Study of the Cosmeceutical Properties of the Plant *Anacyclus monanthos* subsp. *cyrtolepidioides*

Souha Soulef Atig

Dissertation submited to Escola Superior Agrária de Bragança to obtain the Degree of Master in Biotechnological Engineering under the scope of the double diploma with University Moulay Tahar of Saida

Supervised by

Maria João Sousa
Noureddine Halla

Bragança
2024

Acknowledgment

I am very thankful to the Erasmus program, which has allowed me to do this research and expand my horizons. My special thanks to my home university for the support, and to my supervisors professor “Maria João Sousa” and Professor “Noureddine Halla” for their guidance, without which this work would not have been accomplished.

I would like to extend my deepest thanks to my family, for their eternal support. To my grandmother, for the infinite love and encouragement; to my mother “ Hafida Chaib”, who has been a guiding star and firm foundation in my life. My father, “Larbi,” brothers, and sister, “Ritedje,” each one of them is a pillar of strength, for which I equally thank my cousin and the rest of my family for standing by me. Special thanks to my dear friend “Halima” for her invaluable help and friendship, and to all my friends back home in Algeria and those who have left, whose friendship has been an unremitting source of joy and strength.

In the lab, I am especially grateful to my lab mates, Tayna and Beatriz, for making the lab a more supportive and inspiring environment and to Paula Vanessa, our lab technician, for her generous guidance and advice throughout my work in the laboratory. I extend my gratitude to engineer Jorge Sá Morais for his invaluable help with the GC analysis, which was a cornerstone of this research.

I would also like to take this opportunity to express my heartfelt appreciation to the staff of the IPB Library, especially Clarisse, Marina, and all the rest, for their wonderful support and commitment making this journey smoother and more enriching and more rewarding.

I hold in my thoughts and heart my brother and sister in Gaza, who are unable to complete their studies due to the hardships they face. I dedicate part of this work to them, as well as to every innocent child who has lost their life too soon.

Finally, to everyone mentioned here and all others who have been a part of my journey, I love you all and am profoundly grateful for your presence in my life. Thank you for being a part of this experience.

Table of content

Index of Figures

Index of Tables

List of abbreviations

Rpm: rotation per minute

GC: Gas Chromatography

GC-MS: Gas Chromatography-Mass Spectrometry

GAE: Galic acid equivalent

QE: Quercetin equivalent

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

HAE: Hydroalcoholic extract

EO: Essential oil

pH: Potential of hydrogen

UV: ultraviolet

SPF: sun protection factor

RH: Relative humidity

IC50: half maximal inhibitory concentration

LC50: Lethatl concentration 50

FRAP: ferric-reducing antioxidant power

A. salina : *Artemia salina L*

Abstract

Anacyclus monanthos subsp. *cyrtolepidioides* is a plant subspecies endemic to North Africa (specifically Algeria, Tunisia, and Morocco), highly valued in traditional medicine. Recently, it has raised significant interest regarding its potential cosmetic applications. The current study aims to explain the biological and cosmeceutical functions of the plant, together with the bioactive compounds that contribute to its efficacy for the improvement of skin health and aesthetic appeal.

The extraction of the essential oils yielded 0.4%, and its chemical constituents were identified using GC-MS, showing 69.9% of the total volatile compounds such as Fragranyl 2-methyl butyrate (32.9%), cis-Chrysanthenyl acetate (24.6%) and trans- chrysanthenyl acetate (9.8%). The quantification of total phenolic and flavonoid content gave values of 27.47 ± 0.58 mg GAE/g for the total phenolic compounds and 8.40 ± 0.021 mg QE/g for flavonoids, reflecting remarkable antioxidant activities. On the fat content analysis, a percentage of 0.13% was obtained, and the fatty acid profile showed major components to be Methyl butyrate 69.30% and Methyl palmitate 7.12%. Protein content determination indicated 13.69%, which adds weight to the nutritional value of this plant. Assays of minerals showed a significant result for the micronutrient, indicating the richness of minerals within the plant.

Bioactivity assays, which investigated the plant's potential advantages, gave notable antioxidant properties through the DPPH radical scavenging activity assay with an IC50 value of 0.57 ± 0.01 mg/mL. The reducing power assay confirmed this result by showing considerable electron donation capabilities of 0.55 ± 0.076 mg/mL. Toxicity testing was carried out using the brine shrimp test and recorded LC50 values of 0.016 mg/mL for the essential oil and 0.038 mg/mL for the hydroalcoholic extract, the sun protection factor was measured with a value of 22.42 ± 1.42, hence making it suitable for use as an ingredient in sunscreen preparation.

A cosmetic cream was formulated using botanical extracts, essential oil, and camel fat as emollients and subjected to comprehensive stability assessments. The cream demonstrated a smooth, consistent texture with a stable pH of 5.16 and maintained uniform density. It showed resistance to intense light, high temperatures, and humidity, with constant viscosity for easy application. Spectrophotometric analysis confirmed the presence of UV-absorbing compounds, validating its potential as a sunscreen. Safety testing, including the HET-CAM ocular irritancy test, indicated low irritation, and microbial tests confirmed effective preservation against fungal

growth. This study establishes *Anacyclus monanthos* subsp. *cyrtolepidioides* as a valuable cosmeceutical ingredient, highlighting its bioactive properties and contributing to the field of natural skincare. As the first comprehensive analysis of this plant, this research provides a solid foundation for future studies in cosmeceutical and bioactive applications.

Keywords: Cosmetic formulation, Biological activities, chemical analysis, Antioxidant properties, Camel fat

Resumo

Anacyclus monanthos subsp. *cyrtolepidioides* é uma subespécie de planta endêmica do Norte da África (especificamente Argélia, Tunísia e Marrocos), altamente valorizada na medicina tradicional. Recentemente, despertou um interesse significativo devido às suas potenciais aplicações cosméticas. O presente estudo tem como objetivo explorar as funções biológicas e cosmecêuticas da planta, juntamente com os compostos bioativos que contribuem para sua eficácia na melhoria da saúde da pele e do apelo estético.

A extração dos óleos essenciais apresentou um rendimento de 0,4%, e seus constituintes químicos foram identificados por GC-MS, indicando 69,9% do total de compostos voláteis, com os principais sendo: Fragranyl 2-methyl butyrate (32,9%), cis-Chrysanthenyl acetate (24,6%) e trans-Chrysanthenyl acetate (9,8%). A quantificação dos compostos fenólicos totais e flavonoides resultou em valores de 27,47 ± 0,58 mg GAE/g e 8,40 ± 0,021 mg QE/g, respectivamente, refletindo notáveis atividades antioxidantes. Na análise de gordura, foi encontrado um teor de 0,13%, com os principais ácidos graxos identificados sendo Methyl butyrate (69,30%) e Methyl palmitate (7,12%). A determinação do teor de proteína indicou um valor de 13,69%, destacando também o valor nutricional da planta. O ensaio mineral revelou uma rica composição de micronutrientes.

Os ensaios de bioatividade demonstraram propriedades antioxidantes notáveis por meio do teste de atividade sequestrante do radical DPPH, com um valor de IC50 de 0,57 ± 0,01 mg/mL. O ensaio de poder redutor confirmou esses resultados, apresentando uma considerável capacidade de doação de elétrons com um valor de 0,55 ± 0,076 mg/mL. O teste de toxicidade realizado com Artemia salina registrou valores de LC50 de 0,016 mg/mL para o óleo essencial e 0,038 mg/mL para o extrato hidroalcoólico. O fator de proteção solar (FPS) foi determinado como 22,42 ± 1,42, destacando o potencial da planta como ingrediente em formulações de protetores solares.

Foi desenvolvido um creme cosmético contendo extratos botânicos, óleo essencial e gordura de camelo como emoliente, submetido a rigorosos testes de estabilidade. O creme apresentou uma textura suave e consistente, com pH estável de 5,16 e densidade uniforme. Demonstrou resistência à exposição à luz intensa, altas temperaturas e umidade, mantendo viscosidade constante para facilitar a aplicação. A análise espectrofotométrica confirmou a presença de compostos capazes de absorver radiação UV, validando seu potencial como protetor solar. Ensaios de segurança, incluindo o teste ocular HET-CAM, demonstraram baixa

irritabilidade, enquanto os testes microbiológicos comprovaram a eficácia do sistema conservante contra o crescimento de fungos.

Este estudo posiciona Anacyclus monanthos subsp. cyrtolepidioides como uma planta de valor significativo no campo cosmecêutico, destacando suas propriedades bioativas e contribuindo para o desenvolvimento de formulações naturais para cuidados com a pele. Como a primeira análise abrangente desta planta, esta pesquisa estabelece uma base sólida para futuras investigações em cosmecêuticos e compostos bioativos.

Palavras-chave : Formulações cosméticas, Atividades biológicas, Análise química, Propriedades antioxidantes, Gordura de camelo

1. Introduction

1.1. Framework

The winds of social change are blowing strongly in the 21st century, affecting cosmetic applications everywhere worldwide (Saraf & Saraf, 2015). The general public's need for safe personal care and cosmetics products has expanded significantly (Shim et al., 2024). Today's globe is seeing a major shift in the cosmetics industry as ethical consumerism and environmental sustainability become more refined (Man & Rahman, 2019). Sustainable marketing strategies are becoming increasingly important in the cosmetics industry in order to solve issues. While there are numerous aspects to sustainable marketing, the three basic ones are ethical purchasing practices, environmental responsibility methods, and open customer communication (Yang & Hamid, 2024).

The term “cosmetic” is derived from the Greek word “kosmeticos,” which means “to adorn” (Manzoor, 2024). The preparation used for this purpose is called a cosmetic. The term “cosmetic” can be defined as “an external preparation intended for application on the skin, hair, and nails of the body for purposes such as coloring, covering, softening, cleaning, nourishing, waving, setting, preserving, removing, and protecting,” among other uses (Khan & Alam, 2019). Items meant to be applied to poured, sprinkled, sprayed, or otherwise rubbed on the human body or any portion of it in order to clean, beautify, enhance attractiveness, or change the look is also a definition of cosmetics (Pandey et al., 2024). The regulatory frameworks for cosmetics differ significantly between the US and the EU, with the EU focusing more on safety and certainty, while the US emphasizes freedom and responsibility (Ferreira et al., 2022). The EU Cosmetic Products Regulation ensures safety by harmonizing classification, labeling, and packaging rules for hazardous substances and mixtures. It mandates classification and notification obligations for manufacturers, importers, and suppliers and establishes a harmonized list and inventory of substances. It excludes finished products intended for the final user (Rasmussen & Mech, 2014).

The use of cosmetics began around 6,000 years ago and has since expanded throughout the world. The purpose of these products was to adorn and scent the body, not alter the makeup or characteristics of the skin (Pereira & Pereira, 2018). The evolution of society is intimately linked to the research and development of cosmetic products (Infante et al., 2018). Cosmetic use dates back to ancient civilizations, with significant milestones such as the use of kohl in Ancient Egypt and the introduction of synthetic ingredients in the 20th century (Blanco-Dávila, 2000).

Another way to display one's social standing is through cosmetics. The cost of the goods and the usage of particular brands can reveal this status (Neibecker & Imdahl, 2022). Social media plays an important role in shaping beauty trends by increasing the transparency and accessibility of cosmetic operations (Hassan et al., 2021). The emergence of social media beauty influencers highlights a significant shift in how beauty trends are created and shared. With expanding trends in media and technology, men and women have adapted to the beauty and skincare sector (Castillo et al., 2022). Even when customers seek medical advice, the negative consequences of cosmetics and toiletries are reportedly underestimated (Toklu et al., 2019); this is why it's imperative that a methodical vigilance system be established and implemented in the home products and cosmetics industry. This is a practical approach for producers to collect information, enhance their goods, and meet regulations pertaining to authority transparency and customer safety (Kornfeld-Lecanu et al., 2010).

Research has shown that cosmetics products include a variety of harmful or toxic compounds that can have negative effects on the skin, such as allergic responses (Khan & Alam, 2019). The pharmaceutical sector started investing in innovative active ingredients and cosmetic production technologies as our understanding of the physiology of skin and its constituent elements expanded. Consequently, updated quality control tests in the manufacturing of such cosmetics are also required to ensure the safety of utilizing such compounds (Dreno et al., 2014). Consumers are increasingly demanding cosmetics with natural and organic ingredients (Faria-Silva et al., 2020), and seeking for transparency and eco-friendly products within the beauty and cosmetic industry. This increasing demand is pushing companies to be more honest and sustainable in their practices (Kaur & Subburayan, 2024). Consumers today show a strong preference for natural over synthetic ones because of health and environmental aspects (Hinčica et al., 2024) Additionally, many consumers believe that natural ingredients are gentler on the skin and more sustainable, aligning with their values of environmental consciousness (Luchs et al., 2010).

The global natural cosmetics market shows a current and widening growth, impelled by consumer demand for naturally ingredient-based products that open the door for innovative and sustainable formulations (Cavinato et al., 2017). This shift is driven by an increased understanding of the risks associated with synthetic chemicals and the health advantages of products generated from plants and other natural resources (Chang, 2011). Plant-based ingredients in cosmetics offer a multitude of benefits due to their rich content of vitamins, antioxidants, and other bioactive compounds. These natural components are well-known for

their potent photoprotective properties and capacity to protect and nourish the skin. They are appropriate for sensitive skin and lower the chance of irritation because they frequently include soothing and antibacterial properties. These components may also have antiaging and depigmenting effects (Binic et al., 2013). Additionally, plant-based ingredients can improve the hydration of the skin with natural moisturizing agents, elasticity, and young appearance by stimulating collagen production while fighting oxidative stress (Dini & Laneri, 2021).

This study explores a North African plant species, traditionally valued in traditional medicine, that has gained interest from the skincare perspective. It focuses more on its composition and natural compounds involved in possibly increasing the vitality and resilience of the skin. The study was done on some unique properties of this plant that make it relevant in addressing concerns in skin health, including hydration support, antioxidant benefits, and protection against environmental stressors. Results have shown compatibility with modern preferences for natural and effective ingredients, further establishing this plant as promising for developing new skincare products. Such research could deepen the present knowledge on plant-based skincare products and open a framework for new, nature-inspired formulations.

1.2. Objectives

General objectives

This proposed work aims to investigate the biological and cosmeceutical properties of *Anacyclus monanthos* subsp. *Cyrtolepidioides*, a plant endemic to the desert regions of Algeria.

This thesis will contribute to a better understanding of the biochemical properties of *Anacyclus monanthos* subsp. *cyrtolepidioides* and camel fat, as well as explore their potential as ingredients in the field of cosmeceutical products. The findings could have practical applications in the cosmetics industry by offering natural and effective alternatives for skin care.

The specific objectives are:

- Analyze the chemical composition of the plant *Anacyclus monanthos*, with a focus on bioactive compounds.
- Evaluate the antioxidant and other potential properties of this plant for skin health
- Examine the chemical composition of camel fat
- Assess the moisturizing, nourishing, and regenerative properties of camel fat for the skin.
- Develop and characterize a cosmetic cream incorporating *Anacyclus monanthos* and camel fat.
- Evaluate the combined effects of *Anacyclus monanthos* and camel fat on the skin.
- Conduct *in vitro* and *in vivo* experiments to assess the effects of plant and camel fat formulation stability
- Study the stability of this formulation under storage and usage conditions.

2. Literature Review

2.1. Cosmetic products

Globally, there are a few minor variations in the definitions of cosmetics (Kipgen et al., 2021). Such definitions are based on the product's functions, the portions of the body to which it is administered, the manner of application, the indication of use, claims, and consumer views. However, products are regulated and classified differently depending on the country (Ferreira et al., 2022). In Europe, according to (Regulation (EC) No 1223/2009) Cosmetic Products are defined as *"Any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours"*. Cosmetics may also be determined based on their product form and application area. Cosmetics can be liquids, cream emulsions, powders (pressed and loose), dispersions, and anhydrous creams or sticks (Mohiuddin, 2019). According to (Mitsui, 1997) Cosmetics can be classified into various classes, as illustrated in Table 1.

Table 1. Cosmetics classification (Mitsui, 1997).

Classification		Usage	Main products
Forskin	Skin care cosmetics	Cleansers	Face cleansing creams and foams
		Conditioners	Lotions, packs, and massage creams
		Protectors	Milky lotion, Moisture cream
	Makeup cosmetics	Base makeups	Foundations, Face powders
		Point makeups	Lipstick, Blushers, Eyeshadow, Eye liners
		Nail care	Nail enamels, Nail polish removers
	Body cosmetics	Bath	Soaps, Liquid cleansers, Bath preparations
		Sun cares and sun tans	Sunscreen creams, Sun oils
		Antiperspirants and deodorants	Deodorant sprays

		Bleaching, Depilatory	Bleaching creams, Depilatory creams
		Insect repellents	Insect repellent lotions and sprays
For hair and scalp	Hair care cosmetics	Cleansing	Shampoos
		Treatments	Rinses, Hair treatments
		Hair styling	Hair mousses, liquids and pomades
		Permanent waves	Permanent wave lotions
		Hair colors and bleaches	Hair colors, Hair bleaches, Color rinses
	Scalp care cosmetics	Hair growth promoters	Hair growth promoters, Hair tonics
		Treatments	Scalp treatments
For Oral	Oral care cosmetics	Toothpastes	Toothpastes
		Mouthwashes	Mouthwashes
	Fragrances	Fragrances	Perfumes, eau de cologne

Cosmetic care products are growing more popular among dermatologists. They are used to reverse aging, improve skin quality, increase facial structures, and enhance the patient's overall look. They can also help with certain dermatoses (Bennett & Henderson, 2003). Moreover, they are widely used around the world. An increasing number of chemical compounds are being added to the formulation of cosmetic products, such as additives, perfumes, preservatives, stabilizers, surfactants, dyes, and shine, to improve their quality, properties, and shelf life (Bilal et al., 2020). As the global demand for cosmetics rises, so does the number of chemical compounds employed in manufacturing. As a result, the danger of intoxication, allergic reactions, prolonged chemical exposure, adverse effects, and indiscriminate use increases (Pereira & Pereira, 2018). Thus, consumers' growing interest in natural products, and producers were looking for novel methods for natural cosmetic production (Hinčica et al., 2024).

2.2. Types of cosmetic formula

Among the most popular cosmetic formulas are emulsions, gels, lotions, creams, and ointments. To maximize application and product contact with the skin, topical vehicles are selected according to the type of skin, region to be applied in, as well as its size (Barnes et al., 2021).

