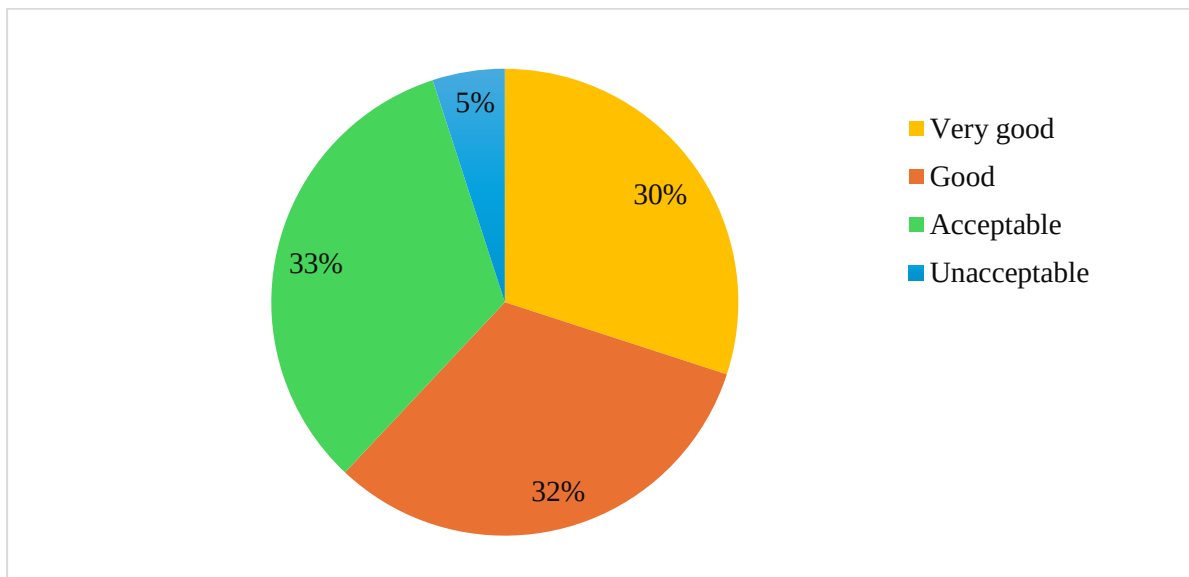


Figure 36: Evaluation of makeup removers with carrot seed extract

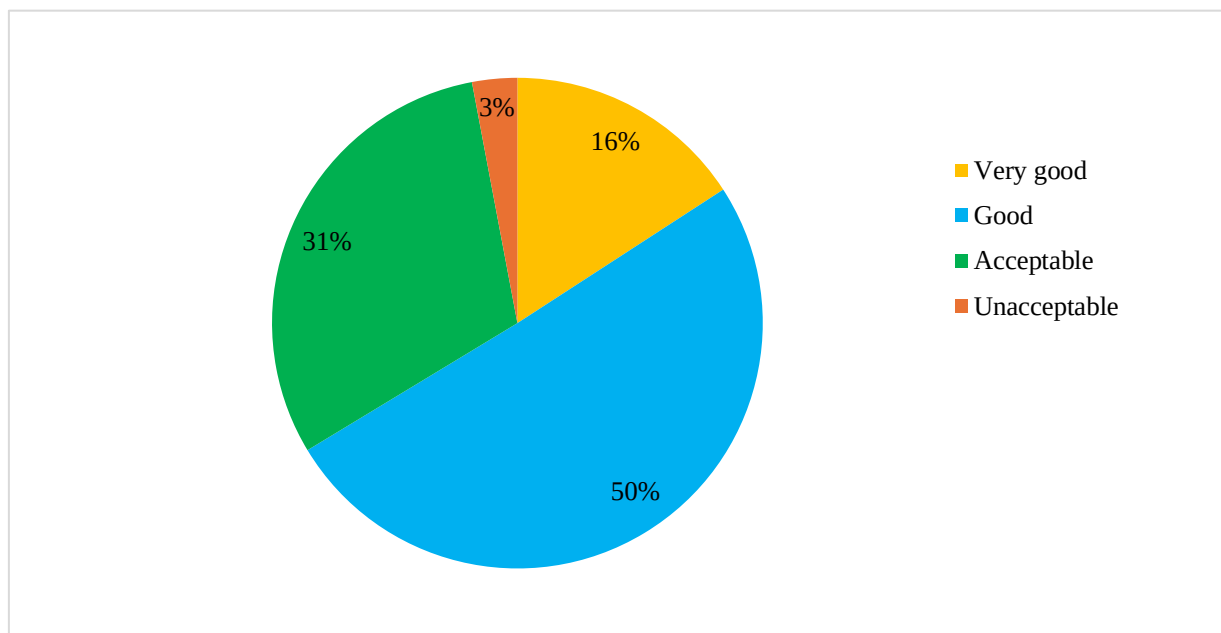


Figure 37: Evaluation of makeup removers with licorice extract

I. Evaluation of the scent of makeup remover with extracts

In makeup remover with carrot seed we see that the answers are "very good" (9%), "good" (40%), "acceptable" (45%), and "unacceptable" (7%). But in makeup remover with licorice, we see that the answers are "very good" (6%), "good" (48%), "acceptable" (42%), and "unacceptable" (5%).