2.2.1. Emulsions

Emulsions are stable mixtures of one immiscible liquid with another. While water-in-oil (w/o) emulsions have water droplets in oil, oil-in-water (o/w) emulsions contain oil droplets scattered in water (Sakamoto et al., 2017). Emulsions are colloidal systems that are used in a wide range of scientific and industrial fields. Emulsions stabilized by fine solid particles are known as solids-stabilized emulsions. Certain finely separated particles help produce the emulsion and/or increase its stability (Lopetinsky et al., 2006). An emulsion's composition has a significant impact on its physical characteristics, which in turn influence how the product behaves during production, packaging, and use (Dubuisson et al., 2018). The selection of the emulsion formulation ingredients determined the emulsion ionicity, type, and form according to the effectiveness, look, and viscosity of the ingredients used in the cosmetics. An emulsion experiences features such as separation, coagulation, sedimentation, change in viscosity, and loss of homogeneity as it loses stability. Although improper formulation is the main culprit, emulsions can also become unstable due to light and temperature (Iwata & Shimada, 2012).

2.2.2. Gels

A gel is a semisolid mixture of a liquid and a gelling agent, or at least two interpenetrating phases. Hydrogels are gels that contain water, whereas organogels are gels that contain an organic substance. In its broadest definition, hydrogels are composed of a matrix of water-soluble substances, such as natural gums and cellulose derivatives (Das et al., 2009). Because the gels are easier to apply and have better percutaneous absorption than other semisolid preparations, they are growing in popularity. Gels can take on the contour of the treated region and withstand the physiological stress brought on by skin flexion, blinking, and mucociliary movement (KP et al., 2010). Furthermore, it is widely recognized that gel formulations are made to effectively transport polar active chemicals while being less greasy, sticky, and easily washable (Krongrawa et al., 2018). Gel formulations can include semi-synthetic materials like

methyl cellulose, hydroxy ethyl cellulose, hydroxyl propyl methyl cellulose, carboxy methyl cellulose, and a synthetic polymer called carbopol, as well as inorganic substances like aluminum salts and organic polymers of natural or synthetic origins like natural gums, tragacanth, carrageen, pectin, agar, and alginic acid (Aruna et al., 2015).

2.2.3. Creams

Cosmetic creams are a particular category of skin care emulsions that take care of and protect the skin. Their raw materials are of vegetable origin, essentially. It is from these extracts, after chemical transformation, that one can obtain active principles, thickening agents, or emulsifiers. Cosmetic creams make active ingredients-water and oil-soluble-available to hydrate and smooth the skin with satisfaction. The hydro-lipid layer they form is preventive, improves skin texture, and protects against all sorts of influences from the outside. Their formulation affects even texture, absorption, and, finally, efficacy (Rähse, 2019).

A cream typically describes an emulsion that is highly viscous, needs a jar or tube, and does not flow easily with gravity. A lotion is the term typically used to describe an emulsion that has relatively low viscosity, flows with gravity, and can be pumped out or poured from a bottle. Cosmetic creams are composed of several key ingredients that generally carry out some useful functions. Among them, the category of emulsifiers includes lecithin and hydrogenated lecithin, which stabilize the emulsion and maintain the pH. They are also helpful in the delivery of actives into the skin without disrupting its barrier properties. One class of ingredients that includes emollients, silicones, natural oils, and hydrocarbons has a great impact on the cream's feel and application, balancing the feel and hence enhancing delivery. Actives principles include active sun screening agents, such as octinoxate; anti-acne agents, such as salicylic acid; and skin-whitening agents, such as hydroquinone. All these have specific therapeutic functions. Properties normally provided by the humectants, usually the glycols, are those attracting and retaining moisture, hence supplementing skin hydration and maintaining the stability of the emulsion. Its viscosity and stability control the application and performance of the cream, such as in acrylic-based polymers, predominantly carbomers (Palefsky, 2022). Creams are often thermodynamically unstable and consist of two-phase systems (oil in water or water in oil), with one spread in tiny droplets throughout the other (Djiobie Tchienou et al., 2018). Because they tend to be thicker, more occlusive, and consequently more effective, creams are preferred (Moldovan & Ciortea, 2010).

2.3. Cosmetics raw materials

Cosmetics contain key components for efficacy and safety, such as water, oils, preservatives, surfactants, and ingredients to enhance acceptability, including scents. Ingredients are mentioned on the product (or label) or container in descending order of concentration (MacFarlane, 2019).

2.3.1. Humectants

Humectants are water-soluble organic compounds, usually polyhydric alcohols (polyols) that absorb water. Glycerol is the most popular moisturizer, although other options include sorbitol, propylene glycerol, butylene glycol, urea, sodium lactate, and sodium pyrollidone carboxylic acid (PCA), which are created by the stratum corneum itself. The topical application of humectants is certainly effective in improving the symptoms of dry skin (Rawlings et al., 2002). Humectants may be one of the most essential ingredients in cosmetics. Humectants are hygroscopic chemicals that retain moisture in a product while in use and alter skin moisture levels. The ability of a humectant to attract water is determined by its chemical composition. The humectant's ability to moisturize the skin is also determined by its penetration qualities. Large polymers, such as hyaluronic acid and collagen, will remain on the outside, whereas smaller molecules, such as urea, glycerin, and lactic acid, are more likely to enter the tissue. Humectants have an impact on several additional stratum corneum features, including flexibility, desquamation, radiance, permeability, and microbial colonization (Lodén & Alander, 2022).

2.3.2. Oily materials

Cosmetic formulations rely on fats, oils, waxes, and esters for emollient, nourishing, solubilizing, consistency, and dispersing properties. Mineral or synthetic oils are commonly used due to their consistent composition, purity, refractive index, lack of color, slipperiness, and softness on the skin. Neutral mineral waxes are also used for consistency. These waxes include ceresin, ozokerite, and polyethylene (Archambault & Bonté, 2021). The choice of oily materials depends on the product's functional requirements, product attributes, and user options.

Oily components typically perform the following functions. emulsions benefit from their softening impact on waxy components, resulting in desired consistency, appearance, and temperature stability. They are very compatible with other fats and contribute to formula consistency. These include ceresin, ozokerite, and polyethylene waxes. Furthermore, oily

components serve as solvents for lipid-soluble compounds. They operate as a carrier for active chemicals to penetrate deeper layers of the skin, which is desirable (Kroke, 1978).

2.3.3. Surface active agents

A surfactant, also known as a surface-active agent, is a substance that can adsorb onto a system's surfaces or interfaces and significantly alter their free energies when present at low concentrations. The term interface refers to the border between any two immiscible phases, whereas the term surface refers to an interface where one phase is a gas, usually air (Rosen & Kunjappu, 2012). Surfactants are used in cosmetics to provide detergency, wetting, emulsifying, solubilizing, dispersion, and foaming properties (Rieger, 2017)**.** Surfactants are amphiphilic chemical substances with hydrophobic and hydrophilic moieties that lower surface tension and create emulsions between liquids of different polarity. Almost half of surfactants produced are for the washing and cleaning industries (Daniel et al., 1998). Long-term use of chemical surfactants can pose considerable environmental risks (Mann & Bidwell, 2001). Natural surfactants are those derived from natural sources (Holmberg, 2001). Natural surfactants are safer, odorless, colorless, and pure, making them ideal for usage as multipurpose cosmetic components with minimal environmental impact (Rieger, 2017). Surfactants in cosmetics are normally classified as ionic that is, anionic and cationic or non-ionic, which provide different functional properties. Mostly, anionic and non-ionic surfactants are being used because of their emulsifying, cleansing, and dispersing properties, hence adding high commercial value to products. A good number of materials like methylcellulose, sodium carboxymethyl cellulose, polyvinyl alcohol, polyvinylpyrrolidone, Gum arabic, and starch act as effective surfactants. Although cationic and amphoteric surfactants are also used, they are mainly valued for their bactericidal action and their ability to condition hair. (Matsumoto, 1973).

2.3.4. Polymers

Cosmetic products contain several types of polymers in their formulations, depending on their intended uses (Patil & Ferritto, 2013). Polymers serve various functions in cosmetic formulations, including rheological modification, emulsification, stimuli responsiveness, conditioning, film formation, fixation, foam stabilization, enhancement of skin feel, and antimicrobial activity (Lochhead, 2007). Cosmetic polymers are categorized into four types: synthetic polymers, polysaccharide-based polymers, proteins, and silicones (Ricapito et al., 2016). Synthetic polymers are appealing as an excipient for cosmetic formulations because they

can be customized for specific purposes. They are frequently less expensive than the other polymers, can be manufactured on a big scale, and have a long shelf life. The most typically found synthetic polymers in cosmetics include acrylic acid-based polymers, polyacrylamides, and alkylene oxide-based homopolymers and copolymers (Alves et al., 2020). Cosmetically, the formulation of polysaccharides involves their incorporation into other cosmetic ingredients like surfactants, salts, and other polymers. Generally, these polymers perform the following functions in personal care products: thickening, stabilization, skin cleansing, sun screening, and moisturizing. All polysaccharides are usually classified into five categories: anionic, cationic, non-ionic, amphoteric, and hydrophobic. Today, these products are attracting interest as active skin care agents in the prevention of inflammatory diseases and skin aging (Goddard & Gruber, 1999). Silicones have high thermal and chemical stability, bio-inertness, and low dependency on temperature in their physico-mechanical properties, which predetermines their use in cosmetic products. Besides that, the compatibility of silicones with components like vitamins, essential oils, and others expands their functionality in a number of applications where silicone derivatives act as antistatic, binding, and film-forming agents (Ivanova et al., 2023). Proteins polymers are very important in cosmetic formulation, relating to skin and hair restoration by acting in the place of lost proteins through aging and damage. More common examples include collagen, elastin, fibronectin, and keratin, besides proteins of milk and silk. Such advanced technologies, including microbial fermentation and genetic engineering, have made it possible to produce proteins to meet the increased demand for animal-free raw materials and ethically sourced cosmetics ingredients (Dai & Hansenne-Cervantes, 2024).

2.3.5. Antioxidants

Antioxidants are used to protect human skin from UV radiation (Kusumawati & Indrayanto, 2013), and they are used in cosmetics to reduce oxidative damage, making them an excellent choice for treating and preventing premature aging (Ratz-Lyko et al., 2012). The antioxidant components are also used to avoid oxidation of the oily content in the formulation and to prevent or decrease oxidative degradation of the cosmetic's active ingredients (de Lima Cherubim et al., 2020). Antioxidants, whether synthetic or natural, are employed in cosmetic products. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate, are commonly employed due to their low production costs (Hoang et al., 2021). Natural substances extracted from plants, such as polyphenol compounds, can serve as safe and effective natural antioxidants (He et al., 2021). Many

antioxidants are inherently unstable; therefore, sustaining efficacy during a product's shelf life necessitates careful optimization of their stability and concentration. To ensure consistent product performance and consumer benefit, formulations must be rigorously assessed and controlled for antioxidant activity and chemical stability (Kusumawati & Indrayanto, 2013).

2.3.6. Preservative

It is well acknowledged that some cosmetics formulations can support microbial growth, which may result in spoilage issues. The microbial activity could cause visible growth or chemical changes such as hydrolysis, oxidation, and reduction in cosmetics products that could manifest as off-odors, color changes, pH shifts, emulsion breakdown, and textural changes (Croshaw, 1977). The European Union Cosmetics Directive 76/768/EEC defines preservatives as "*substances which may be added to cosmetic products for the primary purpose of inhibiting the development of micro-organisms in such products*," which excludes antioxidants and light absorbent substances (Polati et al., 2007). Preservatives are an essential constituent in most cosmetic formulations to inhibit microbial growth and ensure product safety; considering the large number of formulations that contain water and organic materials at high percentages, this inhibition should be effective throughout a broad activity spectrum and last longer than the cosmetic product itself, corresponding to the estimated shelf-life plus the consumption time (Halla et al., 2018). A variety of preservative classes are available; the first classes include organic acids and salts, such as benzoic acid, effective under acidic conditions; alcohols and derivatives, including benzyl alcohol, with a mild antimicrobial activity; formaldehyde releasers, like imidazolidinyl urea, active against all types of microorganisms but have health considerations. Isothiazolinones and halogenated compounds, such as triclosan, provide broad-spectrum efficacy, while quaternary ammonium compounds, like benzalkonium chloride, provide both preservative and conditioning benefits in hair and skincare products. All types of preservatives selected are effective, have the necessary regulatory status, and serve formulation needs (Alvarez-Rivera et al., 2018).

2.4. Cosmetic products stability (physicochemical stability and organoleptic stability)

Stability testing in cosmetic formulation is necessary to ensure that a product's key attributes, such as appearance, texture, and fragrance, remain consistent over time. The process

will monitor any change in the critical characteristics and establish acceptable limits, detecting any possible problems at an early stage. Stability data act as a valuable early warning system, guiding formulation or packaging changes to maintain product quality and consumer satisfaction (Romanowski & Schueller, 2001). Stability tests consist of organoleptic characteristics (color, odor, and appearance), spreadability, pH, viscosity, and mechanical tests, among others (Fernandes et al., 2013). Microbial testing is also essential, as it ensures that cosmetic formulations remain free from harmful microorganisms throughout their shelf life.

Maintaining and protecting normal/physiological skin pH is a critical duty, external influences influence skin pH, and topically applied items must be considered as facilitators of this process. Cosmetics, due to their widespread use, may help to maintain skin health by regulating skin pH. Furthermore, in certain skin problems, the use of topical agents that can regulate skin pH should be part of the treatment plan, each topically applied product's pH and buffering ability should be carefully addressed. There is broad agreement that topical products should be acidic, with pH levels ranging from 4 to 6 (Lukić et al., 2021).

In cosmeceutical formulations, organoleptic agents such as colors and texturing agents are stable in the product and enhance consumer appeal. Together with active ingredients, they contribute to the integrity of the finished product, maintaining its quality. Stability testing for organoleptic properties is essential and should be conducted under conditions that reflect the physical and chemical characteristics of the cosmeceutical formulation, as well as anticipated changes during storage. These tests, which use validated analytical methods, assess critical attributes, including microbiological stability, preservative effectiveness, and the physical, chemical, and organoleptic stability of the product (Patil et al., 2018).This viscosity is one of the crucial physico-chemical characteristics of a product and plays an essential role in rheological studies since it exerts a direct impact on the evaluation of stabilizers usually employed to reduce or halt sedimentation or aggregation processes in dispersed liquid systems. The viscosity also affects product consistency, which depends upon adhesion and cohesion forces, among other factors. Thus, rheological studies offer important insights into maintaining the stability of the product and desired texture (Franzol et al., 2021).

Performance testing for mechanical stability of cosmetic formulations may be viewed as one of the many facets of performance testing of products under normal market conditions of storage and use. As such, realistic environments that give a view on formulation integrity and quality over time will be emulated. Other important characteristics of mechanical stability studies involve the determination of physical properties, in which various factors like

formulation components, processing conditions, and environmental changes could be changed to study their interaction with product stability. The formulator will, therefore, be able to make some specific formula adjustments in view of enhancing performance and shelf life (Cekić et al., 2023).

Cosmetic products can be contaminated by microorganisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, which can alter the product's properties and pose health risks. The Challenge Test, a widely used method in the cosmetic industry, evaluates the antimicrobial protection of products by simulating microbial contamination during their lifecycle. This test helps determine preservative efficacy, ensures regulatory compliance, and protects consumer safety. According to EC Regulation no. 1993/2009, cosmetic products must be safe for use, and preservatives used must be effective against a broad spectrum of microorganisms. The Challenge Test involves inoculating products with specific pathogens and monitoring microbial reduction over time, ensuring that products remain safe and stable throughout their shelf life (Giorgio et al., 2018).

2.5. Cosmetic product safety

Laws in major markets around the world require cosmetic and personal care products in commerce to be safe for use by the consumer. In order to be certain of safety, a complex process of risk assessment is utilized throughout the development and marketing cycle for cosmetic and personal care products. The common definition of risk is "the probability of an adverse effect occurring under defined exposure conditions." This approach would take into consideration the probability of exposure and the nature and severity of such hazards, with an assurance to safeguard the consumer and to ensure the safe use of cosmetic products (Engasser et al., 2007). Cosmetic products must be seriously studied to decide whether these products are safe or not. Successive assessment phases usually include preliminary toxicity and irritancy tests, skin reaction tests, and further studies under actual use conditions. This will help determine reliable data concerning the product's safety, efficacy, and performance. Manufacturers could make claims about a product, and simultaneously, it would mean the requirements of the regulation are complied with in view of a structured analysis of the risks, exposure conditions, and degree of risk severity. Structured analysis renews the commitment to consumer protection, which prolongs the confidence that cosmetic products placed on the market are safe (Edward & Norman, 1982). Cosmetic safety involves not only avoiding forbidden chemicals and high

concentrations of approved ingredients but also ensuring acceptable microbiological purity and stability. Consequently producers use preservatives often utilize synthetic preservatives such as parabens (Matwiejczuk et al., 2020).

2.6. Cosmetic product toxicity

Toxicology is a branch of science that studies the negative effects of chemicals on living organisms. Toxicity is one of the major problems in the field of cosmetic product safety, in which toxicological properties of each component have to be assessed by considering its chemical structure and the level of human exposure. Assessment includes conditions of exposure under different conditions relating to the target group and area of application for which the product is intended (Demir et al., 2019). Cosmetic ingredient toxicology thus involves many aspects in the pursuit of ensuring cosmetic product safety on skin irritation, eye irritation, acute toxicity, genotoxicity, and teratogenicity, each describing a different type of possible harm. Skin and eye irritation tests, on the other hand, classify an ingredient based on its ability to directly destroy tissues at the point of contact, whereas acute toxicity tests quantify direct toxic effects upon exposure. Testing for genotoxicity finds out whether there is capability to cause DNA damage which could result in a mutation, while teratogenicity testing looks at the potential to cause birth defects. Other long-term effects that may be included are skin sensitization, carcinogenicity, and sub-acute or sub-chronic toxicity, which can show their potential cumulative or delayed toxic effects with long-term use (Luco et al., 2019).

2.7. Cosmeceutical properties of natural products

Natural components have been used in cosmetics for ages, and they are becoming more common in modern formulas (Thiyagarasaiyar et al., 2020). The term "natural" refers to a substance that is obtained directly from plants or animal products and is produced or found in nature (Veeresham, 2012). The natural compounds might come from herbs, fruits, flowers, leaves, minerals, or soil. The efficacy of natural substances in skincare products depends on their *in vitro* and *in vivo* efficacy, as well as the type of formulation base into which they are mixed. Plants have long been utilized medicinally; before the use of synthetic chemicals with similar qualities, plants were the primary source of all cosmetics (Baumann et al., 2009).

Natural product-based ingredients have drawn much interest from consumers and researchers alike because of the widespread perception that they are safe (Alves et al., 2020). According to European cosmetic standards, natural components in cosmetics refer to their origin and production process (Bruusgaard-Mouritsen et al., 2020). The recent years have shown that the cosmeceutical industry can have a place for innovative applications of natural ingredients in anti-inflammatory properties of skin conditions, anti-acne effects, antioxidant activity for anti-aging, and maybe even for cancerous skin lesions (Juhász et al., 2018).