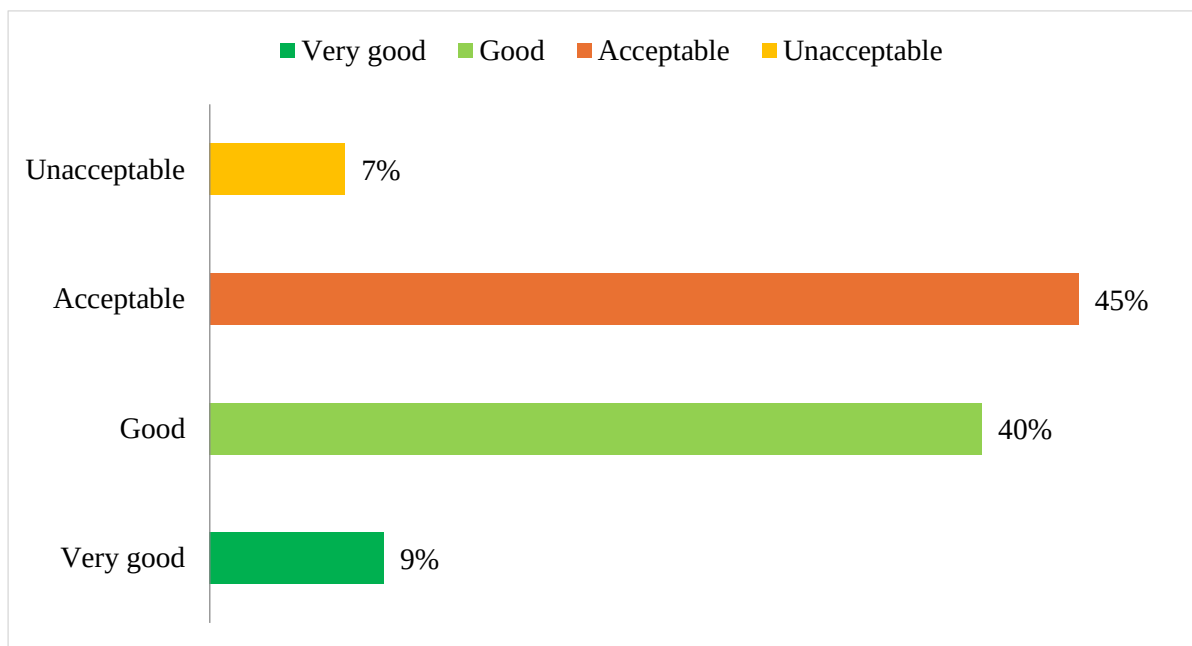


Figure 38: Evaluation of the scent of makeup remover with carrot seed extract

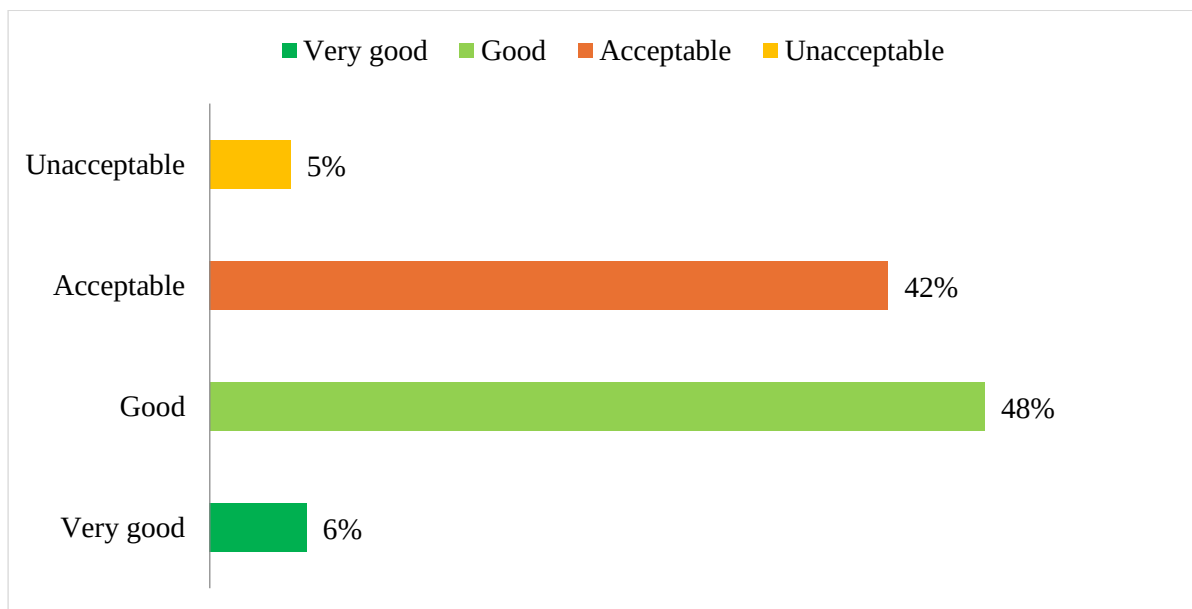


Figure 39: Evaluation of the scent of makeup remover with licorice extract.

m. Feeling irritated or sensitive when using makeup remover extracts

In carrot seed make-up remover, we see that the answers are "yes" (6%), "sometimes" (68%), "maybe" (26%). In make-up remover with licorice, we can see that the response "no" received the largest percentage (70%), followed by: "maybe" (23%), and "yes" (7%).

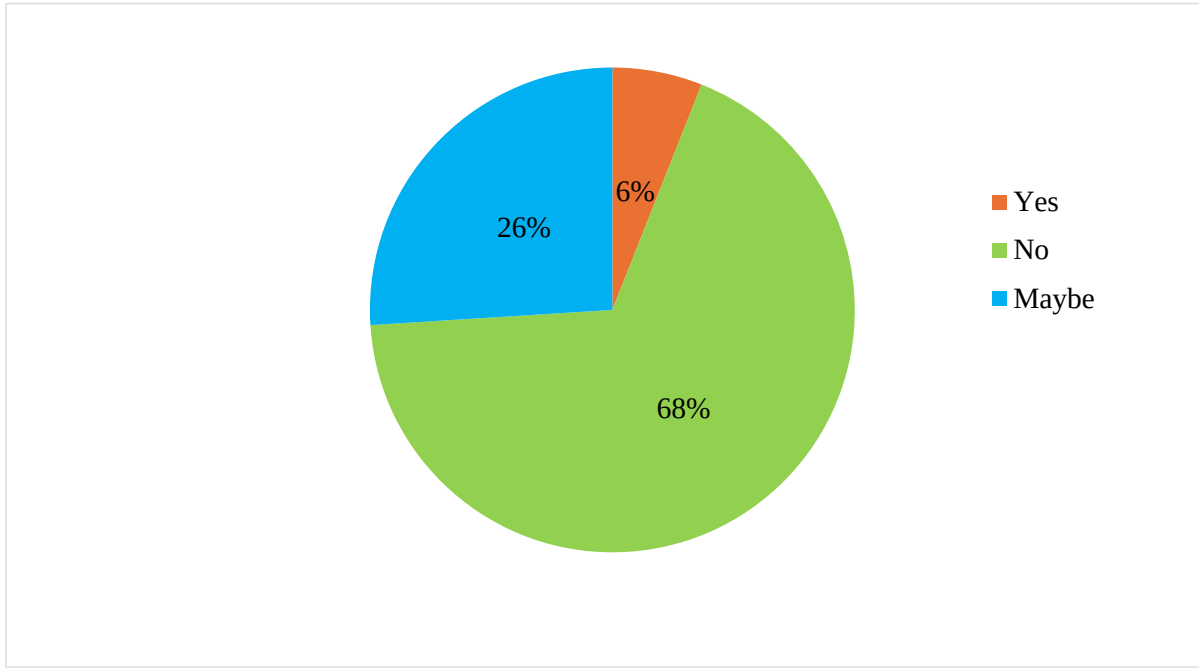


Figure 40: Feeling irritated or sensitive when using makeup remover with carrot seed.