Botanical extract is one of the most natural compounds used in cosmetics, valued for its powerful cosmeceutical properties, such as antioxidant activity, anti-aging effects, and antimicrobial action. Study by Thring et al. (2009) demonstrates that several plant extracts exhibit strong anti-elastase, anti-collagenase, and antioxidant activities due to their high phenolic content. Another study by Herman et al. (2013) reveals that certain plant extracts and essential oils possess potent antimicrobial activity, indicating their potential as natural alternatives in preservative-free or self-preserving cosmetics. Further work by (Ribeiro et al., 2015) explores the effects of certain plants as tyrosinase inhibitors, highlighting their potential to reduce pigmentation and manage hyperpigmentation disorders.

The Asteraceae family is one of the biggest plant families and offers numerous species recognized for their beneficial bioactive compounds. Various botanical extracts from this family are utilized in cosmetics. For instance, *Achillea millefolium L.* and *Arnica montana L.* are used toward skin-soothing and anti-inflammatory purposes, and other species are used for antioxidant and anti-wrinkle effects. These plants have been incorporated into different commercial products, substantiating their efficacy and safety in cosmetic use (Dorni et al., 2017). The plant used in our study is *Anacyclus monanthos* subsp. *Cyrtolepidioides* which belongs to the *Asteraceae* family

2.8. *Anacyclus monanthos* subsp. *cyrtolepidioides*

2.8.1. Overview

Anacyclus monanthos subsp. *cyrtolepidioides*, commonly called “Tafsa”, a name given by the Algerian dialect, is a flowering plant belonging to the *Asteraceae* family, characterized by the presence of compound capitula. This subspecies is particularly remarkable for its particular adaptations and morphology which distinguish it from other species of the genus *Anacyclus*. Occurring in particular ecological niches, it proliferates in varied environments, demonstrating its resilience and adaptative capability. Native to the desert areas of North Africa, it is found in Algeria, especially in Bechar, Bayed, and El M'sila, but also in Tunisia, Morocco, and Libya (Sarri et al., 2018). It grows under extreme desert conditions, being resistant under lack of water, reaching its proper development under arid conditions, and spontaneously propagating itself without human intervention. The species grows on semi-arid soils, starts germinating in spring, and is harvested in summer. Interest in this subject has not only been for its special morphological features but also for its possible applications in therapy and ecology, which are very important focuses of investigations within both botany and phytochemistry.

Anacyclus monanthos subsp. *cyrtolepidioides* has been used in traditional medicine because of its therapeutic values. In the treatment of different diseases, it is widely used for stomach pain, earaches, and sexual dysfunction, among others, in its medicinal benefits. The preparation mode normally involves boiling the plant in water to make an infusion to be taken as a drink. This traditional use illustrates the importance of this plant to the local healing practices of the regions where the plant is found.

2.8.2. Taxonomic Classification

Anacyclus monanthos subsp. *cyrtolepidioides* belongs to the category of flowering plants, respectively belonging to Kingdom Plantae and Phylum *Streptophyta*. Under the Subclass *Magnoliidae*, it belongs to Class *Equisetopsida*. This specific species is pertaining to Order Asterales and linked with the family *Asteraceae*, or commonly known as special composite flower heads. The genus *Anacyclus* holds plants utilized for medicine purposes, among which is the highly featured *Anacyclus monanthos* and its subspecies *cyrtolepidioides* with remarkable characteristics (*Plants of the World Online | Kew Science*).

2.8.3. Morphological features

Anacyclus monanthos subsp. *cyrtolepidioides* is a pubescent annual plant, sometimes with ligules. It generally has central florets with 2 erect teeth and 3 spreading teeth, and outer achenes with a marked crown. The capitula are homogamous discoids with grayish leaves and flexible stems. Leaves are 1–2 pinnatipartite with elongated, linear segments. Flowers group in capitula on short terminal peduncles, all tubular and 5-toothed (Fodil et al., 2019). This plant exhibits a small, herbaceous perennial habit with finely divided, feathery pinnate leaves. The solitary flowering heads consist of both ray and disc florets typical of the *Asteraceae* family; ray florets are usually white or light-colored, encircling yellow disc florets (Figure 1 shows a dried specimen of the plant). These characteristics enhance its aesthetic appeal and are vital for its reproduction and survival in native habitats.

Figure 1. *Anacyclus monanthos* subsp. *cyrtolepidioides* collected from Bechar, Algeria, in July 2023

2.9. Camel fat

The two domesticated species that are considered "*camelids*" are the two-humped, shorter-legged camel (*Camelus bactrianus*) and the one-humped camel (*Camelus dromedarius*). Central Asia is home to the two-humped camel, whereas the hot, dry regions of the Middle East, Africa, and Eastern Asia are home to the one-humped camel. In the Arabian Desert, the domesticated one-humped camel, *Camelus dromedarius* is one of the most prevalent, accounting for 90% of the species Camelus (Jassim et al., 2018). Since camels (*Camelus dromedarius*) can tolerate high temperatures and conditions of food scarcity, they are one of the most significant providers of meat and fat in the desert. They have been utilized as treatments for a variety of illnesses since ancient times and serve as a mode of transportation (Benyagoub & Mammeri, 2023). Animal fat plays an important role in human nutrition. Insufficient fat intake may cause many physiological disorders (Fleming, 2002), camel fat is a very important health enhancer and implementer, obtained particularly from a camel's hump. Rich in saturated fatty acids such as palmitic and stearic acid, it is used in skin-related treatments, amongst other areas of health concerns. Its uniqueness constitutes skin protection against UV radiation, healing burns, and the treatment of skin conditions like eczema and age spots. The blood flow improves because camel fat is anti-inflammatory in nature; it helps the skin regain its natural appearance. Because of this, cosmetic and topical applications have made it good for skin tightening, anti-aging, and as a natural moisturizer (JASSIM et al., 2020), it contains all three types of fatty acids, namely, omega-3, omega-6, and omega-9, in sizeable proportion; these are well-known anti-inflammatory factors and are promoters of good cardiac health. In addition to a rich lipid profile, camel fat is a very good source of Vitamin E; the antioxidant guards the cells against damages caused by oxidation and maintains skin health. Such high concentrations of these useful lipids make camel fat an important constituent in both traditional and modern dietaries (Jassim et al., 2018).

3. Material and method

3.1. Sampling

3.1.1. *Anacyclus monanthos* subsp. *Cyrtolepidioides*

Anacyclus monanthos subsp. *cyrtolepidioides* plant was collected from the region of Bechar, south Algeria region, from different cultivars in July 2023; upon the collection, the plant aerial parts was dried in the sun for between 7 to 14 days, and the result whole was stored in normal condition (temperature between 30°C to 40°C, low humidity below 60%, with a protection from direct sunlight and strong artificial light, adequate airflow to avoid moisture buildup, and in airtight containers to safeguard against moisture, air, and light), and sent from Algeria to Instituto Politécnico de Bragança – IPB, Portugal where lyophilization was performed before to further analysis(see Figure 2 for the dried plant upon arrival from Algeria).

Figure 2. Dried *Anacyclus monanthos* subsp. *cyrtolepidioides*.

3.1.2. Essential oil extraction

The Essential oils were extracted from plant aerial parts. The hydro-distillation process was used with Clevenger Apparatus equipment for extraction, as shown in Figure 3 (Wany et al., 2014), 70 g of Dried plant was placed in 800 ml of ultra-pure water. The hydro-distillation lasted 3 hours, and the resulting oil was stored in the freezer at -20°C in the dark until the analysis.

Figure 3. Essential oil extraction by Clevenger apparatusus.

3.1.3. Hydroalcoholic extraction

A hydroalcoholic extraction was conducted using hydroalcoholic mixtures of ethanol 80:20 (v/v), 5g of the plant samples were agitated at 25 °C and 150 rpm for 30 minutes (Figure 4.a), and this procedure was carried out three times (Nicuță et al., 2024). Subsequently, 300 ml of the extract was collected, filtered using Whatman paper, and transferred into glass flasks (Figure 4.b). The extract was then stored at 4 °C in a refrigerator until further use.

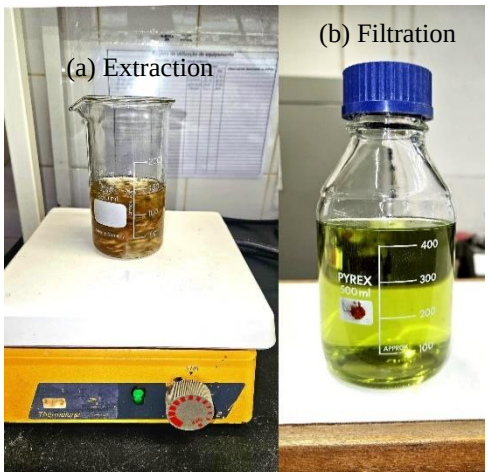

Figure 4. Hydroalcoholic extraction of *Anacyclus monanthos* subsp. *cyrtolepidioide*s. (a) The extraction process, (b) After the Filtration process.

3.1.4. Camel fat

The camel fat was carefully collected from El Bayed region in Algeria, assuring cleanliness and limiting contamination at all stages. The fat samples were subsequently placed in plastic bags to retain purity and then kept in a refrigerator set at 4°C to keep the temperature steady. This meticulous storage was important to maintaining the sample intact until it was ready to send. The samples were shipped in May. However, throughout transit, despite ambient temperatures ranging from 24°C in Algeria to 22°C in Portugal, the shipping business failed to maintain sufficient refrigeration, damaging the quality of the camel fat. This omission occurred during transit and profoundly impacted the preservation efforts. Although contamination was not visible to the naked eye, it was later detected through microbial testing of the cream formulation (see Figure 5 for an image of the camel fat after its arrival).

Figure 5. Camel fat after the arrival from Algeria.

3.2. Chemical analysis

3.2.1. Essential oil by Gas Chromatography and Gas Chromatography-mass spectrometry (GC and GC-MS)

The GC-MS unit consisted of a Perkin. Elmer Autosystem XL (Perkin Elmer, Shelton, Connecticut, USA) gas chromatograph, equipped with DB-1 fused-silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm) (J & W Scientific, Inc.), and interfaced with a Perkin-Elmer Turbomass mass spectrometer (software version 4.1, Perkin Elmer, Shelton, Connecticut,

USA). Injector and oven temperatures were as above; transfer line temperature, 280°C; ion trap temperature, 220°C; carrier gas, helium, adjusted to a linear velocity of 30 cm/s; split ratio, 1:40; ionization energy, 70 eV; ionization current, 60 μA; scan range, 40-300 amu; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C9-C17 n-alkane indices and GC-MS spectra from a homemade library, constructed based on the analyses of reference oils, laboratory-synthesized components and commercially available standards. The volume of injection was 0.2 μl of a pentane-oil solution. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from each essential oil, without using correction factors.

3.2.2. Total phenolic compounds determination

3.2.2.1. Standards and blank solution

To determine the phenolic compounds presented in hydroalcoholic extracts we followed the protocol described by Singleton et al. (1999). The total extractable phenolic compounds were calculated using the Folin–Ciocalteu reaction technique using the extract obtained from the hydroalcoholic extraction (0.5 ml of sample was added to an assay tube with an initial concentration of 2.996 mg/mL, then 2 mL of 75% sodium carbonate was added, followed by 2.5 mL of 10 % Folin-Ciocalteu reagent) in triplicate. The resulting solution was left to stand for 2 hours in the dark at room temperature, and then the absorbance was measured at 760 nm. The blank was also performed in the same manner without a sample.

Gallic acid was used as the reference standard to establish the calibration curve for total phenolic analysis. The stock solution was prepared by dissolving 0.1 g of gallic acid in 100 ml of deionized water. Various concentrations were prepared using measured volumes of 0.1, 0.25, 0.5, 0.6, 0.75, 1, 1.5, and 4 ml of the stock solution in 25 ml volumetric flasks, which were then topped up with deionized water. The blank was prepared by adding 0.5 ml of deionized water to the reagents (2.5 ml of Folin-Ciocalteu and 2 ml of sodium carbonate). After 2 hours in the dark, the absorbance was measured at 760 nm.

3.2.2.2. Determination of method performance

The calibration curve was determined, as shown in Figure 6, with seven concentration levels of standards of gallic acid ranging from 0.004 to 0.006 mg/mL. The curve equation was y = 10.218 x – 0.01 and the R^2 = 0.9999. The concentration was calculated as mg/mL of gallic acid, considering the dilution factors.

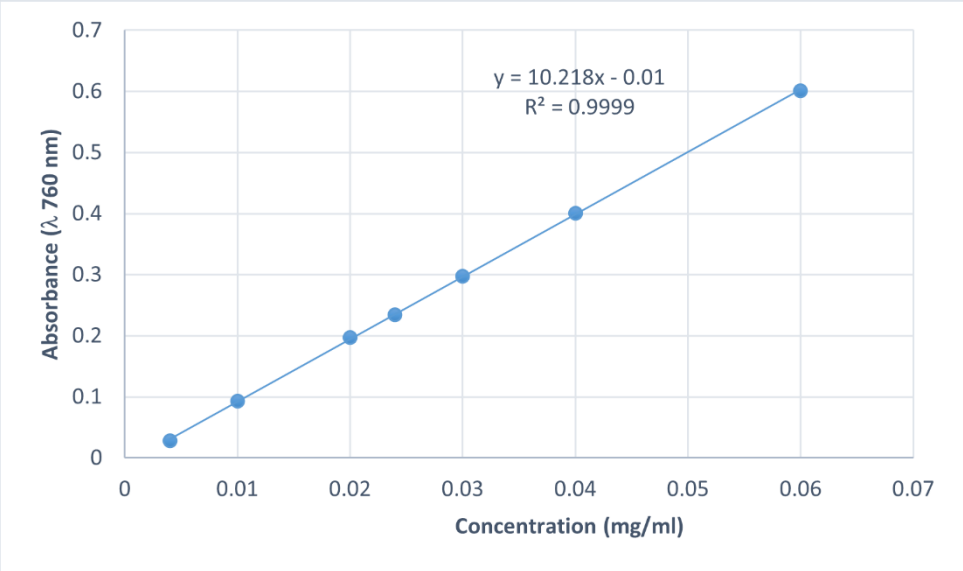

Figure 6. Gallic acid calibration curve determination.

3.2.3. Total flavonoids determination

3.2.3.1. Solution preparation

The total flavonoids were estimated following the method described by Woisky & Salatino, (1998), the experimental procedure commenced with adding 2.5 mL of plant hydroalcoholic extract (HAE) with 2.996 mg/mL as the starting concentration into assay tubes in triplication. Subsequently, 2.5 mL of AlCl3 (2%) ethanol solution was added to each tube. The samples were then incubated in the dark at room temperature for 1 hour. Following incubation, the absorbance of the samples was measured using a spectrophotometer at a wavelength of 420 nm.

3.2.3.2. Determination of method performance

The calibration curve was determined, as shown in Figure 7, with six concentration levels of standards of Quercetin ranging from 0.001 to 0.002 mg/mL. The curve equation was

y = 22.444 x + 0.0014 and the R^2 = 0.9989. The concentration was calculated as mg/mL of Quercetin, considering the dilution.

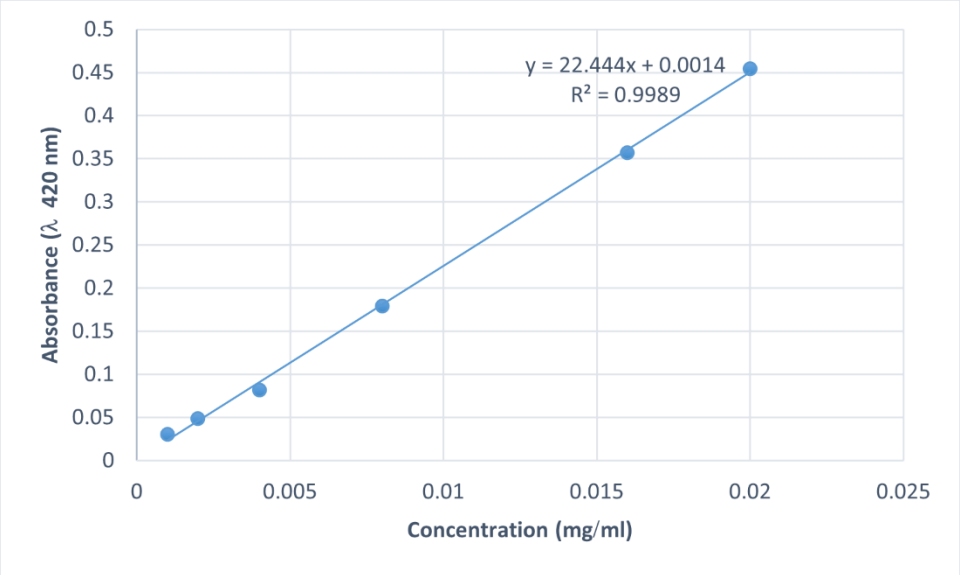

Figure 7. Calibration curve of Quercetin with different concentrations.

3.2.4. Fat content determination

3.2.4.1. Plant fat content determination

Figure 8 presented the Soxhlet extraction method, in which 5g of the crushed plant sample was accurately weighed into a mortar. Subsequently, 250 mL round-bottom flasks, pre-dried in an incubator at 105°C for 1 hour, were placed in a desiccator to cool and then weighed and market each flask. A filter paper cartridge was constructed, and the plant samples were transferred into it. The cartridge was then placed in a Soxhlet extractor at 50°C, and 175 ml of hexane was added. After 12 hours, the cartridge was extracted, and the solvent was recovered. The flasks were placed into a desiccator until they were cold and weighed again.

The crude fat percentage was determined using equation (1) referred to Latimer, (2023).

$$\textbf{crude fat}\% = \frac{\textbf{W2}-\textbf{W1}}{\textbf{S}} \times \textbf{100} \quad \textbf{(1)}$$

When W2= Weight of the flask and extracted fat (g), W1= Weight of empty flask (g), S= Weight of the sample(g).

Figure 8. Soxhlet method equipment.

3.2.4.2. Plant fatty acids profile

After Soxhlet extraction of plant material, fatty acids were converted into fatty acid methyl esters (FAME) for further analysis using gas chromatography (Figure 9). For this purpose, 2 mL of heptane was added to the round-bottom flask containing crude fat extract, followed by the addition of 200 µL of 2 M methanolic KOH solution. The flask was tightly closed and vigorously shaken for 30 seconds. The mixture was allowed to rest until the upper phase became clear, indicating phase separation. An appropriate amount of anhydrous sodium sulfate was added to the solution to absorb residual water. The clear upper phase was transferred to a syringe fitted with a nylon membrane filter. Finally, the solution filtered through was collected in a vial and stored in a refrigerator until gas chromatography analysis.

Figure 9. FAME procedure of *Anacyclus monanthos* subsp. *cytrolipiodioide*.

3.2.4.3. Camel fat determination

To determine camel fat, 0.1 g of sample was introduced into a test tube, and the mass was weighed. After that, 2 mL of n-Hexane was added by a graduated pipette, and 0.3 mL of KOH in methanol solution was added with a micropipette. The mixture was vortexed for 3 min and left over 45 min. To absorb the water, 2 spatulas of anhydrous sodium sulfate were added, and the mixture was vortexed for an additional minute. The n-Hexane phase was transferred into a syringe fitted with a nylon filter and filtering the solution into a vial. The group number was labeled on the vial, which was then sealed tightly to avoid evaporating the fatty acids and refrigerated or frozen until ready for gas chromatography analysis (this method was adapted from (Shingfield et al., 2006)).

3.2.4.4. Fatty acids determination

Gas chromatographic analyses were performed using a Dani GC 1000 gas chromatograph equipped with one flame ionization detector (FID), a data handling system, and a vaporizing injector port into which one column: a Zebra ZB-FAME column (30 m x 0.25 mm ID., film thickness 0.20 μm) (Phenomenex). The oven temperature was programmed as follows: 100°C initial temperature, with a hold time of 2 min, then with a heating rate of 10°C/min until 140°C, followed by a heating rate of 3°C/min until 190°C, and finally a heating rate of 30°C/min until 260°C with a hold time of 2 min. The injector and detector temperatures were set at 250°C and 260°C, respectively. The carrier gas, hydrogen, was adjusted to a flow of 1.1 mL/min. For the detector, the pressures were set at 0.6 bar for the make-up gas, 0.91 bar for air, and 0.7 bar for hydrogen. The samples were injected using a split sampling technique with a ratio of 1:50. The volume of injection was 1 μl of a heptane-oil solution for the plant sample and 1 μl of n-hexane-oil solution for the camel fat samples. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of six repetitions for each plant extract and three repetitions for the camel fat samples.

3.2.5. Protein content determination

To determine the global protein presented in our plants, especially vegetables, as a nutritional analysis we followed Kjeldahl method described by (Kambhampati et al., 2019). Which is a method that effectively converts nitrogen in proteins to ammonium sulfate through digestion with concentrated sulfuric acid, hence enabling the quantification of protein content using nitrogen-to-protein conversion factors. It is formalized into a standard (AOAC 978.04) by (Latimer, 2023), which describes the protocols to be followed for valid protein analysis. For the procedure, 1 g of minced and homogenized plant material was placed in each test tube, followed by adding two catalyst tablets (catalyst with 0.1% Se) with 13 mL of concentrated sulfuric acid (H_2SO_4) at a 96% concentration. Gently, the tubes were shaken to mix their contents and then were put into the digester at heat for 60 minutes at 420°C. The digestion tubes, after cooling, were transferred to the Kjeldahl apparatus, where distillation for 4 minutes at 100% steam power was carried out by using 75 mL of distilled water, 30 mL of boric acid (H_3BO_3), and 50 mL of sodium hydroxide (NaOH) (Figure 10). After distillation, the titration step was performed by adding the standard solution of HCl for the titration of ammonia obtained. In addition, 10 drops of Tashiro's indicator were added to the distillate tubes to follow the course of the reaction. The HCl in the burette was added slowly, and the tubes were swirled gently to ensure that mixing occurred. Titration was continued until a distinct color change marked the endpoint of the titration (Figure 11), showing that all ammonia had reacted with the acid.

Figure 10. Kjeldahl digestion and distillation equipment.

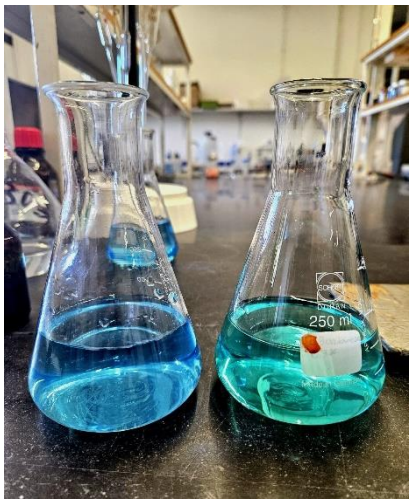

Figure 11. The titration step of the Kjeldahl method.

Once the titration was completed, the volume of HCl consumed at the endpoint was used to determine the amount of nitrogen present in the sample. The moles of HCl added are equivalent to the moles of ammonia NH_3 that were distilled and trapped since each mole of NH_3 reacts with one mole of HCl. The amount of nitrogen in the sample is determined using the molarity of the HCl solution and the measured volume. This value is then used to determine the percentage of nitrogen by mass, which can be further applied to determine the protein content, if necessary, based on a known conversion factor specific to the type of sample(Aguirre, 2023). The percentage of proteins can be calculated with the following equations (2), (3):

$$\textbf{Nitrogen } \% = \frac{\mathbf{V} \times [\mathbf{HCL}] \times \mathbf{m_{a(N)}}}{\mathbf{m}} \times \mathbf{100} \quad \textbf{(2)}$$

Where V= volume of HCL used in titration endpoint, m_a= the atomic mass of Nitrogen, m= sample weights in gram

$$\% \textbf{ of proteins} = \% \textbf{ of Nitrogen } \times \textbf{correction factor } \textbf{(3)}$$

3.2.6. Mineral assay

Boron is an essential element for plants, and its deficiency affects plant growth and yield (Endo et al., 2013). To determine boron content in plant tissues, 1 gram of dried and ground plant material is weighed into a porcelain crucible, which 0.2 grams of finely ground calcium

oxide is added. The mixture is mixed well until homogeneous before being heated on a preheated hot plate at 200°C until completely charred. The charred sample is cooled and transferred to a muffle furnace (Thermolune 6000, Furnance), where it is heated to 500°C for 90 minutes. After cooling, 10 ml of 0.5 M sulfuric acid is added to the sample. Subsequently, 1 ml of tampon solution and 2 ml of azomethine-H are added to the mixture. The mixture is then allowed to rest for 30 minutes with occasional stirring. After resting, the product is filtered into polyethylene tubes and the quantification of boron by means of the Azomethine-H method is carried out (Sah & Brown, 1997). The absorbance of the sample was measured using a spectrophotometer (Thermo Spectronic, Genesys 6) at 420 nm.

For other minerals determination, such as azote (N), potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu), the protocol outlined by Brandão et al. (2023).

3.3. Bioactivity assays

3.3.1. Antioxidant activity

3.3.1.1. DPPH

The antioxidant activity was analysed through free radical scavenging activity or 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method described by Hatano et al. (1988) with modifications. A mother solution of DPPH (6×10^{-5} M) was prepared. Dilutions (C1 to C12) of the hydroethanolic extract of *Anacyclus monanthos* subsp. *Cyrtolepidioides* were prepared, and a total volume of 30 µL of each of the different concentrations of extract and ethanol was mixed with 270 µL of ethanolic DPPH solution, homogenized, and placed in the dark for 1 hour. Half maximal inhibition concentration (IC50) was then calculated after measuring the absorbance at 517 nm using a microplate reader spectrophotometer (Thermo Scientific, Multiskan GO). (Figure 12 represent DDPH microplate assay after 1 hour in the dark)

Equation (4) was used to calculate the DPPH inhibition percentage. The results were expressed as IC50, which is the extract concentration required to inhibit DPPH by 50%.

$$\% \textbf{ of inhibition} = \frac{\mathbf{A_{DPPH} - A_A}}{\mathbf{A_{DPPH}}} * \mathbf{100} \quad (4)$$

Equation 4 - Percentage of DPPH inhibition (A_{DPPH} corresponds to the absorbance of the control and A_A to the absorbance of the sample)

Figure 12. DDPH microplate assay.

3.3.1.2. Reducing power

To evaluate the reducing power of the plant *Anacyclus monanthos* subsp. *cyrtolepidioides*, a series of dilutions (C1 to C12) was made in a microplate, starting with a concentration of 25.97 mg/mL (performed in triplicate), as shown in Figure 5. In the first well of the microplate, 100 µL of plant hydroalcoholic (HAE) was added, followed by serial dilutions of 50 µL to well C12. Then, 50 µL of sodium phosphate buffer solution (pH 6.6; 200 mmol/L) and 50 µL of (1% w/v) potassium ferricyanide were added to each well. The mixtures were homogenized, and the microplate was incubated at 50°C for 20 min. Then, it was added 50 µL of 10% (w/v) trichloroacetic acid and 16 µL of 0.1% iron chloride. Finally, the absorbance was measured at 690 nm using a microplate reader (Thermo Scientific, Multiskan GO). The blank was prepared through the same procedure and reagents, except that the plant HAE was omitted. (Figure 13 represents reducing power evaluation

Eventually, the concentration of the extract solution corresponding to 0.5 of reducing power (IC50) was calculated by interpolation from the absorbance graph as a function of the concentration of the plant HAE extract.

Figure 13. Reducing power evaluation.

3.3.2. Toxicity assay using brine shrimp (*Artemia salina* L.)

Sorgeloos et al. (1986) described the detailed development of *Artemia salina L.* eggs in artificial seawater conditions in an Erlenmeyer (Table 2). Brine shrimp eggs, incubated for 48 hours under room temperature conditions of 25-30°C, are transferred in artificial seawater in an Erlenmeyer, and aeration and lighted conditions are applied. The freshly hatched larvae (*Nauplius*) are phototropic to one side of the Erlenmeyer and withdrawn using a pipette.

Table 2. Artificial seawater medium composition used in *Artemia salina L.* hatching.

Medium composition	Amount (g/L)
NaCl	3.0
$MgSO_4$	1.3
$MgCl_2$	1.0
$CaCl_2$	0.3
KCl	0.2
$NaHCO_3$	2.0

Bioactivity of *Anacyclus monanthos* subsp. *cyrtolepidioides* of two samples were assayed in terms of the hydroalcoholic extract (HAE) and the essential oil (EO). Regarding the former mentioned by Sorgeloos et al. (1986), a direct exposure of successive concentrations of the extract was done to *A.salina*. As far as the EO was concerned, it was first necessary to select

a solvent that would ensure that the oil was adequately mixed within the aqueous medium that included the *nauplii*. The concentration of the solvent was tested accurately, so its toxicity subtrated of the final value, that mean that only the toxicity of the oil was determined. A series of concentrations of HAE (0.15%, 0.075%, 0.035%, 0.009%) and EO (0.1%, 0,03%, 0.015%, 0.009%, 0.008%) were prepared and the final volume was adjusted with artificial seawater to 20 mL. Thereafter, each of the prepared concentrations was filled up to 1.5 mL in six wells of a microplate, and twenty *nauplii* were carefully picked, transferred, and released into each of the six sample wells assigned to it, as shown in Figure 14. After an hour, we counted the number of *nauplii* placed in each well to verify our count. Counting of both living and dead *nauplius* in each well was done and cross-checked during the observation of the microplates under magnification glass after 24 hours. If the *nauplii* did not show any external movement after several seconds of observation, they were considered dead (between counts, the plate must be in a dark environment with a temperature equal to 25ºC).

Experiments were conducted with two controls: the negative control, which was the artificial seawater with tween 80 (1.5% v/v), and the positive control, which was potassium dichromate ($K_2Cr_2O_7$) with a series of dilutions (10%, 5.6%, 3.2%, 1.8%, 1%) in 6 wells for each concentration with a total *Artemia* initial number of 20 *nauplii*.

Previous research by Naidu et al., (2014) offered the following equation (5) for calculating the percentage of mortality (M%)

$$\mathbf{M\%} = (\mathbf{Survival\ naupulis\%\ in\ the\ Blank\ control}) - (\mathbf{Survival\ naupulis\%\ in\ the\ samples)\ (5)}$$

Where M%= the percentage of mortality.

The concentration that resulted in 50% lethality to the *nauplii*, referred to as LC50, was accurately determined from the best-fit line derived through linear regression analysis of the relationship between the percentage of mortality and the concentration levels.

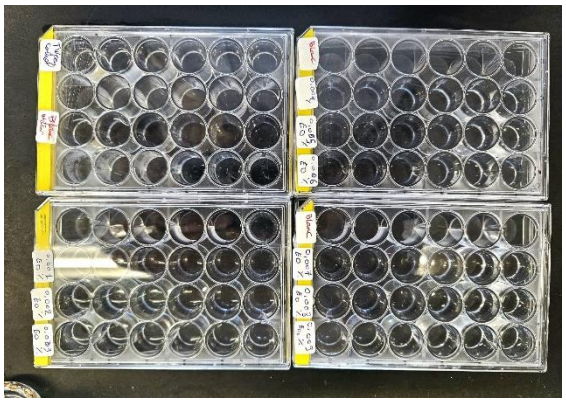

Figure 14. *Artemia salina L.* cytotoxicity assay microplate.

3.4. Sun protection factor

The sun protection factor of *Anacyclus monanthos* subsp. *cyrtolepidioides* HAE was determined to investigate his ability to protect against UV damage (SPF) using the method developed by Mansur et al. (1986). First, the sample was diluted to a concentration of 2000 ppm in absolute methanol. The absorbance was then measured at seven wavelengths, each with a range of 5 nm, ranging from 290 to 320 nm.

The SPF values were calculated using the equation (6), and each measurement was done in triplicate

$$\mathbf{SPF_{spectrophotometric}} = \mathbf{CF} \times \sum_{\mathbf{290}}^{\mathbf{320}} \{\mathbf{EE}(\boldsymbol{\lambda}) \times \mathbf{I}(\boldsymbol{\lambda}) \times \mathbf{Abs}(\boldsymbol{\lambda})\} \quad \mathbf{(6)}$$

Where CF is Correction Factor (10), EE (λ) = Erythrogenic Effect of radiation, I (λ) = Solar Intensity spectrum, Abs (λ) = Spectrophotometric absorbance value, and EE x I values are constant

3.5. Cosmetic formulation preparation and stability

3.5.1. Preparation of cream formulation

The following procedure describes the preparation of a moisturizing cream formulation. Accordingly, various ingredients will be selected and combined appropriately to result in a stable and effective product. Each ingredient is measured and mixed under controlled

conditions to assure reproducibility and quality. The preparation procedure comprises heating, mixing, and cooling stages with specific techniques to enhance the stability and efficacy of the cream. The following Table 3 provides the ingredients used, their respective amounts, and their roles in the formulation.

Table 3. Cream ingredients and % in 100 g of formula.

Ingredients	Function	Percentage (in 100 g)
Glycerin	Humectant	4 %
Lanette wax	Emulsifier	8 %
Cetyl alcohol	Emollient	5%
Camel fat	Emollient	10%
***Anacyclus monanthos* subsp. *cyrtolepidioides* EO**	Preservative agent	0.4%
***Anacyclus monanthos* subsp. *cyrtolepidioides* HAE**	Antioxidant agent	1 %
Distilled water	Diluent	71.6%

To prepare 100 grams of moisturizer cream, we started by mixing the aqueous phase (distilled water and glycerin) in one porcelain crucible. In the other crucible, we put camel fat (10%), Lanette wax (8%), and cetyl alcohol (5%). Both crucibles were placed into the water bath at 65°C, with stirring from time to time, until complete melting and mixing of the ingredients in each of the crucibles occurred. Then, we measured the temperature of the contents in both crucibles to ensure both had attained the same temperature. At this point when both phases had acquired the same temperature, we mixed the two phases vigorously by hand. While mixing, *Anacyclus monanthos* subsp. *cyrtolepidioides* essential oil (0.4%) and HAE (1%) were added, and mixing was continued until a smooth cream texture was attained. To obtain an even softer texture, a blender was used. Finally, the cream was cooled at 4°C and then stored in a sterilized glass jar and placed inside a refrigerator.

3.5.2. Stability essays of the cream formulation

The stability of the formulation was assessed by conducting a series of complete tests, which included the evaluation of physical, chemical, and microbiological features over a certain period. Tests for the full scrutiny of appearance and some physicochemical tests such as centrifugation, mechanical stress, and light exposure. Key parameters like pH, density, and viscosity were measured to check consistency and reliability. Advanced techniques, such as spectrophotometric analysis and accelerated stability testing studies, were conducted to predict the long-term behavior of the formulation. The microbial stability tests were conducted in a very stringent manner to ensure safety and efficacy during the shelf life of the product.

3.5.2.1. Texture and consistency

The evaluation focused on the texture and consistency that are so crucial in a cream formulation for its application and user experience. Assessment of sensory evaluation was conducted on the cream's feel, spreadability, and absorption on the skin (organoleptic performance). The texture analysis that will ensure product conformity and quality, firmness, spreadability, and adhesiveness are some of the key parameters assessed (Kulawik-Pióro et al., 2019). To evaluate the cream's texture, look, and penetration into the skin, it was applied to the palm of the hand and rubbed in circular motions with the fingers.

3.5.2.2. Centrifugation test

The centrifugation test evaluates physical stability through the action of high-speed rotation that greatly accelerates the separation of phases. It helps in predicting long-term stability of the formulation under normal storage conditions (Navarro-Pérez et al., 2021). For this, we took a measured amount of 1 g of the sample formulation into centrifuge tubes (Figure 15) and centrifuged them at 3000 rpm for 30 min with a centrifuge (mini spin Eppendorf). After centrifugation, we visually inspected the samples for any signs of phase separation or sedimentation.

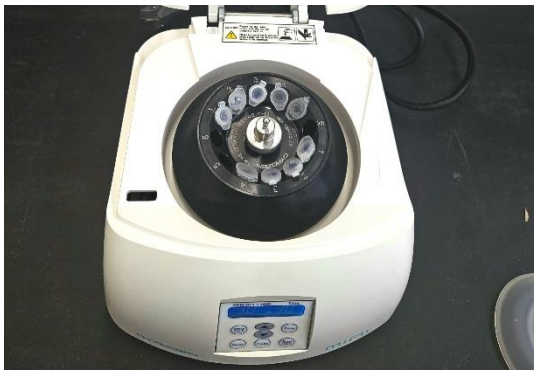

Figure 15. Centrifugation test of the cream.

3.5.2.3. Mechanical vibration test

The mechanical vibration test gives an evaluation of the formulation stability against mechanical vibrations, which may simulate transportation conditions such as mechanical vibrations, which have the potential to provoke phase separation or other instabilities in the product (Mawazi et al., 2022). The formulation was deposited in 2 mL Eppendorf tubes (in triplicate), vortexed for 10 seconds, and inspected for textural changes.

3.5.2.4. pH determination

The pH of a formulation is a critical parameter that affects its stability, efficacy, and skin compatibility. Regular pH measurements ensure that the product remains within the desired pH range throughout its shelf life (Lukić et al., 2021). 1g of the formulation was dissolved in 30 mL of distilled water; then, we calibrated the pH meter (Mettler Toledo) using standard buffer solutions. We then measured the pH by immersing the pH electrode into the sample and recording the pH value as shown in Figure 16.