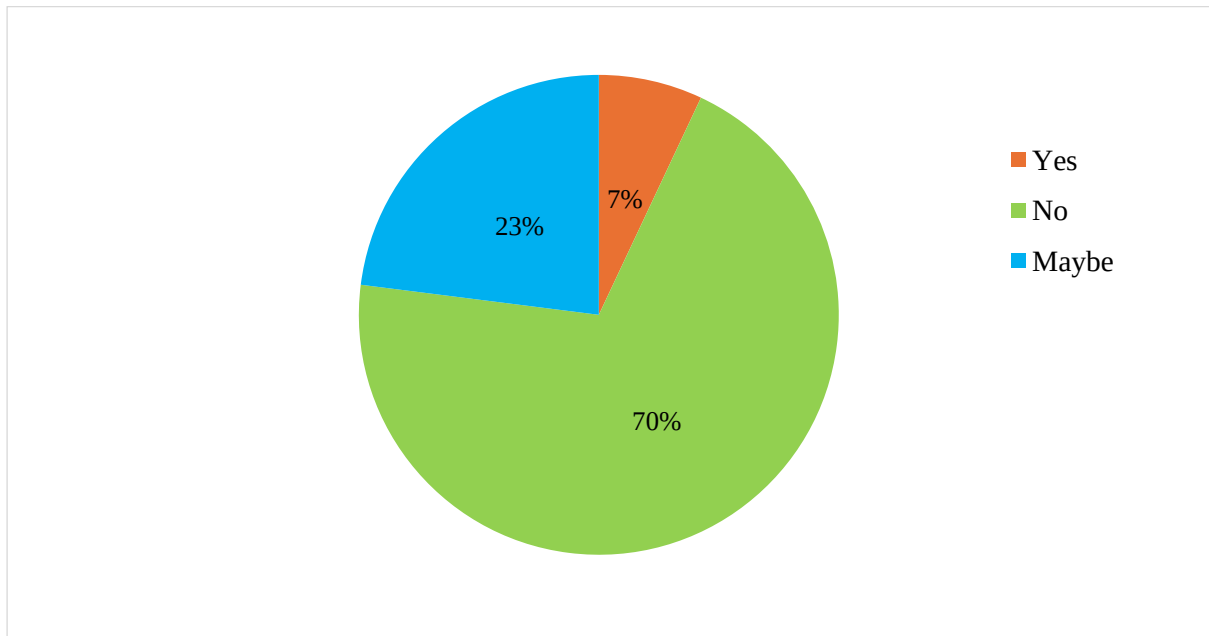


Figure 41: Feeling irritated or sensitive when using makeup remover with licorice.

n. Recommend it to your family and friends

In carrot seed make-up remover, we can see that the response "yes" received the largest percentage (55%), followed by "maybe" (39%), and "no" (7%). In licorice make-up remover we can see that the response "yes" received the largest percentage (49%), followed by "maybe" (43%), and "no" (8%).

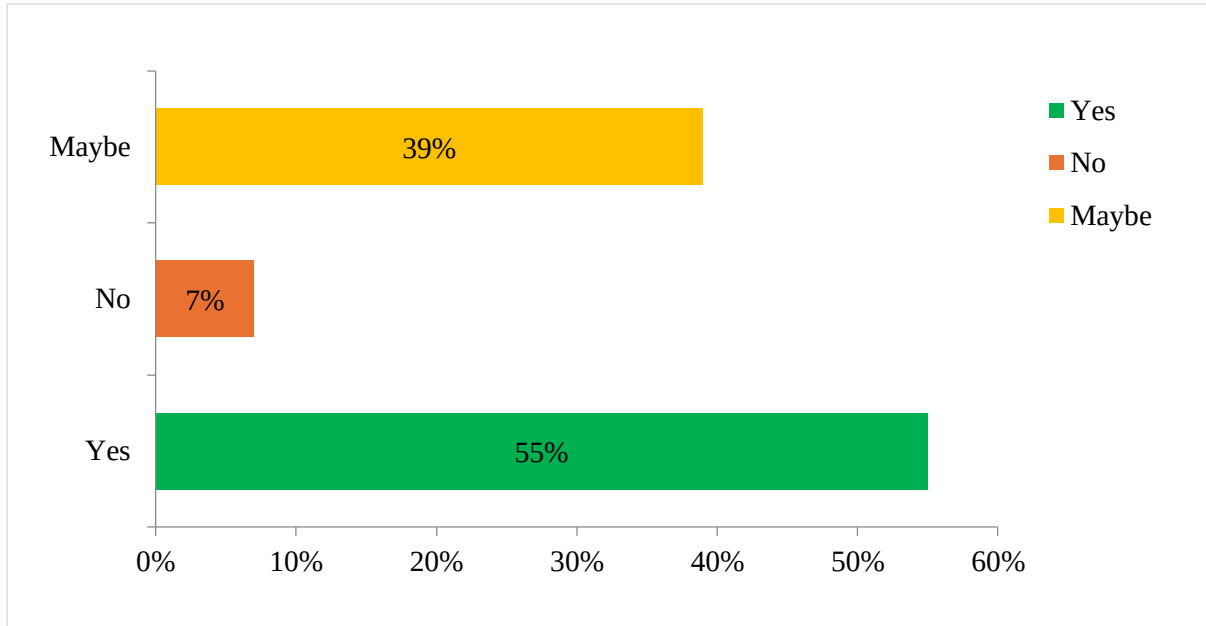


Figure 42: Recommend carrot seed product to family and friends.

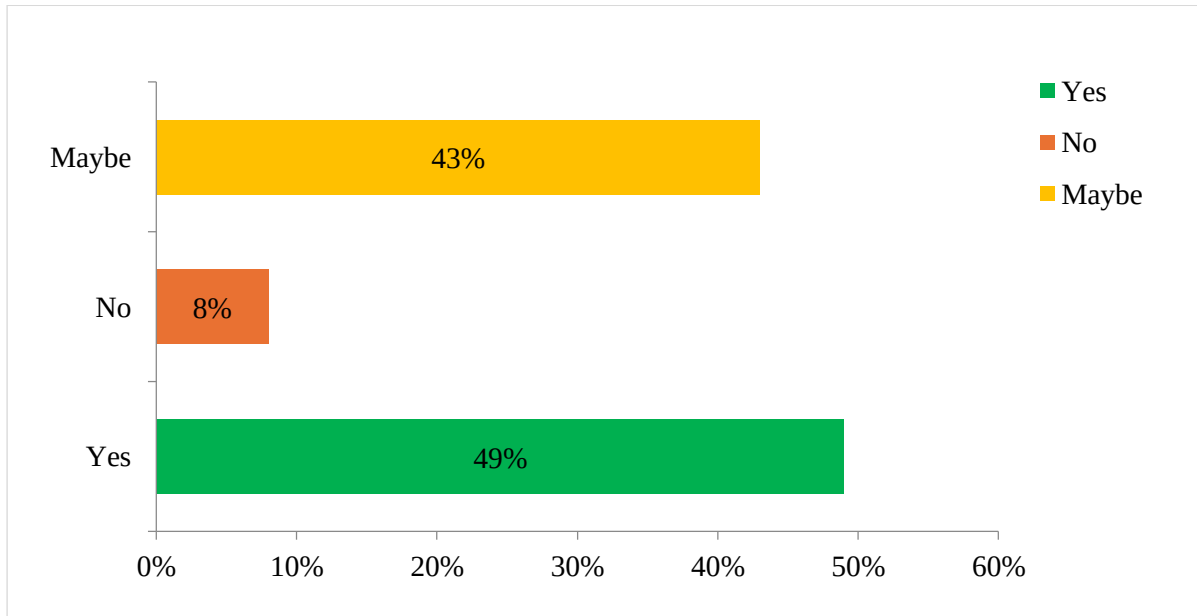
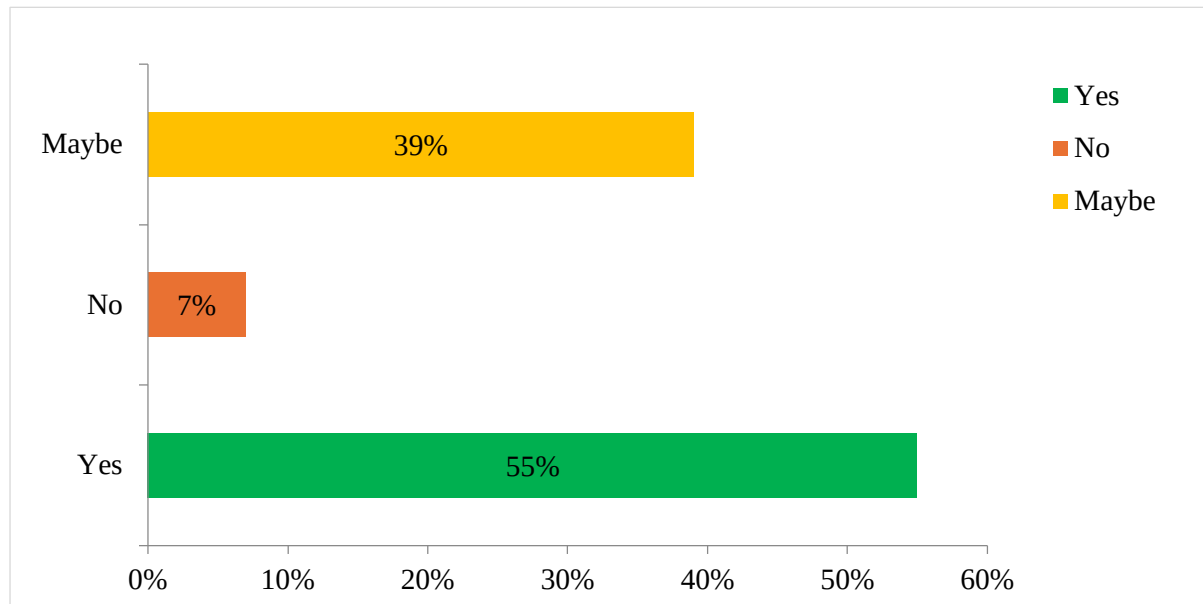


Figure 43: Recommend licorice product to family and friends.

o. Buy the product again

In carrot seed make-up remover, we can see that the response "yes" received the largest percentage (55%), followed by "maybe" (39%), and "no" (7%). But in licorice make-up remover we can see that the response "yes" received the largest percentage (47%), followed



by "maybe" (45%), and "no" (8%).

Figure 44: Buy carrot seed product again.

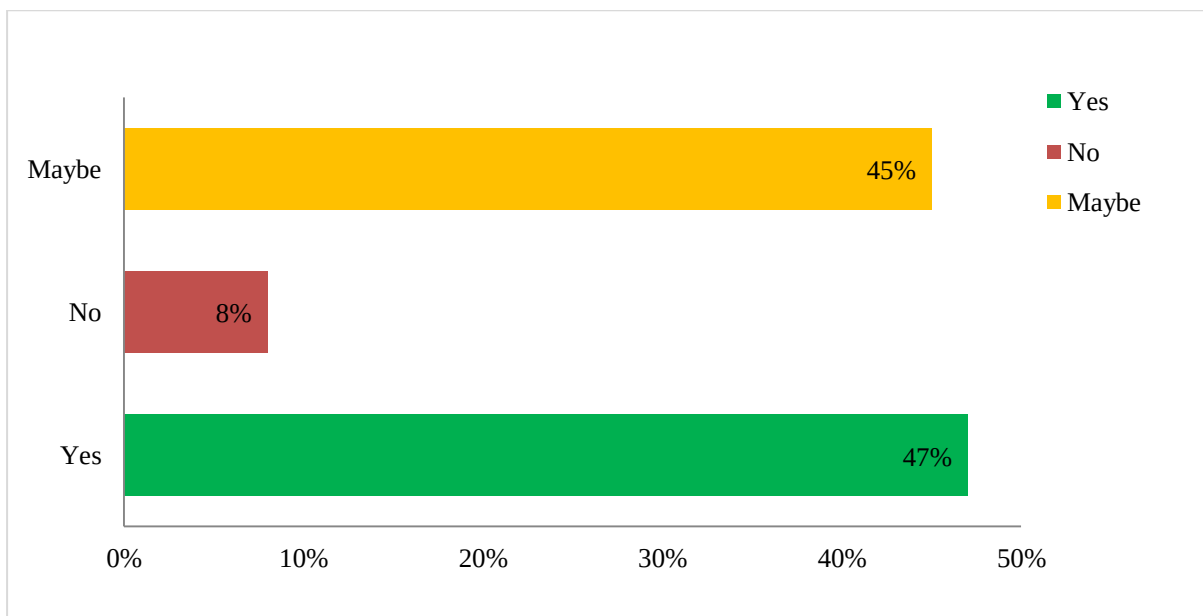


Figure 45: Buy licorice product again.

p. One type makes your skin cleaner than the other

We note that the percentage of yes to makeup remover with carrot seed extract (36%) is greater than the percentage of yes to makeup remover with licorice extract (28%), while the answer “both” was (38%).