Figure 16. pH determination.

3.5.2.5. Density determination

The determination of density helps understand the consistency and uniformity of a formulation. It forms one essential part of quality control and batch-to-batch consistency. According to the National Health Surveillance Agency (2005), the relative density of the formulation was measured by adding 0.8g of cream to 40 mL of distilled water; the final volume was marked. The relative density was then calculated using the formula:

$$\mathbf{D} = \frac{\mathbf{m}}{\mathbf{v_1} - \mathbf{v_2}} \quad \mathbf{(7)}$$

When m =Weigh of the cream (g), v_1 = Volume after the addition of the cream (L), v_2 = Volume before the addition of the cream (L)

3.5.2.6. Light test

The cosmetic formulations were placed in transparent plastic containers and subsequently exposed to an extreme light source for 15 days, using a daylight bulb (Lamp T8 L 30W/77 OSRAM) with a photoperiodicity system (16 hours light and 8 hours dark) as Figure 17 presented. At the end of the exposure period, the samples were examined for any changes in physical properties, such as appearance, clarity, color, and liquefaction. Any phase separation or change in color observed is considered indicative of product instability.

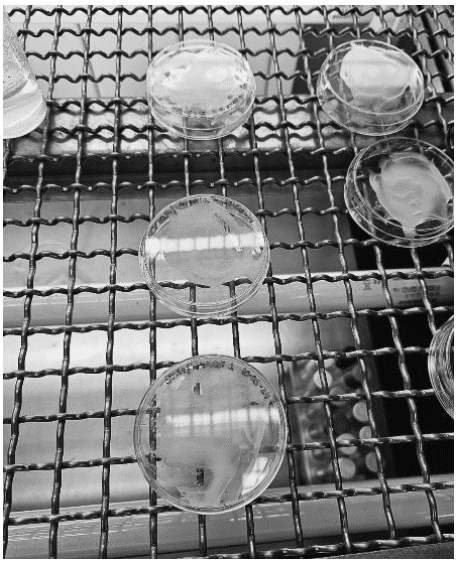

Figure 17. Sample in the dark and light chamber.

3.5.2.7. Viscosity test

Viscosity measurement is crucial for assessing the flow properties of a formulation. It affects the application, spreadability, and stability of the product (Rico et al., 2024). Cream viscosity was measured using a Viscometer (Especialidades Médicas MYR Viscometer, Type V1, Model L, 061-0028) at a temperature of 25 ± 2°C (figure 18). A suitable spindle (L4) was employed, with rotation speeds ranging from 0.3, 0.5, 0.6, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0 rpm. Two measurements were made, one with the rotation rates increasing from 0.3 rpm to 6.0 rpm and the other falling from 6.0 rpm to 0.3 rpm, with a 5-minute rest interval between the two. This procedure assures that the viscosity values are unaffected by any shear-thinning or thickening effects that may occur during the test, resulting in a more accurate evaluation of the formulation's viscosity under varied shear circumstances.

Figure 18. Viscosity measurement with the viscometer.

3.5.2.8. Spectrophotometric test

A cream formulation sample was diluted in ultra-pure water and subjected to spectrophotometric analysis by the spectrophotometer (VWR, UV-VIS 3100PC). The spectrum was traced in the UV-visible region between 210 and 600 nm and compared thereafter to one of the reference samples. The spectrophotometric analysis aims to detect, through the bands, the forms of instability of the formulation, manifested by a change in intensity, or colour, of the band that can be hyperchromic or hypochromic bands (National Health Surveillance Agency, 2005).

3.5.2.9. Accelerate stability test

Accelerated stability tests involve the formulation under elevated temperatures, humidity, and other stress conditions to predict the shelf life of such a formulation. These studies give insight into any potential stability problems in a much shorter timeframe (Navarro-Pérez et al., 2021) have carried out accelerated stability tests in the prediction of the long-term stability of cosmetic emulsions.

An amount of the sample was stored for two weeks in temperatures of 40 ± 2°C at 75 ± 5% RH and 25 ± 2°C with 60 ± 5% RH. Once this period was over, organoleptic criteria were examined, and the pH was valued (National Health Surveillance Agency, 2005).

3.5.2.10. Microbial test (Challenge test)

The microbial test was conducted using the challenge test method (also known as a preservative effectiveness test, PET, or antimicrobial effectiveness test) is a procedure to determine whether a formulated cosmetic is adequately preserved to prevent contamination from raw materials and during consumer use (Russell, 2003). The protocol followed was the one described in (*ISO 11930*, 2019).

3.5.2.11. Evaluation of ocular irritancy test

The hen's egg chorioallantoic membrane (HET-CAM) test was used to assess ocular irritancy. To evaluate the irritation-inducing capability of the produced formulations while in touch with a highly sensitive biological membrane, emphasizing their capacity to generate toxicity in the chorioallantoic membrane of a chicken egg. The procedure followed is the one described by Pinto et al. (2024)

4. Results and discussions

4.1. Chemical analysis

4.1.1. Essential oil chemical composition

The extraction yield of *Anacyclus monanthos* subsp. *cyrtolepidioides* was 0.4 %, and the oil composition was characterized by 72 constituents, which accounted for 69.9% of the total oil. From the total of 72 detected compounds, 68 compounds were identified by GC (Table 4). However, the remaining 4 compounds, two were identified by comparison of retention times through a previous study on the same genus species (UI_A/ UI_D), one was identified through studies on different genus species (UI_D) as shown in Table 5, and one compound remained unidentified. The main compound was Fragranyl 2-methyl butyrate (32.9%), followed by cis-Chrysanthenyl acetate (24.6%). However, it was found that trans- chrysanthenyl acetate was the major compound with (9.8%) in a previous study by Fodil et al. (2019). This discrepancy may be due to geographical variation, as the current study's sample was collected from Bechar region, while the previous study's sample was from the region of M'sila. Environmental factors such as soil composition, climate, altitude, and local flora in these regions can influence the biosynthesis of secondary metabolites, leading to variations in the chemical profile of the essential oil. Also, the chemical analysis of *Anacyclus monanthos* subsp. *cyrtolepidioides* using GC showed that it is rich in oxygen-containing monoterpenes (60.2%), followed by oxygen-containing sesquiterpenes (3.1%), sesquiterpene hydrocarbons (2.6%), and monoterpene hydrocarbons (2.5%). Both phenylpropanoids and fatty acids were present in minor proportions. The extracted essential oil of the plant contains significant levels of α and β-pinene, β-myrcene, myrtenol, terpinen-4-ol, and α-terpinene, indicating possible antibacterial and antifungal properties similar to those seen in essential oils from other plants of the same genus (Aliboudhar et al., 2015).

Table 4. Yields percentage composition of the essential oils isolated from the plant Anacyclus monanthos subsp.cytrolepidioides cyrtolepidioides. with (RI: Retention Index relative to C9-C23 n-alkanes on the DB-1 column, UI: unidentified compounds, * Identification based on mass spectra only, t: trace (< 0.05%)).

Components	RI	Yield (%)
α-Thujene	924	t
α-Pinene	930	1.6
α-Fenchene	938	t
Camphene	938	0.1
Thuja-2,4(10)-diene*	940	t
Sabinene	958	0.2
β-Pinene	963	0.2
β-Myrcene	975	0.4
Benzene acetaldehyde	1002	t
α-Terpinene	1002	t
p-Cymene	1003	t
β-Phellandrene	1005	t
Limonene	1009	t
γ-Terpinene	1035	t
2,5-Dimethyl styrene	1059	t
Linalool	1074	0.1
trans-Pinocarveol	1106	t
1,3,8-p-Menthatriene	1114	0.2
Borneol	1134	0.1
cis-Chrysanthenol	1140	1.5
Terpinen-4-ol	1148	t
Octanoic acid (= Caprylic acid)	1149	t
α-Terpineol	1159	t
Estragole (= Methyl chavicol)	1163	t
Myrtenol	1168	t
trans-Carveol	1189	t
Cuminaldehyde	1200	t
Geraniol	1236	t
cis-Chrysanthenyl acetate	1241	24.6

trans-Anethole	1254	0.4
Nonanoic acid (= Pelargonic acid)	1263	0.3
Cumin alcohol (= p-cymen-7-ol)	1265	t
Bornyl acetate	1265	t
Thymol	1275	0.2
2-Undecanone	1275	t
Carvacrol	1286	0.6
Myrtenyl acetate	1290	t
Eugenol	1327	t
Decanoic acid (= Capric acid)	1356	t
β-Caryophyllene	1414	t
α-Humulene	1447	0.4
ar-Curcumene	1474	1.2
α-Zingiberene	1492	0.3
β-Bisabolene	1500	0.2
trans-Calamenene	1505	t
δ-Cadinene	1505	0.3
Kessane	1517	0.2
β-Eudesmol	1517	0.2
UI_A	1525	0.7
Fragranyl 2-methyl butyrate *	1550	32.9
UI_B	1564	10.9
Humulene epoxide	1580	0.5
UI_C	1626	7.2
UI_D	1632	7.0
α-Bisabolol	1656	0.3
Cedr-8(15)-en-10-ol *	1658	0.2
α-Oxobisabolene	1701	1.9
Hexadecanoic acid (= Palmitic acid)	1908	0.1
n-Eicosane (C20)	2000	t
n-Heneicosane (C21)	2100	t
n-Docosane (C22)	2200	t
n-Tricosane (C23)	2300	t

% identification	69.2
Grouped components	
Monoterpene hydrocarbons	2.5
Oxygen-containing monoterpenes	60.2
Sesquiterpene hydrocarbons	2.6
Oxygen-containing sesquiterpenes	3.1
Phenylpropanoids	0.4
Fatty acids	0.4
Alkanes	t
Others	t

Among the 72 compounds detected, four compounds were unidentified, two were tentatively identified by comparing retention times with a previous study on the same genus species **(**UI_A/ UI_D**),** one was identified through studies on different genus species (UI_C), and one compound remained unidentified UI_B.

Table 5. Identification of the unidentified compounds through a RI literature comparison

Unidentified compounds	RI	Identification of the compound	References
UI_A	1525	Tricosane	(Bergaoui et al., 2011)
UI_B	1564	-	-
UI_D	1632	epi-α-muurolol	(Fodil et al., 2019)
UI_C	1626	Caryophyllene-4(14),8(15)-dien-5α-ol	(Selles et al., 2013)

4.1.2. Total phenolic compounds and flavonoids determination

The determination of total phenolics using the colorimetric Folin-Ciocalteu method with the linear regression of the gallic acid as a reference standard revealed a significant quantity of phenolic compounds in *Anacyclus monanthos* subsp. *cyrtolepidioides* HAE. Upon adding the

Folin-Ciocalteu reagent, a notable color change to blue was observed, indicating the presence of phenolic compounds. This reaction, due to the reduction of the reagent by the phenolics, produced a chromophore with a characteristic blue hue, directly proportional to the phenolic concentration, with absorbance measured at 765 nm, yielding values of 27.47 ± 0.58 mg GAE/g of sample. A similar study by Jawhari et al. (2021) and Cherrat et al. (2017), showed a highest concentration of phenolic compounds of the genus *Anacyclus* with 51.78±0.49 in leaves and 21.84±2.41 in flowers, respectively

4.1.3. Total flavonoids determination

For flavonoid determination, the analytical method utilized consisted of linear regression from a quercetin standard calibration curve. Overall, the principle behind the general methods is derived from the reaction between flavonoids and aluminum chloride, resulting a yellow complex, whereby the degree of yellowness, established by absorbance value, is proportional to the concentration of flavonoids. Flavonoid content is derived from the absorbance measured at 420 nm; the flavonoid value concentration in *Anacyclus mananthos* HAE was 8.40 ± 0.021 mg QE/g. A similar study by Jawhari et al. (2021) and Cherrat et al. (2017), showed the highest concentration of phenolic compounds of the genus *Anacyclus,* with 13.53±0.05 mg QE/g in leaves and 3.04 mg QE/g in flowers, respectively

4.1.4. Fat content determination

4.1.4.1. Plant fatty acids composition

The fatty acid profile of the plant *Anacyclus monanthos* subsp. *Cyrtolepidioides*, with an overall fat content of 0.13%, was examined using gas chromatography to determine the presence of certain fatty acids in the plant sample, as well as their relative proportion. Each fatty acid produced a separate peak at its matching retention time (figure 19), which was then compared to established standards to identify the individual fatty acid contents. The integration of peak areas enabled the determination of the % amount of each fatty acid in the sample. The results are shown in Table 6.

Table 6. *Anacyclus monanthos* subsp. *cyrtolepidioides* fatty acid composition, RT: Retention time of each compound in minute.

Components	RT (min)	% Amount
Methyl butyrate	1.868	69.307
Methyl caproate	3.472	0.154
Methyl decanoate	5.060	0.387
Methyl dodecanoate	6.864	0.266
Methyl tridecanoate	7.956	0.105
Methyl myristate	9.208	0.583
(cis-9) Methyl myristoleate	9.992	0.439
Methyl palmitate	12.284	7.120
(cis-10) Methyl palmitoleate	12.916	0.272
Methyl heptadecenoate	14.044	0.683
Methyl stearate	15.932	1.549
(trans-9) Methyl octadecenoate	16.292	0.732
(cis-9) Methyl oleate	16.404	0.555
(all-cis-9,12) Methyl linoleate	17.528	3.422
(all-cis-9,12,15) Methyl linolenate	18.964	1.255
Methyl arachidate	19.828	1.463
(all-cis-11,14) Methyl eicosadienoate	21.344	0.488
Methyl heneicosanoate	21.788	0.232
(all-cis-8,11,14) Methyl eicosatrienoate	22.124	0.172
Methyl behenate	23.424	6.684
(all-cis-5,8,11,14,17) Methyl eicosapent + (cic-13) Methyl erycate	23.612	0.416
(all-cis-13,16) Methyl docosadienoate	24.040	0.777
Methyl tricosanoate	24.168	0.354
Methyl lignocerate	24.688	2.409
(cis-15) Methyl nervonate	24.856	0.037
(all-cis-4,7,10,13,16,19) Methyl docosahexaenoate	24.908	0.136

The table shows that Methyl butyrate (C4:0) is the major fatty acid at about 69.307%. Such a high proportion of this compound means that Methyl butyrate would make up the majority of the sample and might determine the overall character of the sample, like aroma and flavour. Other major fatty acids include Methyl palmitate (C16:0) at 7.120% and Methyl lignocerate (C24:0) at 2.409%. Both of these are saturated fatty acids that enhance the stability and melting features of the sample. Unsaturated fatty acids like Methyl oleate (C18:1n9c) at 0.555%, Methyl linoleate (C18:2n6c) at 3.422%, and Methyl linolenate (C18:3n3) at 1.255% confer nutritional value because such compounds are known to confer health benefits, such as anti-inflammatory properties. Besides, there is a need for long-chain polyunsaturated fatty acids, among which Methyl docosahexaenoate (C22:6n3) represents 0.136%. Fatty acids may become major ingredients in cosmetics due to their possible functions in cleansing, emulsification, and moisturizing. They may act as surface-active agents, breaking down and carrying away the impurities from the skin in cleansers and soaps. In emulsions, fatty acids may contribute to lowering the interfacial surface tension; thus, they enhance emulsion stability and facilitate a smooth, uniform texture. Moreover, fatty acids can act as emollients and humectants, capable of supplying the skin with hydration and improving its softness (Kelm & Wickett, 2017).

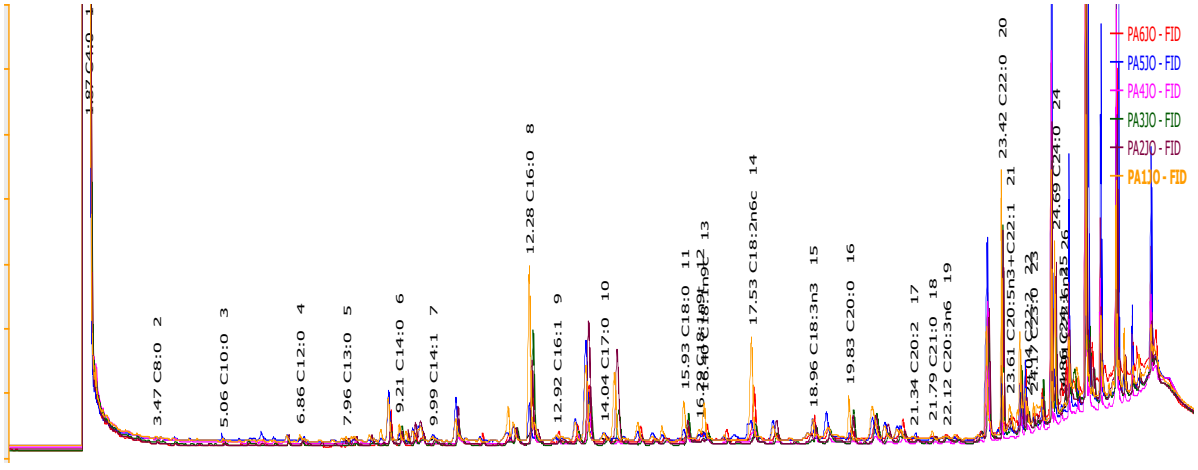

Figure 19. *Anacyclus monanthos* subsp. *cyrtolepidioides* fatty acid chromatographs'

4.1.4.2. Camel fat fatty acid composition

Twenty-two of different fatty acid compounds were identified in the analyzed sample that reflected a great diversity of methyl esters with highly variable relative abundance. All the major compounds found were Methyl palmitate (30.380%), (cis-9) Methyl oleate (31.874%), and (trans-9) Methyl octadecenoate (22.648%). These high concentrations suggest that these fatty acids play a crucial role in defining the sample's characteristics, potentially influencing its physical properties such as melting point and stability. The presence of both saturated (e.g., Methyl palmitate) and unsaturated fatty acids (e.g., (cis-9) Methyl oleate) indicates a balance that could affect the sample's reactivity and nutritional profile. The minor components, although present in smaller amounts, contribute to the overall complexity and functionality of the camel fat sample. with the presence of Methyl myristate 7.802% and of cis-10 methyl palmitoleate 2.861%. Table 7 summarizes camel fat composition

Table 7. Camel fat composition, RT: retention time for each component in minutes.