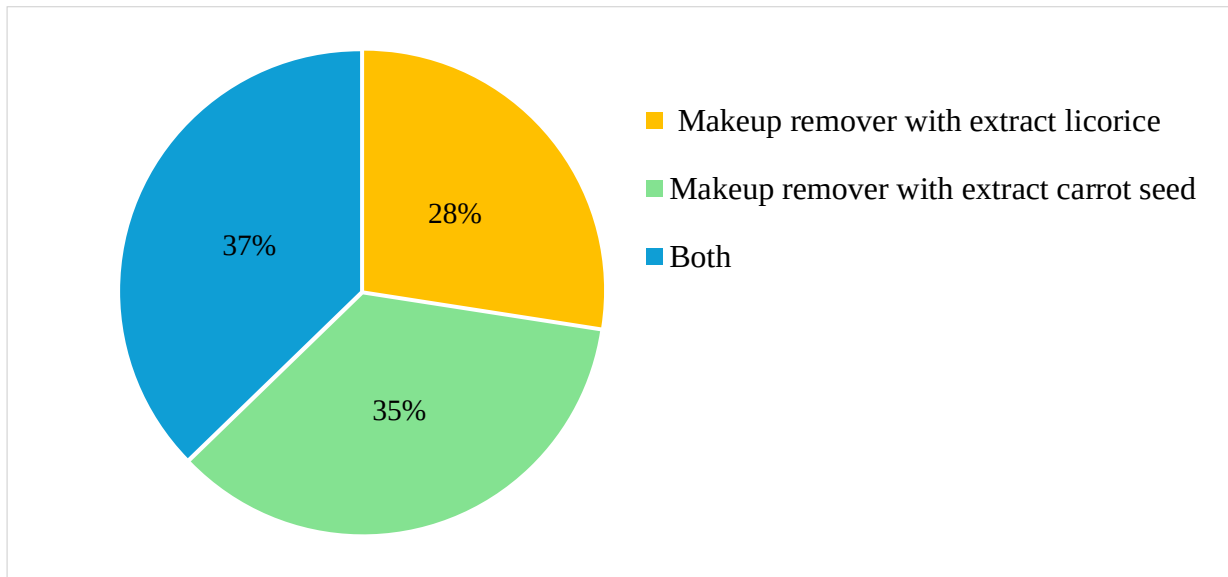


Figure 46: One type makes your skin cleaner than the other.

q. Note the difference in effectiveness between the two types

We can see that the response "yes" received the largest percentage (50%), followed by "maybe" (35%), and "no" (16%).

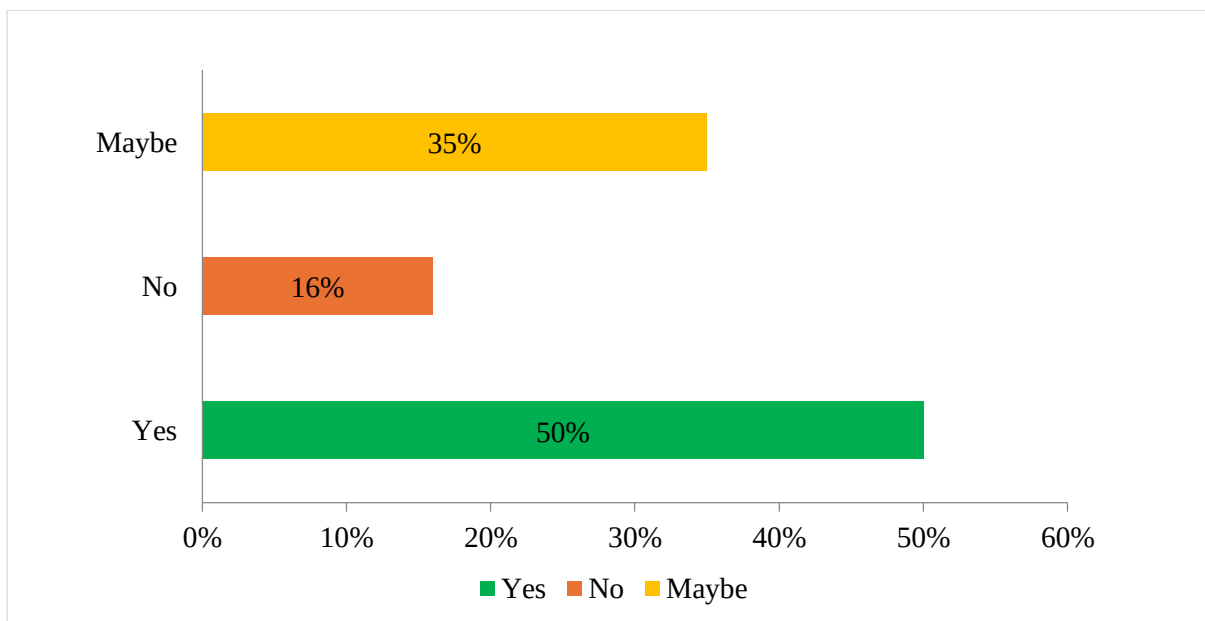


Figure 47: Note the difference in effectiveness between the two types.

II.2. Discussion

II.2.1. Test phytochimique

In this study, the results obtained through phytochemical tests that were conducted made it possible to highlight the different secondary metabolites in *Daucus carota* L seed extract and *Glycyrrhiza glabra* L extract. For *Daucus carota* extract, the results indicate the presence of polyphenols, flavonoids, alkaloids, condensed tannins, glycosides and terpenoids, these results are like those obtained in a study of the aqueous extract of the same species in study of **Latreche and Chaabane (2020)**. The results differed in the study of **Benrabbah and Boucheout (2021)** by the absence of saponins and the presence of alkaloids.

However, for the *Glycyrrhiza glabra* L extract the results indicate the presence of flavonoids, polyphenols, glycosides, alkaloids, tannins, terpenoids, and saponins. Indeed, flavonoids play a role in the coloring of plants (**Makhloufi, 2010**), also they have very important roles in plants, whose plants they protect against water stress and generate plant tolerance to heavy metals present in the soil. Apart from the plant, flavonoids have several pharmacological effects (**Makhloufi, 2010**). The presence of tannins, this compound which gives a bitter taste to the bark or leaves and makes them unsuitable for consumption by insects or livestock (**Eberhard et al., 2005**). Plants can produce phenolic substances (tannins) in response to environmental stress, caused by different factors: nutrient deficiency, drought, overheating (high temperatures) and light intensity (**Rira, 2006**).

These molecules can exhibit numerous properties. Indeed, polyphenols have been associated with a reduced risk of a number of chronic diseases, including cancer, diabetes, cardiovascular disease and neurodegenerative disorders. (**Handore & Khandelwal, 2017; Rasouli et al., 2017**). Polyphenols protect organisms against chronic pathologies by modulating numerous physiological processes, such as cellular redox potential, enzymatic activity, cell proliferation and signaling transduction pathways (**Luca et al., 2019**).

The plants are very rich in saponins. Saponins have anti-inflammatory and anti-edematous properties, they are particularly toxic for fish and other aquatic animals (**Bouhadjera, 2005**). Also, these molecules have properties analgesics (**Roux & Catier, 2007**).