Components	RT	%amount
Methyl caproate	3.496	0.013
Methyl decanoate	5.072	0.050
Methyl dodecanoate	6.892	0.302
Methyl tridecanoate	7.984	0.063
Methyl myristate	9.320	7.802
(cis-9) Methyl myristoleate	9.996	0.421
Methyl pentadecanoate	10.720	1.173
Methyl palmitate	12.636	30.380
(cis-10) Methyl palmitoleate	13.044	2.861
Methyl heptadecenoate	14.164	1.299
(cis-10) Methyl heptadecenoate	14.692	0.691
(trans-9) Methyl octadecenoate	16.540	22.648
(cis-9) Methyl oleate	16.856	31.874
(all-cis-6,9,12) Methyl linolenate	18.364	0.062
Methyl arachidate	19.904	0.150
(all-cis-11,14) Methyl eicosadienoate	21.412	0.013
Methyl heneicosanoate	21.832	0.017

(all-cis-8,11,14) Methyl eicosatrienoate	22.108	0.022
(all-cis-5,8,11,14) Methyl arachidonate	22.620	0.056
Methyl behenate	23.436	0.015
(all-cis-5,8,11,14,17) Methyl eicosapent + (cic-13) Methyl erycate	23.600	0.008
(cis-15) Methyl nervonate	24.776	0.080

Jassim et al. (2018) investigated camel hump fat as a functional ingredient in cosmetics with regards to stability and protective properties upon UVA exposure. Indeed, their study showed that camel fat withstands UVA light exposure with very slight changes in its chemical composition and efficient UVA absorption—clear evidence for resistance to photodegradation. Additionally, the study emphasized the role of omega-3 fatty acids in camel fat, which protect the skin from UV-induced damage and may limit cancer cell formation. These fatty acids enhance skin health, particularly in the epidermis, providing protective and restorative benefits against environmental stressors. Overall, these findings highlight camel fat's potential as a stable, natural ingredient for improving skin resilience in cosmetic formulations.

4.1.5. Protein content determination

Table 8 exhibited the total proteins and nitrogen percentage of *Anacyclus monanthos* subsp. *cyrtolepidioides* determined by Kjeldahl method, the lowered protein content of Sample 2 was 11.60 ± 0.42% compared to Sample 1 with 13.69 ± 0.28%, probably due to the plant recollection time that accounted for the concentrated protein content. This means that factors like maturity at the time of harvest and environmental conditions affect the plant sample concentration.

These results are comparable to the study carried out by Hussain et al. (2011) about Phytochemical analysis of selected medicinal plants, the Kjeldahl method was also used for the protein determination. Protein values of different plant species fall in the range from 0.2857 to 0.5338 mg/100g; the difference in protein values between the studies can be attributed to the way results are reported, the plant species analyzed, and the type of samples

Table 8. Protein % and Nitrogen values obtained from the Kjeldahl method

Samples	% of Nitrogen	% of Proteins
Sample 1	2.23 ± 0.045	13.69 ± 0.28
Sample 2	1.19 ± 0.08	11.60 ± 0.42

Sample 1 represents the plant *Anacyclus monanthos* subsp. *cyrtolepidioides* harvested in 2023, while Sample 2 is from the 2022 harvest of the same plant.

The difference in nitrogen and protein content between Sample 1 and Sample 2 of the same plant subspecies may is due to the variation in environmental conditions, as well as genetic diversity. Other factors, such as soil quality, weather, and timing of harvest, altogether affect the level of nutrients. Underlying these variables helps in optimizing cultivation for a better nutritional quality in future harvests.

4.1.6. Mineral assay

The results of the mineral assay are shown in table 9:

Table 9. Minerals content of *Anacyclus monanthos* subsp. *cyrtolepidioides*.

Minerals	The amount present in the plant
Phosphorus (P) %	0.034
Potassium (K) %	3.097
Calcium (Ca) %	0.952
Magnesium (Mg) %	0.401
Boron (B) [mg kg-1]	77.5
Iron (Fe) [mg kg-1]	883.407
Manganese (Mn) [mg kg-1]	45.378
Zinc (Zn) [mg kg-1]	16.92
Copper (Cu) [mg kg-1]	10.45

Result for boron in *Anacyclus monanthos* subsp. *cyrtolepidioides* was 77.5 mg/L, showing relatively high concentrations of this important micronutrient. Boron plays an

important role during cell wall formation and in the development of the reproductive organs (Vera-Maldonado et al., 2024). The high level of boron in the plant might be modulated by some edaphoclimatic factors like soil type, salinity, and climatic conditions, which may control the uptake and accumulation of boron in plants, particularly under high levels of soil salinity. Boron has been noted to play a significant role in cell wall rearrangement, membrane integrity of the plasma, and also in signaling activities of the hormones. It is also involved in the regulation of membrane transporters' activity in charge of ionic and water homeostasis within the plant, a function that is found to be especially useful under saline conditions (Qu et al., 2024). This micronutrient is essential for regulating H^+-ATPase activity, which influences various ion channels and aquaporin expression, supporting water transport and overall plant adaptation to saline soils. These functions highlight boron's role in plant resilience and may help explain its relatively high levels in *Anacyclus monanthos* subsp. *Cyrtolepidioides*

The phosphorus (P) content of *Anacyclus monanthos* subsp. *cyrtolepidioides* was measured at 0.034%, which is lower than the phosphorus levels reported in other medicinal plants by (Boroomand et al., 2018). This lower concentration may be attributed to the plant's natural adaptation to sandy desert soils, which are typically nutrient-poor and have low phosphorus availability. Study by (Lajtha & Schlesinger, 1988) have shown that soils under arid ecosystems frequently undergo long-term phosphorus depletion as total P decreases gradually through leaching and is not replenished well by organic sources. In these soils, the calcium-bound phosphorus forms generally dominate, whereas the bioavailable forms, such as phosphates associated with iron, aluminium, or organic matter, are in trace quantities. This shortage of available phosphorus could be a reason for the low levels of phosphorus observed in the plant, reflecting the influence of arid soil conditions on nutrient content in desert plants.

The levels of (Fe), (Mn), (Zn), and (Cu) in the plant are within the range of those reported by Başgel & Erdemoğlu. (2006) for other medicinal herbs. This could be seen as a suggestion that *Anacyclus monanthos* subsp. *cyrtolepidioides* mineral composition is very similar to other medicinal plants. The presence of these essential minerals proved that the plant will be able to undertake important physiological functions contributing to its health and medicinally valuable elements. Also, the observed levels of zinc and copper underline its possible protective properties, which give this plant additional value in traditional and modern health practices.

Comparing the (K), (Ca), and (Mg) values with the study by Marume et al. (2017) our results indicate that the level of potassium (K) in our plant specimen is higher than in most plants examined. The high value of K implies greater physiological benefit, as this ion plays a vital role in enzyme activity and osmoregulation. In contrast, magnesium (Mg) and calcium (Ca) levels are in concordance with the findings of Marum et al., which are quite important for chlorophyll production and cell wall integrity. Generally, these mineral profiles enhance the nutritional value of *Anacyclus monanthos* subsp. *cyrtolepidioides* and thereby give credence to traditional and modern applications in health.

4.2. Bioactivity assays

4.2.1. Antioxidant activity

4.2.1.1. DPPH

The antioxidant activity of *Anacyclus monanthos* subsp. *cyrtolepidioides* HAE extracts was evaluated using the DPPH radical scavenging assay. The results showed that the HAE extract exhibited significant free radical scavenging activity, with an IC50 value of 0.57 ± 0.01 mg/mL, indicating a strong antioxidant potential. This result is comparable to that obtained in a previous study by Bouriche et al. (2016), which evaluated the antioxidative capacity of different extracts (methanolic extract, aqueous extract) of *Anacyclus clavatus*, a plant from the same genus as *Anacyclus monanthos* subsp. *cyrtolepidioides*, using the radical scavenging method. The IC50 results of the methanolic extract and aqueous extract were 28.30 ± 3.45 and 68.98 ± 1.64 μg/mL, respectively.

The IC50 value was considerably higher in HAE when compared to the previous study, it still reflects strong antioxidant activity due to the high concentration of active compounds. This high antioxidant activity could be because of phenolic and flavonoid compounds (Zhao et al., 2014), which have been screened for free radical scavenging properties. These findings support that this plant could be an excellent source of natural antioxidants. HAE extract has potential in the prevention of diseases caused by oxidative stress.

4.2.1.2. Reducing power

Reducing power is a significant indicator of antioxidant activity and is generally assessed based on the measurement of the reduction of Fe^{3+} to Fe^{2+} in the presence of

antioxidants (Munteanu & Apetrei, 2021). The reducing power of *Anacyclus monanthos* subsp. *cyrtolepidioides* HAE was assessed using FRAP methods. The results showed that the HAE extract had a notable ferric-reducing antioxidant power activity, expressed in the IC50 value of 0.55 ± 0.076 mg/mL. This result is compared to that obtained in a previous study by Manouze et al. (2017) on *Anacyclus pyrethrum* (a plant from the same genus), where two extracts of *Anacyclus pyrethrum* were tested using FRAP methods, and the IC50 results obtained were 50.89 ± 1.25 µg/mL for methanolic extract and 60.17 ± 4.48 µg/mL for aqueous extract. Both extracts antioxidant potencies were noticeably higher than those of the reference antioxidants quercetin and butylated hydroxytoluene (BHT) used in the same study.

Compared to the reference solution, our results are higher, but still indicating a potential antioxidant activity with good results. This suggests that the HAE extract of *Anacyclus monanthos* subsp. *cyrtolepidioides* may have a higher capacity to donate electrons and neutralize free radicals, which is a crucial mechanism in preventing oxidative stress. Oxidative stress is linked to various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders (Prasad et al., 2010).

4.2.2. Toxicity assay using brine shrimp (*Artemia salina* L.)

The plant EO and HAE toxicity tests were conducted using the brine shrimp (*Artemia salina*) due to its easy handling and incubation (Banti & Hadjikakou, 2021). Studies show the toxic effects of various natural substances on Artemia (Silva & Silva, 2023). The toxicity testing involved counting the number of *A. salina* surviving at zero, one, twelve, and twenty-four hours after exposing the *A. salina* to the toxicity of the EO and HAE. Two types of controls were implemented to improve evaluation: positive and negative. The negative control, which utilizes artificial seawater with tween 80 (1.5%), helps to validate the *A. salina* mortality in non-ideal salinity conditions. The positive control, potassium dichromate ($K_2Cr_2O_7$) with a series of dilutions, ensures that *A. salina* responds predictably to a known toxicant. Table 10 presents the number of deaths after a specific time interval with their % of mortality and probit number for each of the samples

Table 10. The corresponding results of the toxicity assay, Where Ns: total number of surviving *A. salina* after a specific time interval, Nd: total number of dead *A. salina* after 24 hours, control (+): potassium dichromate, control (-): artificial seawater with Tween 80 (1.5%), Blanc: artificial seawater.

Samples		**Ns**				**Nd**	**% of mortality**	**Probit number**
		T0	T1	T12	T24			
EO	0.1 %	20	20	0	0	20	100	8.09
	0.03 %	20	20	0	0	20	100	8.09
	0.01 %	20	20	3	3	17	85	6.04
	0.09 %	21	21	19	19	2	9.52	3.66
	0.08 %	20	20	20	20	0	0	/
HAE	0.15 %	20	20	0	0	20	100	8.09
	0.075 %	20	20	4	4	16	80	5.84
	0.0375 %	20	20	18	18	2	10	3.72
	0.009 %	20	20	19	19	1	5	3.36
Control	(+)	20	20	2	2	18	90	6.28
10%		20	20	7	7	13	65	5.39
	5.6%	19	19	10	10	9	47	4.92
	3.2%	20	20	17	17	3	15	3.96
	1.8%	20	20	20	20	0	0	----
	1%							
Control (-)		20	20	19	19	1	5	3.36
Blanc		20	20	20	19	1	5	3.36

This assay aimed to calculate the LC50 utilizing probit analysis and log10 transformed concentrations of the investigated drugs. The linear regression of probit values versus log10 concentration did not immediately result in a probit value of 5, corresponding to 50% mortality. This indicates that the probit values were not uniformly distributed around this key threshold. To address this, we employed interpolation based on the linear regression equation; by solving for y =5 (which corresponds to 50% mortality) and calculating x, the log10 of the LC50 for

each sample, we determined the concentration at which this occurs. The antilog (inverse log10) was then used to convert x back into the actual concentration. Figures 1, 2, and 3 represent the interpretation of the EO, HAE, and potassium dichromate graphs, respectively, showing the log10 concentration plotted against the probit values and the corresponding regression lines.

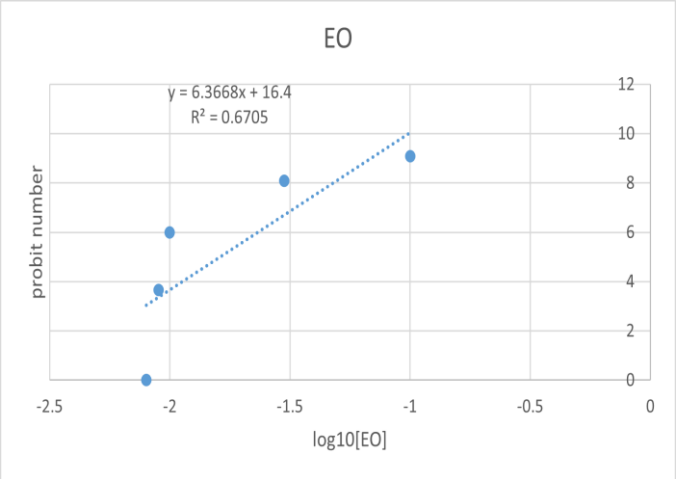

Figure 20. EO Samples Linear Regression of Probit as a Function of Log10 Concentration.

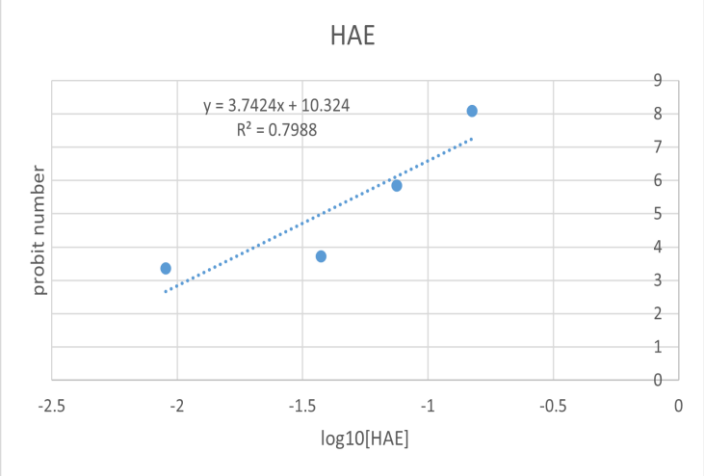

Figure 21. HAE Samples Linear Regression of Probit as a Function of Log10 Concentration.

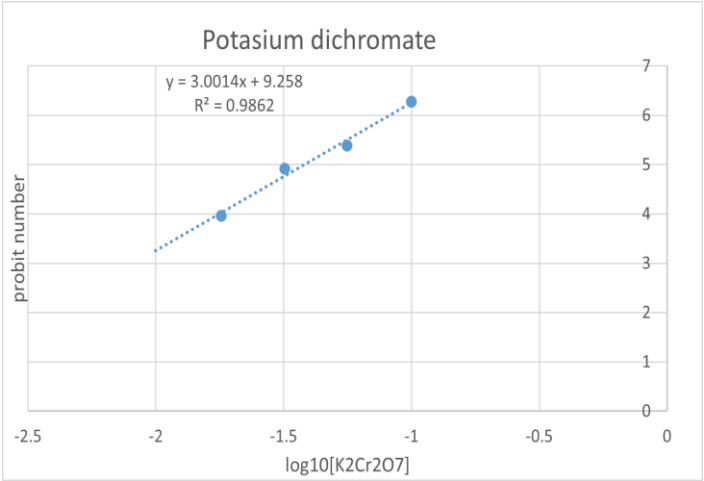

Figure 22. Potassium dichromate Linear Regression of Probit as a Function of Log10 Concentration.

The LC50 values for each sample were established after the relevant calculations were performed. We estimated the log10 values of the LC50 by putting y =5 into the linear regression equations generated from the graphs of each sample. These log10 values were then transformed to their concentrations using the antilogarithm. The determined LC50 values for the EO and HAE samples are summarized below (Table 11), indicating the concentration at which 50% of the *A. salina* experienced mortality.

Table 11. LC50 values for each sample.

	Log10(LC50)	**LC50 (mg/mL)**
EO	-1.79	0.016
HAE	-1.42	0.038
Potassium dichromate	-1.41	0.0389

The LC50 values of EO and HAE in the current study were determined as 0.016 mg/mL and 0.038 mg/mL, respectively, indicating that the toxicity of *Anacyclus monanthos* subsp. *cyrtolepidioides* was much higher than the previous study about the toxicity evaluation of some medicinal plants using *Artemia salina* method, by Arcanjo et al. (2012). This suggests that our plant possesses a potent bioactive potential, which could be advantageous for developing new medicinal applications. Cosmetic applications might be encouraged by their nature as antimicrobial and antioxidant. However, documented toxicity requires further study in order to

establish the appropriate concentrations for topical application. Safety testing is highly necessary to balance effective and safe cosmetic formulations.

4.3. Sun protection factor

Spectrophotometric analysis of *Anacyclus monanthos* subsp. *cyrtolepidioides* HAE showed highly significant properties of UV absorption and hence possesses potential candidates of worth as a natural sunscreen agent. Calculated SPF values showed regular absorption within the studied wavelength. The average SPF value obtained for the triplicate measurements was 22.42 ± 1.42. This finding is higher than the studied vegetable extracts and then chemical sun filters, such as Tinosorb S™, which has an SPF value of 21.01 ± 0.2 studied by Cefali et al. (2019).

4.4. Cosmetic formulation

4.4.1. Texture and consistency

The obtained cream is a moisturizing cream formulation that easily penetrates into the skin to give a smooth, grease-free feel. The cream has a white color. It has a refreshing effect upon application and absorbs quickly, leaving no oily film on its application. It must be applied thoroughly to be wholly absorbed in the skin, as too much application is characterized by fewer flakes, meaning very little quantity can be used to accomplish optimum hydration. The semi-solid nature of the cream makes it easy to apply. This cream has a strong smell because of the addition of the essential oil of *Anacysclus monanthos subsp. cytrolipiodide*; this could be too overpowering and needs further refinement to achieve a more balanced fragrance. Textural properties remained the same during all the storage times, and no phase separation took place; hence, it is stable in formulation. The organoleptic properties of the developed formulation are listed in Table 12

Table 12. Organoleptic properties of the obtained cream formulation.

organoleptic properties	Obtained cream formulation
Color	white
Smell	Noticeable
Texture	Smooth
Consistency	Viscous
Phase separation	No phase separation

4.4.2. Centrifugation test

To anticipate potential disturbances which may affect the cream by differentiating the phases (aqueous and oily), the cream samples were subjected to centrifugation for 30 minutes at 25°C. After centrifugation, Eppendorf tubes containing the cream were meticulously examined individually. No phase separation was observed in the formulation, as shown in Figure 24. Both visual examination by eye and the lack of phase separation observed confirmed the stability of the cream, as no signs of complete or slight phase separation were detected in any samples. This indicates the robustness and uniformity of the emulsification process employed.

Figure 23. Evaluation of the aspect of formulations after centrifugation.

4.4.3. Mechanical vibration test

Mechanical vibration testing showed that, under the applied conditions, the transportation simulation did not disturb the stability of the cream formulation. Immediately after testing in a mechanical vibration test, samples did not exhibit phase separation; that would mean the structural entity of the formulation is strong enough to support mechanical stress. Moreover, further textural changes were not observed; hence, the product is consistent and optimal under more realistic shipping and handling conditions. This stability is important to keep the cream properties for the desired time of its shelf life.

4.4.4. pH determination

For the cream formulation sample, the pH was measured three times in succession for the same cream sample after 1 day, 15 days, and 30 days of cream preparation. The average values obtained in each measurement are as follows 5.16 ± 0.1, 5.19 ± 0.02, 5.4 ± 0.3 for day 1, 15, and 30, respectively. Moreover, the results are shown in the clustered column chart in Figure 25.

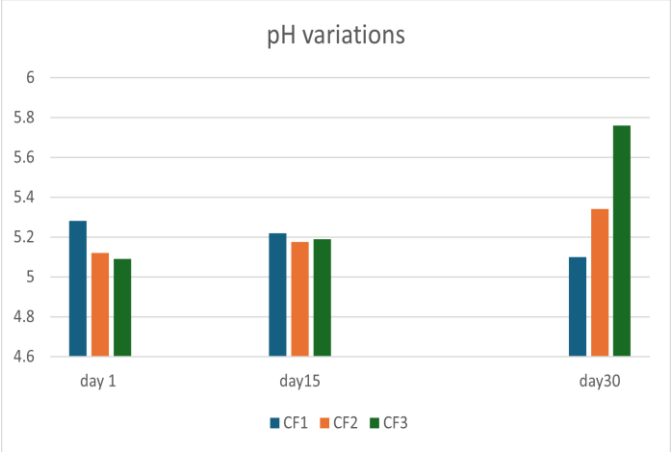

Figure 24. pH measurement of cream formulation during different stages of testing.

The created formulation's pH values are considered appropriate to reduce the danger of irritation upon application to the skin since they are closer to the pH of human skin, which generally ranges from 4 to 6 (Lukić et al., 2021). The pH values showed minimal variation during storage, allowing the prepared formulation to be suitable for topical application.

4.4.5. Density test

The density determination findings on days 1, 15, and 30 were consistent across batches, indicating that the formulation is reliable. On day one, the relative density was 1 ± 0.01. On day 15, the density remained consistent at 1 ± 0.01, and by day 30, it had increased to 1.04 ± 0.04. This continuous density shows that the cream's texture and performance qualities remain stable throughout time. The well-dispersed chemicals in the formulation contribute to its stability. These results demonstrate that the cream satisfies the essential requirements for product consistency and dependability, assuring a high-quality final product throughout its shelf life

4.4.6. Light test

After a 15 days exposure to an intense light source using a daylight bulb with a photoperiodicity system (16 hours light and 8 hours dark), the cosmetic formulation showed no changes in its physical characteristics. There were no modifications regarding aspect, clarity, color, or liquefaction. There was no evidence of any phase separation, and the color remained unaltered as shown in Figure 26, which may point to the product being stable for the period of the test performance. This represents excellent light stability for the cream, establishing its resistance to light exposure and ensuring that under such conditions, it will maintain its effectiveness and quality. The stability demonstrated in this test is indicative of the formulation's strengths, making it suitable for long application and storage.

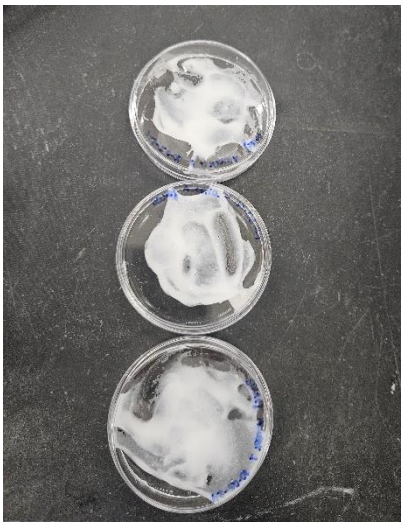

Figure 25. cosmetic formulation samples after exposure to the light.

4.4.7. Viscosity test

The viscosity of the cream formulation was measured for its stability at different rotational speeds and for a long period of time. The obtained results showed some fluctuation but within a very narrow range. Viscosity readings initially (in the first week) were quite variable from each rotation speed (Figure 27), hence the formulation was considered stable .

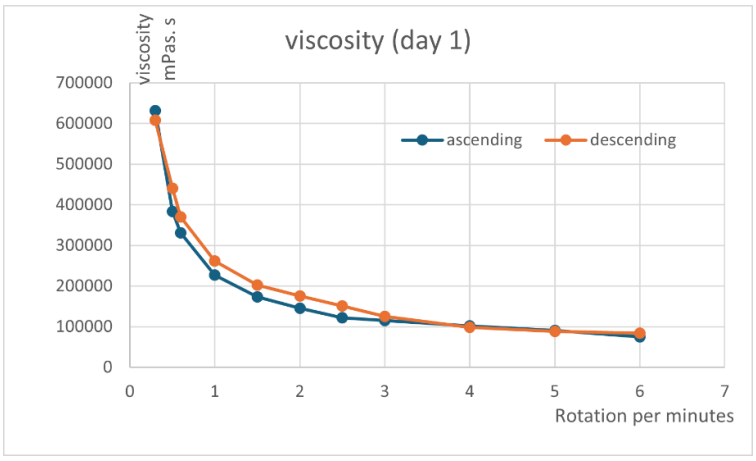

Figure 26. Evaluation of the viscosity of the cream formulation after one day from the preparation.

On the other hand, viscosity increases slightly in the second and fourth weeks, as shown in Figure 28. The smooth increase, though small, insinuates that some structural rearrangement in the cream formulation takes place, which could go further with settling or minor losses of water that increase its thickness. Viscosity changes remained within a range that would assure proper application and spreadability to be effective. This is assured because of the consistency taken during the test period, confirming the stability of the formulation both for long-term use and storage. These facts are reflected in the related graphs, which give evidence to prove that the cream formulation keeps up the intended attributes within the testing period.

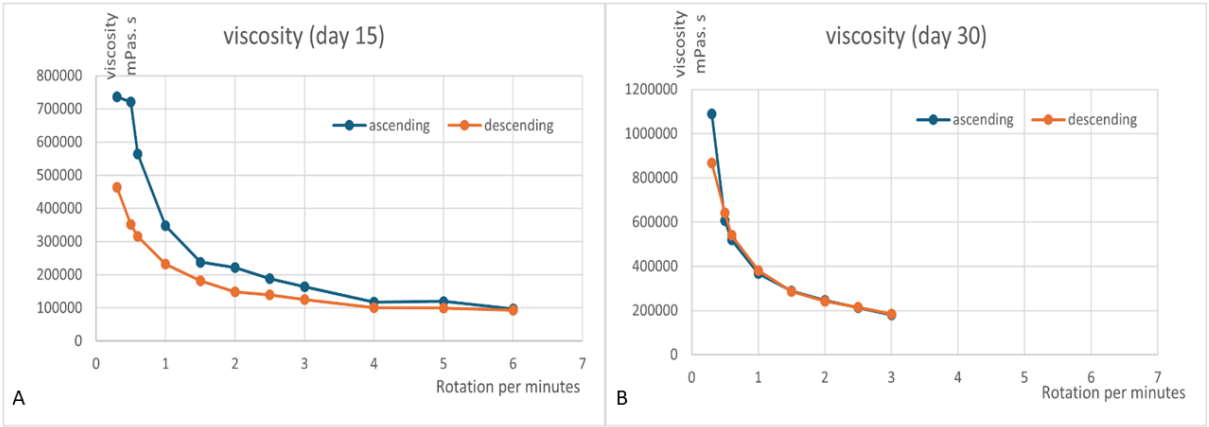

Figure 27. Evaluation of the viscosity of the cream formulation. where (A) is the viscosity after 15 days and (B) is the viscosity after 30 days from the preparation.

4.4.8. Spectrophotometer test

Results of the spectrophotometry assay are presented in Figure 29, which represents the absorbance pattern of cosmetic cream formulation in continuous function of wavelength. In such context, it was noted that the high absorbance values of the product lay within the ultraviolet range, precisely between 200 and 300 nm, including UVA and UVB regions. UVA and UVB radiation, by the wavelength of 320-400 and 280-320 nm, respectively, can cause damage to the skin (Khunkitti et al., 2014), and the high absorbance in this range shows the presence of ingredients that can absorb and probably block these harmful rays.

Beyond 300 nm, the curve shows a considerable fall in absorbance, leveling off beyond 400 nm at much-reduced values. This would suggest that the formulation of the cream is effective in absorbing UV radiation, particularly within the UVA and UVB spectra, which is desirable in an effective sunscreen. Uniformity in absorption patterns across all samples attests to uniformity in dispersion and stability of UV-absorbing agents in the formulation.

The results of this study confirm the cosmetic cream in terms of stability and efficacy in its functions of ultraviolet protection, thus maintaining its quality and effectiveness for a long time. The uniformity and stability expressed by the spectrophotometric analyses emphasized formulation resistance and ensured reliability during sun protection throughout its application.

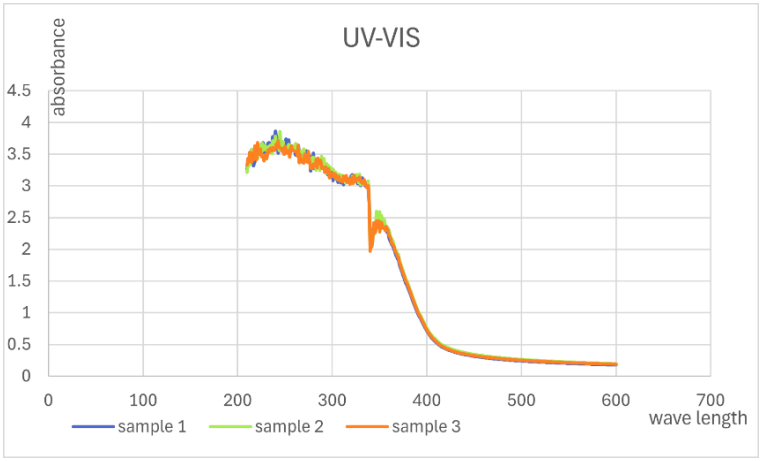

Figure 28. Spectrophotometric analysis of the formulation.

4.4.9. Accelerate stability test

Accelerated stability study conducted for cream formulation provided sufficient evidence about its long-term stability. Samples were kept in environmental conditions of 40 ± 2°C at 75 ± 5% RH and 25 ± 2°C at 60 ± 5% RH for two weeks. Studies after storage revealed changes in the organoleptic characters of the formulation. The pH of the formulation increased from 5.16 to 5.89, which revealed a slight shift towards alkalinity. Whereas the odor gradually became perceptible. Also, the color changed to slight yellowish; while all other properties, such as viscosity, did not change, no phase separation could be perceived. Therefore, indicating the structure of formulation integrity. Results indicate that, though the physical stability is maintained throughout at high temperatures and humidity, slight changes both in its chemical nature and its sensory properties can be observed. Increased pH, as well as changes in odor and color. Obtained results are listed in Table 13.

Table 13. Results of accelerating testing (pH and organoleptic properties).

organoleptic properties and pH	accelerated test cream formulation
pH	5.89
Color	Slightly yellowish
Smell	Noticeable
Texture	Smooth
Consistency	Viscous
Phase separation	No phase separation

4.4.10. Microbial test (Challenge test)

Table 14 below summarizes the results obtained in each analysis of the cream samples, showing the number of CFU concentrations calculated and log reduction in microbial counts for each microorganism during T0, T7, T14, and T28.

Table 14. CFU concentration and $\mathbf{R_X}$ results for the cream formulation for each microorganism and at different times.

	Log reduction values ($\mathbf{R_X = \log N_0 - N_X}$)									
	Pseudomonas aeruginosa		*Staphylococcus aureus*		*Escherichia coli*		*Candida albicans*		*Aspergillus brasiliensis*	
	CFU/mL	R_X	CFU/mL	R_X	CFU/mL	R_X	CFU/mL	R_X	CFU/mL	R_X
T0	1744000	1.75	56900000	0.24	43600000	0.3	149000	>1	12000	>1
T7	900000	2.04	61600000	0.21	71000	>3	129000	>1	1100	>1
T14	154000	2.81	150000	2.82	162000	2.79	138000	>1	600	>1
T28	216000	2.66	268000	2.57	66000	>3	50000	>1	200	>1
Acceptable criteria	Does not meet any criteria		Does not meet any criteria		Criteria B		Criteria A		Criteria A	

The challenge test for the cosmetic formulation was conducted over a 28 days period following ISO 11930:2019 guidelines to assess the antimicrobial efficacy of the product's preservative system. This test involved inoculating the product with five microorganisms commonly associated with cosmetic contamination: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus brasiliensis*. Colony-forming units per milliliter (CFU/mL) were measured at four-time points (T0, T7, T14, T28) to observe changes in microbial populations. A reduction factor (R_X) was calculated for each microorganism at each time point using the following equation:

$$R_X = \log N_0 - N_X \quad (8)$$

Where N_0 represents the initial microbial count (10^8 for the bacterial count and 10^7 for fungal count), and N_X is the count at time X.

The results showed a reduction in the microbial count for both *Aspergillus brasiliensis* and *Candida albicans*, achieving Criteria A according to the established ISO 11930:2019. Meeting Criteria A indicates that the microbiological risk associated with those fungi in the formulation is well under control, attributed to the antifungal properties of essential oils within the product. This, therefore, supports the conclusion that the preservative system is adequate to provide control against fungal contamination throughout the period tested.

For *Escherichia coli*, the microbial reduction met the requirements of Criteria B. ISO 11930:2019 stipulates that Criteria B means that there is a certain level of control, but further measures may be needed to ensure adequate protection against this bacterium. This might imply that the preservative system exhibits moderate efficacy against *E. coli* but possibly needs further reinforcement to ensure long-term stability and safety.

In contrast, *Pseudomonas aeruginosa* and *Staphylococcus aureus* did not meet either Criteria A or B, indicating that the microbiological risk associated with these bacteria under the current formulation is not adequately controlled. Such ineffectiveness against these micro-organisms would, therefore, mean that the preservative system may need tighter control measures or the inclusion of more potent antibacterial agents in the formulation to impart adequate protection. However, the high bacterial counts may also be the result of accidental contamination or procedural inconsistencies during testing. Additional studies or repetition of the experiments under strictly controlled conditions may go a long way toward confirming the findings of preservative efficacy and ensuring that the results are due to the intrinsic antimicrobial properties of the formulation itself, free from extraneous interferences.

4.4.11. Evaluation of ocular irritancy test

The HET-CAM test findings showed that the negative control (NaCl 0.9%) caused no irritation, but the plus control (0.1 NaOH) caused severe irritation after being applied to the egg membrane. However, the cream formulation caused no irritation on the chorioallantoic membrane. There were no observations of vascular damage, reaction, or lysis, as well as no hemorrhage, all of which are common markers of irritation or toxicity. These findings indicate that the cream formulation is mild and safe to use near sensitive regions such as the eyes. The results are presented in figure 30

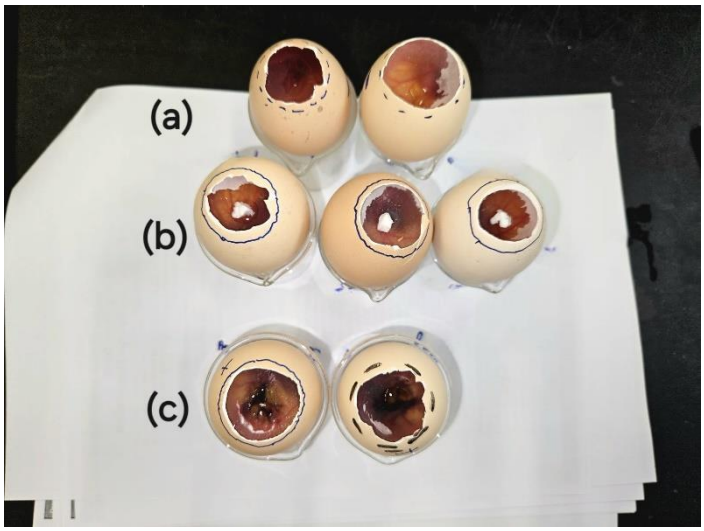

Figure 29. Evaluation of ocular irritancy of cream formulation, where (a): Negative control (NaCl) with no irritation of CAM, (b): Cosmetic formulation with no irritation of CAM, and (c): Positive control (NaOH) with irritation of CAM.

The lack of irritation in the HET-CAM test can most probably be explained by the particular components of the formulation: essential oil, botanical extract, and camel fat. It is common for essential oils and botanical extracts to contain natural anti-inflammatory and soothing compounds that may reduce irritation; on the other hand, camel fat is very rich and moisturizing, able to support skin barrier integrity. Together, these ingredients may contribute to the formulation's gentle effect on the chorioallantoic membrane, supporting its suitability for use around sensitive areas.

5. Conclusion

This study highlights *Anacyclus monanthos* subsp. *cyrtolepidioides*, a plant with great potential to become an important players in the cosmeceutical industry. This detailed analysis shows the complex phytochemical composition, including essential oils, phenolic compounds, and flavonoids responsible for its high antioxidant activity and efficiency in improving skin health. These results hint that this plant is a very promising natural photoprotective agent, and therefore it would be a good ingredient to include in sunscreen products. Furthermore, the successful development of a cosmetic cream using botanical extracts, essential oil, and camel fat demonstrates the practical applications of a formulation that is stable, minimally irritating, and has good antimicrobial activity. This will add to the literature knowledge on plant-based cosmeceuticals and open more horizons for further studies on the discovery of novel skincare solutions based on endemic plants. As such, this work provides a solid foundation for the enhancement of sustainable practices in the cosmetic sector and sparks further research on the wide applications of *Anacyclus monanthos* subsp. *cyrtolepidioides* and allied species.