II.2.2. Extraction yield

The crop varies with the plant species, the organ used, the drying conditions, the content of each plant type in metabolites and the nature of the solvent used. The results obtained show that the highest yield of *Daucus Carota L* extract, which was estimated by 13.2%, these results are like those obtained by **Chabbane and Latreche (2020)**. *Glycyrrhiza glabra L* extract gave low extraction yield (10%), and this was very low compared to that which was found by **Hammadou and Ourouba (2019)** with 17.60% collected from a university city approach to the one which got by **Messaoudi and Dilekh (2020)**.

These differences are due to several factors: the geographical origin, the environmental factors in particular (temperature and humidity) (**Granger et al., 1973**), the same plant species, the plant organ, the growth phase, the harvesting period, the conservation of plant materials, and the partial drying method used. Study and extraction method. (**Sefidkon et al., 2007**).

II.2.3. Estimation of total polyphenols contents

Phenolic compounds (PCs) form a wide group of compounds originating from the secondary metabolism of plants found in different natural sources such as fruits, vegetables, tea and honey (**Lima et al., 2019**). These compounds possess antioxidant, antimicrobial, anti-cancer, anti-inflammatory properties and inhibit lipid peroxidation (**Salehi et al., 2019**). This is the reason why, the estimation of the total phenols of our plant was carried out in our study.

By following the Ciocalteu-Folin method, the amount of total polyphenolic acids was estimated using the calibration curve for gallic acid, where the phenolic content was expressed in the number of milligrams equivalent to gallic acid per gram of extract (mg AGE/g Ext).

According results on the calibration curve, the content of total phenolic compounds in our sample is estimated at 55.78 ± 0.35 mg EAG/gE for *Daucus carota L* extract, and 108.46 ± 0.89 mg EAG/gE for *Glycyrrhiza glabra L* extract. On the other hand, the study of *Daucus carota L* by (**Dahmane, 2018**) found a content of $72,54 \pm 1,31$ mg EAG/gE. This result is more than that found in our study.

After extrapolation of the O.D. results on the calibration curve, the content of total phenolic compounds in our sample is estimated at 55.78 ± 0.35 mg EAG/gE for *Daucus carota* L extract, and 108.42 ± 0.89 mg EAG/Eg for *Glycyrrhiza glabra* L extract.

On the other hand, the study of *Daucus carota* L by **Dahmane (2018)** found a content of $72,54 \pm 1,31$ mg EAG/gE. This result is more than that found in our study. Also the study of *Glycyrrhiza glabra* L by **Rahmouni and Reghis (2016)** found a content of $118,75 \pm 23,68$ mg EAG/gE. This result is more than that found in our study.

The distribution of secondary metabolites may change during plant development. This may be linked to hostile climatic conditions (high temperature, solar exposure, drought, salinity) which stimulate the biosynthesis of secondary metabolites such as polyphenols. Indeed, the phenolic content of a plant depends on a certain number of intrinsic (genetic) and extrinsic factors (climatic conditions, cultural practices, maturity at harvest and storage conditions) (**Falleh et al., 2008**), also depend on the organ analyzed, and the sampling conditions, the different diseases that can affect the plant (**Park & Cha, 2003**).

II.2.4. Estimation of Flavonoid contents

The quantitative results of flavonoids reveal that their content is high in the extracts; We note that plants react to environmental aggression by increasing the production of polyphenols, especially flavonoids, these phenolic compounds can be subject to significant fluctuations in the face of these aggressions (**Young et al., 1997; Charpenter & Smith, 1975**).

The high contents of phenolic compounds compared to flavonoids are logical given that flavonoids represent the majority compounds of polyphenols (**Boussahel, 2011**). This can also be explained by an increase in the phenolic metabolism of the plant; in addition, the existence of a link with unfavorable climatic conditions and collection conditions such as high temperatures, duration of solar exposure, nature of the soil and the growing season (**Djeridane et al., 2005**).

Based on our discoveries, the flavonoid content of *Glycyrrhiza glabra* L water extract was determined to be 76.28 mg EQ/gE. In comparison to a study (**Hammadou & Ourouba, 2019**) which found a greater rate of flavonoids than ours, it was estimated at 102.8 mg EQ/gE.

The flavonoids obtained from the aqueous extract of *Daucus Carota L* are estimated to 37.73 mg EQ/gE. While **(Dhmane, 2018)** obtained a higher percentage, estimated at 72.54 mg EQ/gE. Secondary metabolites may change during plant development. This can be linked to harsh climatic conditions (high temperature, sun exposure, drought, salinity), which stimulate the biosynthesis of secondary metabolites such as flavonoids **(Fallah et al. 2008)**. Indeed, the phenolic content of a plant depends on a certain number of intrinsic (genetic) and extrinsic (climatic conditions, cultural practices, maturity at harvest and storage conditions) factors **(Falleh et al. 2008)**.

II.2.5. Estimation of condensed tannins contents

Chemically, condensed tannins are defined as polymeric flavonoids **(Hagerman, 1988; Asadi et al., 2019)**. However, they can appear as oligomers as well, when they are composed of two to ten monomeric units **(Haslam, 2007; Asadi et al., 2019)**. In the form of polymeric flavonoids, they have limited to no solubility in water, whereas in oligomeric form, they are water soluble **(Bennick, 2002; Asadi et al., 2019)**.

Estimation of *Glycyrrhiza glabra L* extract implies a greater tannin content than obtained **(Soulef et al., 2023)**. While the determination of the aqueous extract of *Daucus Carota L* indicates a lower tannin content than what was obtained **(Shyamala & Jamuna, 2010)**.

We can explain the high level of tannins in *Glycyrrhiza glabra L* extract due to whatever the mode of extraction, the water records the highest of condensed tannins. Water could solubilize proanthocyanidins. But the problem is that water, especially at high temperatures, also extract unwanted substances such as proteins, lipids and non-phenolic. The extraction of condensed tannins depends on their chemical nature, the solvent used and the operating conditions **(soulef et al.,2023)**.

II.2.6. Evaluation of biological activities

II.2.6.1. Antioxidant activity

As the phenolic substances make one of the major groups of compounds acting as primary antioxidants or free radical scavengers, it was reasonable to determine their antioxidants activity in the selected plant extracts **(Li et al., 2008)**.

There is not a universal method by which antioxidant activity can be measured quantitatively in a very precise way. Most often it is necessary to combine the answers of different and

complementary tests to have an indication on the antioxidant capacity of the sample to be tested (Tabart *et al.*, 2009).

II.2.6.1.1. DPPH radical trapping test

The anti-radical activity is carried out by the method of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) which is a method frequently used for its simplicity. The inhibition of discoloration of the DPPH radical is depending on the concentration of the extracts used. (Favier, 2003).

Glycyrrhiza glabra L extract had inhibitory activity against the DPPH radical closely related to *Daucus carota L* extract. These two results compared to standard ascorbic acids and gallic acids are related to the proportions of polyphenols and flavinoids. The value of *Glycyrrhiza glabra L*, which was estimated at 22.09 mg/ml, is considered low compared to the study (Guerda *et al.*, 2023) for the same type of study, Comparing the *Daucus carota L* extract value of 19.35 mg/ml with another study of the same type, which was completely different from our result. Which amounted to 85.00 ml/mg. (Latreche & Chaabane 2020).

Evidence indicates a positive relationship between phenol level Compounds and antioxidant activity in plants (Savadi *et al.*, 2020).

The antioxidant mechanism of phenolic compounds can be summarized based on transport. on hydrogen atoms or the transfer of one electron through protons, the antioxidant potential of phenolic compounds in plant extracts depends on their type (Yan *et al.*, 2020). There is a strong relationship between antioxidant activity of phenolic acids and number/position of hydroxyl groups in compound. The antioxidant efficiency of monophenols is strongly enhanced Insertion of a second hydroxyl group at the orthologous or isotopic positions (Pereira *et al.*, 2009).

II.2.6.1.2. Ferric reducing antioxidant power (FRAP)

Ferric reducing power is linked with the antioxidant potential of a compound so it may serve as a good indicator of antioxidant activity. In this assay, reducing power assay is based on the reduction of Fe^{3+} to Fe^{2+} by antioxidant compounds visible in changing the yellow color of the test solution to various shades of green and blue, depending on the reducing power of antioxidants. Increased absorbance at 700 nm indicates high ferric reductive ability (Basu & Maier, 2016).

Based on the data we obtained for the aqueous extract of *Glycyrrhiza glabra L*, it was higher compared to that obtained (**Ben belabbas&Abeoub,2021**). Also the case with the aqueous extract of *Daucus Carota L* where our results were lower than those obtained (**Latrache & Chabbane,2020**).

The reducing power of *Glycyrrhiza glabra L*. species is probably due to the presence of hydroxyl groups in phenolic compounds which can serve as electron donors. Therefore, antioxidants can be considered as reductants and inactivators of oxidants (**Siddhuraju & Becker, 2007**). Some previous studies have also shown that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity (**Jeong et al., 2004**). Most non-enzymatic antioxidant activities such as scavenging free radical, and the inhibition of peroxidation is implemented by the redox reaction. the aqueous extract had a strong antioxidant capacity, the aqueous extract is more polar (**Zhu et al., 2002**).

II.2.7. Photoprotective Activity

We might conclude that our aqueous extracts of *Glycyrrhiza glabra L* and *Daucus Carota L*. yielded lower results than (**Lefahal et al.,2018**) methanolic extracts. The study of **Lefahal et al. (2018)** explained that photoprotective potential of the methanolic extract could be explained by its flavonoids content, the methanolic extract exhibited antioxidant effects, is having a possibility to be used as sunscreen in pharmaceuticals or cosmetics formulations (The methanolic extract is more effective).

II.2.8. Anti-inflammatory activity

Our results showed that *Daucus carota L* seeds can be considered an effective anti-inflammatory, as confirmed by the results of (**Mani et al., 2006**). This was also confirmed by (**Leite et al., 2022**), (**Dheeraj, B. et al., 2022**) regarding *Glycyrrhiza glabra L*.

Anti-inflammatory screening showed promise results of aqueous extract of *Glycyrrhiza glabra L* and *Daucus carota L* And show its potential as an effective source of combat inflammatory medications. This source can be blocked Denaturation of albumin- causing heat. The extract contains many phenolic compounds, including gallic acid and coumaric acid, which are known for their powerful anti-inflammatory properties Links. In addition, the extract contains high levels of alkaloids and saponins in *Glycyrrhizaglabra L*, both of which have been shown to have anti-inflammatory effects (**Nile et al.,2016; Surendran et al.,2021**).

II.3. Investigation relating to the application of make-up remover

The findings of the questionnaire according to question (1) revealed that the group of young people between the ages of 21 to 35 years has the highest participation rate because they use makeup and makeup removers more frequently than other groups, while the educational level the responses to question (2), university students have the highest involvement rate due to their extensive knowledge of skin care products. According to the responses to question (3), women who remain at home had the greatest participation rate because they wear makeup more than other categories.

Also, the responses to question (4) revealed that we had participants with various skin types, with combination skin being the most common, followed by oily skin and dry skin. While many of the participants said sometimes, followed by those who answered yes, a few of them answered no, indicating that the majority of the participants were not unaware of the beauty industry and utilized makeup removers, according to their responses to question (5).

Because of their interest in cosmetics and high cultural level, most participants examined ingredient labels for skin care products. Those who replied no may not have been interested in reading the contents of skin care products, but we cannot confirm their lack of understanding about cosmetics. The answers to question (6) supported the use of skin care products. Most participants support the use of skin care products, with some responding occasionally and a few saying no (question 7).

The responses to question (8) revealed that most participants enjoy using makeup removers because they are interested in skin care products, particularly those containing natural extracts, such as those utilized in our study. Most people use makeup remover one to three times every week, according to the responses to question (9). Although the rest of us use it four to six times, which is why we underline that most individuals use makeup remover.

According to the responses to question (10), most participants attempted makeup removers with both extracts because they wanted to test both types. While testing the makeup remover with carrot seed extract, the responses varied from good to very good to acceptable, indicating that the product was well liked by the majority (question11). While the aroma of makeup remover with carrot seed extract was analyzed, the responses ranged from good to acceptable, indicating that many consumers enjoy the scent of the product (question 12).

According to the responses to question (13), makeup remover containing carrot extract did not cause irritation or allergy in many participants since the carrot seed plant contains anti-inflammatory properties, according to a study (**Mani et al., 2006**). While most participants are pleased with the product, the majority also stated that they would recommend it to their families, indicating that they loved it and would like to purchase it again (questions 14,15,16).

A makeup remover containing licorice extract was also tested, and participants' ratings varied from good to very good to acceptable, showing that the product was well welcomed by the majority. Many participants liked the fragrance of the product. Most people had no irritation or allergies because of the licorice root plant's anti-inflammatory characteristics according to a study (**Niel et al.,2016**). For Questions (17, 18, and 19) the majority answered that they would recommend it to their families, and this indicates that they liked the product as the majority would like to buy the product again (questions 20,21).

Many participants liked both products, but they noticed a difference in effectiveness and answered that the makeup remover with carrot seed extract is better and has better skin cleansing effectiveness. This is because carrot seeds are rich in polyphenol compounds, as antioxidants help maintain skin texture (**Latrache & Chabbane,2020**) (questions 22,23).

Conclusion

Phytochemical and to evaluate the antioxidant activity of the aqueous extracts prepared by maceration method of *Daucus Carota L* and *Glycyrrhiza glabra L* collected from El Oued. This plant has a therapeutic property for skin. Phytochemical examination revealed the presence of polyphenols, flavonoids, saponosides, tannins, alkaloids, terpenoids and glycosides for *Daucus carota L* and *Glycyrrhiza glabra L*, and our selected plants were found to be rich in these metabolites secondary.

The total polyphenol content of the two extracts was estimated using the Folin-Ciocalteu colorimetric method. The results showed that the *Glycyrrhiza glabra L* extract contains a high level of polyphenols, estimated by $(108.42 \pm 0.89 \text{ mg EAG/Eg})$ compared to the *Daucus carota L* extract, which is estimated by $(55.78 \pm 0.35 \text{ mg EAG/gE})$.

While flavonoids were estimated using the aluminum trichloride (AlCl_3) method, the results showed that *Glycyrrhiza glabra L* extract contains a high level of flavonoids, estimated at $(76.28 \pm 0.37 \text{ mg EQ/gE})$ compared to the extract of *Daucus carota L*, estimated at $(37.73 \pm 0.34 \text{ mg EQ/gE})$. On the other hand, the content of condensed tannins in *Glycyrrhiza glabra L* extract $(13.99 \pm 0.63 \text{ mg EC/gE})$ is lower than that of *Daucus carota L* extract $(12.33 \pm 0.48 \text{ mg EC/gE})$.

DPPH and FRAP results for both extracts have been shown to have antioxidant capacity. *Glycyrrhiza glabra L* extract has the same antioxidant activity. The anti-inflammatory activity was evaluated, the results showed that *Glycyrrhiza glabra L* extract has been reported to be more active than *Daucus carota L* extract.

The photoprotective activity results showed that *Glycyrrhiza glabra L* and *Daucus carota L* extracts both have good photoprotective capacity.

Finally, to clarify this valuable botanical model, we recommend in the future an in-depth analytical study for active ingredients, *in vivo* anti-inflammatory activity and photoprotective activity of this plant to enrich the field of cosmetology using natural extracts.

Finally, after having prepared the product (make-up remover) based on plant extracts with the properties studied, the product was tested on a sample of 100 female volunteers from El-Oued. The questionnaire results indicate the following:

- The make-up removers containing carrot seed extract and licorice root extract are efficacious and well-received by El-Oued women.

- Most people did not experience irritation or allergies.
- The majority expressed a desire to purchase again.

We encourage the advancement of this field because we believe it has promising potential for the future.

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Annexes

Annexe 01**Questionnaire**

This survey aims to collect information about cosmetics and skin cleaning products and evaluate our natural bio products made using licorice and carrot seeds in the EL Oued region.

Part 1. Personal information

Q1/how old are you?

Less than 20 years ☐

Between 21 years and 35 years ☐

Between 36 years and 50 years ☐

Older than 50 ☐

Q2/What is your educational level?

Uneducated ☐

Baccalaureate level ☐

Graduation level ☐

Q3/What is your professional category?

Student ☐

Employed ☐

Housewife ☐

Part 2. Information about Your Skin

Q1/What is your skin type?

Fatty ☐

Mixed ☐

Dry ☐

Q2/Are you a user of care and beauty products?

Yes ☐

No ☐

Sometimes ☐

Q3/ Have you ever read the list of ingredients on a product?

Never ☐

Sometimes ☐

Daily ☐

Q4/Do you support the idea of using beauty Bioproducts?

Yes ☐

No ☐

Sometimes ☐

Part 3. Information about makeup removers

Q1/ Do you like to use makeup removers?

Yes ☐

No ☐

Sometimes ☐

Q2/ How many times did you use each remover per week?

3_1times ☐

6_4times ☐

More than 6 times ☐

Q3/ What kind of makeup remover did you use?

Makeup remover with extract licorice extract ☐

Makeup remover with extract carrot seed extract ☐

Both together ☐

Part 4. Evaluation of Makeup remover Bioproduct with CARROT extract

Q1 / How do you evaluate the quality of Makeup remover Bioproduct with carrot extract?

Very good ☐

Good ☐

Acceptable ☐

Unacceptable ☐

Q2/How do you rate the smell of this Makeup remover Bioproduct?

Very good ☐

Good ☐

Acceptable ☐

Unacceptable ☐

Q3/Did you feel any irritation or sensitivity when using it?

Yes ☐

No ☐

Maybe ☐

Q4/Are you satisfied with the product?

Very satisfied ☐

Satisfied ☐

A little ☐

Not satisfied ☐

Q5/ Would you recommend this product to your family and friends?

Yes ☐

No ☐

Maybe ☐

Q6/Would you buy the product again?

Yes ☐

No ☐

Maybe ☐

Part 5. Evaluation of Makeup remover Bioproduct with LICORICE extract

Q1 / How do you evaluate the quality of Makeup remover Bioproduct with Licorice extract?

- Very good ☐
 Good ☐
 Acceptable ☐
 Unacceptable ☐

Q2/How do you rate the smell of this Makeup remover Bioproduct?

- Very good ☐
 Good ☐
 Acceptable ☐
 Unacceptable ☐

Q3/Did you feel any irritation or sensitivity when using it?

- Yes ☐
 No ☐
 Maybe ☐

Q4/ Would you recommend this product to your family and friends?

- Yes ☐
 No ☐
 Maybe ☐

Q5/Would you buy the product again?

- Yes ☐
 No ☐
 Maybe ☐

Part 6. Deference Between the Tow Makeup remover Bioproduct

Q1/ Have you noticed a difference in effectiveness between the two types?

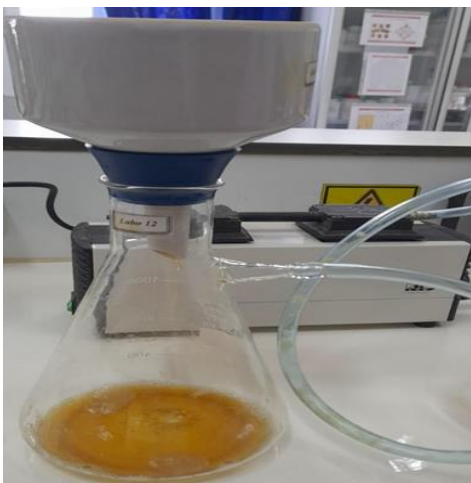
- Yes ☐
 No ☐
 Maybe ☐

Q2/ Do you think that one type leaves your skin clean over the other?

- Yes, Makeup remover with extract licorice extract ☐
 Yes, Makeup remover with extract carrot seed extract ☐
 Both, licorice carrot extracts ☐

Annex 02

The devices used during the experiment.

**Filtration****Rota-vapor****Centrifuge****Water bath**