6. References

Aguirre, J. (2023). Important Topics Related to the Kjeldahl Method. In J. Aguirre (Ed.), *The Kjeldahl Method: 140 Years* (pp. 123–145). Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-31458-2_7

Aliboudhar, H., Tigrine-Kordjani, N., & Youcef Meklati, B. (2015). Competition of microwave-assisted hydro-distillation in highlighting volatile phytochemicals of Anacyclus clavatus species. *Journal of Essential Oil Research*, *27*(4), 355–362. https://doi.org/10.1080/10412905.2015.1029083

Alvarez-Rivera, G., Llompart, M., Lores, M., & Garcia–Jares, C. (2018). Preservatives in cosmetics: Regulatory aspects and analytical methods. In *Analysis of cosmetic products* (pp. 175–224). Elsevier. https://www.sciencedirect.com/science/article/pii/B9780444635082000096

Alves, A., Sousa, E., Kijjoa, A., & Pinto, M. (2020). Marine-Derived Compounds with Potential Use as Cosmeceuticals and Nutricosmetics. *Molecules*, *25*(11), Article 11. https://doi.org/10.3390/molecules25112536

Alves, T. F. R., Morsink, M., Batain, F., Chaud, M. V., Almeida, T., Fernandes, D. A., da Silva, C. F., Souto, E. B., & Severino, P. (2020). Applications of Natural, Semi-Synthetic, and Synthetic Polymers in Cosmetic Formulations. *Cosmetics*, *7*(4), Article 4. https://doi.org/10.3390/cosmetics7040075

Anacyclus monanthos subsp. Cyrtolepidioides (Pomel) Humphries | Plants of the World Online | Kew Science. (n.d.). Plants of the World Online. Retrieved October 22, 2024, from http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:876414-1

Arcanjo, D. D. R., Albuquerque, A. C. M., Melo-Neto, B., Santana, L., Medeiros, M. G. F., & Citó, A. (2012). Bioactivity evaluation against Artemia salina Leach of medicinal plants used in Brazilian Northeastern folk medicine. *Brazilian Journal of Biology*, *72*, 505–509. https://doi.org/10.1590/S1519-69842012000300013

Archambault, J.-C., & Bonté, F. (2021). Vegetable fats in cosmeticology. *Revista Boliviana de Química*, *38*(2), 68–79.

Aruna, M. S., Sravani, A., Resshma, V., Priya, N. S., Prabha, M. S., & Rao, N. R. (2015). Formulation and evaluation of herbal acne gel. *World J Pharm Res*, *4*(5), 2324–2330.

Banti, C. N., & Hadjikakou, S. K. (2021). Evaluation of Toxicity with Brine Shrimp Assay. *Bio-Protocol*, *11*(2), e3895. https://doi.org/10.21769/BioProtoc.3895

Barnes, T. M., Mijaljica, D., Townley, J. P., Spada, F., & Harrison, I. P. (2021). Vehicles for Drug Delivery and Cosmetic Moisturizers: Review and Comparison. *Pharmaceutics*, *13*(12), Article 12. https://doi.org/10.3390/pharmaceutics13122012

Başgel, S., & Erdemoğlu, S. B. (2006). Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. *Science of the Total Environment*, *359*(1–3), 82–89.

Baumann, L., Woolery-Lloyd, H., & Friedman, A. (2009). "Natural" ingredients in cosmetic dermatology. *Journal of Drugs in Dermatology*, *8*(6 Suppl), s5-9.

Bennett, M. L., & Henderson, R. L. (2003). Introduction to cosmetic dermatology. *Current Problems in Dermatology*, *15*(2), 43–83. https://doi.org/10.1016/S1040-0486(03)70003-9

Benyagoub, E., & Mammeri, A. (2023). Physicochemical, biochemical and microbiological quality of dried and salted camel meat (kadid) from the southwestern regions of algeria. *Fresenius Environmental Bulletin*, *32*, 3370–3386.

Bergaoui, A., Hammami, S., & Gannoun, S. (2011). Activity-guided separation of chemical constituents from Tunisian Anacyclus cyrtolepidioïdes chloroformic extract. *Tunisian Journal of Medicinal Plants and Natural Products*, *5*, 33–39.

Bilal, M., Mehmood, S., & Iqbal, H. M. N. (2020). The Beast of Beauty: Environmental and Health Concerns of Toxic Components in Cosmetics. *Cosmetics*, *7*(1), Article 1. https://doi.org/10.3390/cosmetics7010013

Binic, I., Lazarevic, V., Ljubenovic, M., Mojsa, J., & Sokolovic, D. (2013). Skin ageing: Natural weapons and strategies. *Evidence-Based Complementary and Alternative Medicine: eCAM*, *2013*, 827248. https://doi.org/10.1155/2013/827248

Blanco-Dávila, F. (2000). Beauty and the body: The origins of cosmetics. *Plastic and Reconstructive Surgery*, *105*(3), 1196–1204. https://doi.org/10.1097/00006534-200003000-00058

Bouriche, H., Kherbache, A., Kada, S., Senator, A., & Demirtas, I. (2016). Phenolic content, anti-inflammatory and antioxidant activities of Anacyclus clavatus extracts. Environ Exp Biol, 14, 127-135.

Boroomand, N., Sadat-Hosseini, M., Moghbeli, M., & Farajpour, M. (2018). Phytochemical components, total phenol and mineral contents and antioxidant activity of six major medicinal plants from Rayen, Iran. *Natural Product Research*, *32*(5), 564–567. https://doi.org/10.1080/14786419.2017.1315579

Brandão, A. S., Caleja, C., Dias, M. I., ben Salha, A., Rezouga, F., Rodrigues, P., Ferreira, I. C. F. R., Barros, L., & Santos, J. M. R. C. A. (2023). Valorization of pomace from craft cider: Nutritional value, chemical composition, and phenolic and mineral profiles. *eFood*, *4*(3), e85. https://doi.org/10.1002/efd2.85

Bruusgaard-Mouritsen, M. A., Johansen, J. D., Zachariae, C., Kirkeby, C. S., & Garvey, L. H. (2020). Natural ingredients in cosmetic products-A suggestion for a screening series for skin allergy. *Contact Dermatitis*, *83*(4), 251–270. https://doi.org/10.1111/cod.13550

Castillo, R. A., Jaramillo, C. Z., & Sy, L. (2022). The Effectiveness of Social Media Influencers in the Cosmetic and Skincare Industry to the Purchase Intention of the Generation Z Filipinos. *Journal of Business and Management Studies*, *4*(2), Article 2. https://doi.org/10.32996/jbms.2022.4.2.14

Cavinato, M., Waltenberger, B., Baraldo, G., Grade, C. V. C., Stuppner, H., & Jansen-Dürr, P. (2017). Plant extracts and natural compounds used against UVB-induced photoaging. *Biogerontology*, *18*(4), 499–516. https://doi.org/10.1007/s10522-017-9715-7

Cefali, L. C., Ataide, J. A., Fernandes, A. R., Sanchez-Lopez, E., Sousa, I. M. de O., Figueiredo, M. C., Ruiz, A. L. T. G., Foglio, M. A., Mazzola, P. G., & Souto, E. B. (2019). Evaluation of In Vitro Solar Protection Factor (SPF), Antioxidant Activity, and Cell Viability of Mixed

Vegetable Extracts from Dirmophandra mollis Benth, Ginkgo biloba L., Ruta graveolens L., and Vitis vinífera L. *Plants*, *8*(11), 453. https://doi.org/10.3390/plants8110453

Cekić, N., Savić, S., & Savić, S. (2023). Stability evaluation of emulsion-based topical preparations: A valuable potential of dynamic-mechanical thermoanalysis (DMTA) test as a rapid rheological alternative to conventional freeze-thaw test. *Archives of Pharmacy*, *73*(Notebook 5), Article Notebook 5. https://doi.org/10.5937/arhfarm73-46319

Chang, Y. H. (2011). Consumer and Formulator of Natural Cosmetics: Understanding and Integrating Each Other's Needs. In *Formulating, Packaging, and Marketing of Natural Cosmetic Products* (pp. 15–26). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118056806.ch2

Cherrat, A., Amalich, S., Regragui, M., Bouzoubae, A., Elamrani, M., Mahjoubi, M., Bourakhouadar, M., & Touria, Z. (2017). Polyphenols Content And Evaluation Of Antioxidant Activity Of Anacycluspyrethrum(L.) Lag. From Timahdite A Moroccan Middle Atlas Region. *International Journal of Advanced Research*, *5*, 569–577. https://doi.org/10.21474/IJAR01/3546

Croshaw, B. (1977). Preservatives for cosmetics and toiletries. *Journal of the Society of Cosmetic Chemists*, *28*(1), 3–16.

Dai, L., & Hansenne-Cervantes, I. (2024). Protein-Based Materials in Cosmetics. In F. R. Maia, J. M. Oliveira, & R. L. Reis (Eds.), *Handbook of the Extracellular Matrix: Biologically-Derived Materials* (pp. 357–379). Springer International Publishing. https://doi.org/10.1007/978-3-031-56363-8_18

Daniel, H.-J., Reuss, M., & Syldatk, C. (1998). Production of sophorolipids in high concentration from deproteinized whey and rapeseed oil in a two stage fed batch process using Candida bombicola ATCC 22214 and Cryptococcus curvatus ATCC 20509. *Biotechnology Letters*, *20*(12), 1153–1156. https://doi.org/10.1023/A:1005332605003

Das, K., Dang, R., & Machale, M. U. (2009). *Formulation and evaluation of a novel herbal gel of Stevia extract*. https://www.sid.ir/EN/VEWSSID/J_pdf/90420095003.pdf

Lima Cherubim de, D. J ., Buzanello Martins, C. V., Oliveira Fariña, L., & da Silva de Lucca, R. A. (2020). Polyphenols as natural antioxidants in cosmetics applications. *Journal of Cosmetic Dermatology*, *19*(1), 33–37. https://doi.org/10.1111/jocd.13093

Demir, Y., Uckaya, M., & Demir, N. (2019). Evaluation of the efficacy in cosmetic products safety: Comparison with biochemical substrates. *Regulatory Toxicology and Pharmacology: RTP*, *104*, 56–58. https://doi.org/10.1016/j.yrtph.2019.03.001

Dini, I., & Laneri, S. (2021). The New Challenge of Green Cosmetics: Natural Food Ingredients for Cosmetic Formulations. *Molecules*, *26*(13), Article 13. https://doi.org/10.3390/molecules26133921

Djiobie Tchienou, G. E., Tsatsop Tsague, R. K., Mbam Pega, T. F., Bama, V., Bamseck, A., Dongmo Sokeng, S., & Ngassoum, M. B. (2018). Multi-Response Optimization in the Formulation of a Topical Cream from Natural Ingredients. *Cosmetics*, *5*(1), Article 1. https://doi.org/10.3390/cosmetics5010007

Dorni, A. C., Amalraj, A., Gopi, S., Varma, K., & Anjana, S. N. (2017). Novel cosmeceuticals from plants—An industry guided review. *Journal of Applied Research on Medicinal and Aromatic Plants*, *7*, 1–26. https://doi.org/10.1016/j.jarmap.2017.05.003

Dreno, B., Araviiskaia, E., Berardesca, E., Bieber, T., Hawk, J., Sanchez-Viera, M., & Wolkenstein, P. (2014). The science of dermocosmetics and its role in dermatology. *Journal of the European Academy of Dermatology and Venereology: JEADV*, *28*(11), 1409–1417. https://doi.org/10.1111/jdv.12497

Dubuisson, P., Picard, C., Grisel, M., & Savary, G. (2018). How does composition influence the texture of cosmetic emulsions? *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *536*, 38–46.

Edward, M. J., & Norman, F. M. T. R. (1982). The controlled use test in a cosmetic product safety substantiation program. *Journal of Toxicology: Cutaneous and Ocular Toxicology*, *1*(2), 117–132. https://doi.org/10.3109/15569528209051517

Endo, M., Yoshikawa, E., Nemoto, S., Takahashi, Y., Sakai, K., Mizuguchi, H., Sasaki, A., Sugawara, K., Sato, K., & Ihara, T. (2013). Simple and Rapid Determination of Boron in the Wastewater with Azomethine H Using Accelerating Effect of Ammonium Ion. *Journal of Water and Environment Technology*, *11*(4), 355–365. https://doi.org/10.2965/jwet.2013.355

Engasser, P., Long, T., McNamee, P., Schlatter, H., & Gray, J. (2007). Safety of cosmetic products. *Journal of Cosmetic Dermatology*, *6*(s1), 23–31. https://doi.org/10.1111/j.1473-2165.2007.00317.x

Faria-Silva, C., Ascenso, A., Costa, A. M., Marto, J., Carvalheiro, M., Ribeiro, H. M., & Simões, S. (2020). Feeding the skin: A new trend in food and cosmetics convergence. *Trends in Food Science & Technology*, *95*, 21–32. https://doi.org/10.1016/j.tifs.2019.11.015

Fernandes, A. R., Dario, M. F., Pinto, C. A. S. de O., Kaneko, T. M., Baby, A. R., & Velasco, M. V. R. (2013). Stability evaluation of organic Lip Balm. *Brazilian Journal of Pharmaceutical Sciences*, *49*, 293–299. https://doi.org/10.1590/S1984-82502013000200011

Ferreira, M., Matos, A., Couras, A., Marto, J., & Ribeiro, H. (2022). Overview of Cosmetic Regulatory Frameworks around the World. *Cosmetics*, *9*(4). Scopus. https://doi.org/10.3390/cosmetics9040072

Fleming, R. M. (2002). The Effect of High-, Moderate-, and Low-Fat Diets on Weight Loss and Cardiovascular Disease Risk Factors. *Preventive Cardiology*, *5*(3), 110–203. https://doi.org/10.1111/j.1520-037X.2002.01231.x

Fodil, H., Sarri, M., Hendel, N., Maggi, F., & Sarri, D. (2019). Essential oil composition of aerial parts from Algerian Anacyclus monanthos subsp. Cyrtolepidioides (Pomel) Humphries. *Natural Product Research*, *33*(2), 292–295. https://doi.org/10.1080/14786419.2018.1443094

Franzol, A., Banin, T. M., Brazil, T. R., & Rezende, M. C. (2021). Assessment of kinetic stability of cosmetic emulsions formulated with different emulsifiers using rheological and sensory analyses. *Journal of Sol-Gel Science and Technology*, *99*(3), 469–481. https://doi.org/10.1007/s10971-021-05587-x

Giorgio, A., Miele, L., De Bonis, S., Conforti, I., Palmiero, L., Guida, M., Libralato, G., & Aliberti, F. (2018). Microbiological stability of cosmetics by using challenge test procedure. *Journal of Pure and Applied Microbiology*, *12*(1), 23–28.

Goddard, E. D., & Gruber, J. V. (1999). *Principles of Polymer Science and Technology in Cosmetics and Personal Care*. CRC Press.

Halla, N., Fernandes, I. P., Heleno, S. A., Costa, P., Boucherit-Otmani, Z., Boucherit, K., Rodrigues, A. E., Ferreira, I. C. F. R., & Barreiro, M. F. (2018). Cosmetics Preservation: A Review on Present Strategies. *Molecules*, *23*(7), Article 7. https://doi.org/10.3390/molecules23071571

Hassan, S. H., Teo, S. Z., Ramayah, T., & Al-Kumaim, N. H. (2021). The credibility of social media beauty gurus in young millennials' cosmetic product choice. *PLOS ONE*, *16*(3), e0249286. https://doi.org/10.1371/journal.pone.0249286

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root. Their relative astringency and radical scavenging effects. *Chemical and Pharmaceutical Bulletin*, *36*(6), 2090–2097. https://doi.org/10.1248/cpb.36.2090

He, H., Li, A., Li, S., Tang, J., Li, L., & Xiong, L. (2021). Natural components in sunscreens: Topical formulations with sun protection factor (SPF). *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *134*, 111161. https://doi.org/10.1016/j.biopha.2020.111161

Herman, A., Herman, A. P., Domagalska, B. W., & Młynarczyk, A. (2013). Essential Oils and Herbal Extracts as Antimicrobial Agents in Cosmetic Emulsion. *Indian Journal of Microbiology*, *53*(2), 232–237. https://doi.org/10.1007/s12088-012-0329-0

Hinčica, V., Řezanková, H., Macias, K., & Schulzová, M. (2024). Perception of Natural Cosmetics Among Central European Consumers. *Central European Economic Journal*, *11*(58), 233–251. https://doi.org/10.2478/ceej-2024-0016

Hoang, H. T., Moon, J.-Y., & Lee, Y.-C. (2021). Natural Antioxidants from Plant Extracts in Skincare Cosmetics: Recent Applications, Challenges and Perspectives. *Cosmetics*, *8*(4), Article 4. https://doi.org/10.3390/cosmetics8040106

Holmberg, K. (2001). Natural surfactants. *Current Opinion in Colloid & Interface Science*, *6*(2), 148–159.

Hussain, I., Ullah, R., Ullah, R., Khurram, M., Ullah, N., Abdul, B., Khan, F., Khattak, M., Zahoor, M., Khan, J., & Khan, Dr. N. (2011). Phytochemical analysis of selected medicinal plants,. *AFRICAN JOURNAL OF BIOTECHNOLOGY*, *10*, 7487–7492.

Infante, V., Melo, M., & Campos, P. (2018). The social and scientifical evolution of the cosmetic science – a brasilein view: A evolução social e científica da ciência cosmética – uma visão brasileira. *Journal Biomedical and Biopharmaceutical Research*, *15*, 84–95. https://doi.org/10.19277/bbr.15.1.177

ISO 11930:2019. (2019). ISO. https://www.iso.org/standard/75058.html

Ivanova, E. V., Minyaylo, E. O., Temnikov, M. N., Mukhtorov, L. G., & Atroshchenko, Y. M. (2023). Silicones in Cosmetics. *Polymer Science, Series B*, *65*(5), 578–594.

Iwata, H., & Shimada, K. (2012). *Formulas, Ingredients and Production of Cosmetics: Technology of Skin- and Hair-Care Products in Japan*. Springer Science & Business Media.

Jassim, S. A. A., Aldoori, A. A., AbdulMounam, M. A., Faraj, B. R., AbdulHameed, F. F., & Limoges, R. G. (2018). Photoprotection Comprising Oil Derived from Dromedary Camel Hump Fat. *Annual Research & Review in Biology*, *27*(3), 1–11. https://doi.org/10.9734/ARRB/2018/42132

Jassim, S. A. A., Doori, A., & Limoges, R. G. (2020). *Composition comprising camel hump fat and uses thereof* (European Union Patent EP2964236B1). https://patents.google.com/patent/EP2964236B1/es

Jawhari, F. Z., Moussaoui, A. E. L., Bourhia, M., Imtara, H., Saghrouchni, H., Ammor, K., Ouassou, H., Elamine, Y., Ullah, R., Ezzeldin, E., Mostafa, G. A. E., & Bari, A. (2021). Anacyclus pyrethrum var. pyrethrum (L.) and Anacyclus pyrethrum var. depressus (Ball) Maire: Correlation between Total Phenolic and Flavonoid Contents with Antioxidant and Antimicrobial Activities of Chemically Characterized Extracts. *Plants*, *10*(1), Article 1. https://doi.org/10.3390/plants10010149

Juhász, M. L., Levin, M. K., & Marmur, E. S. (2018). The use of natural ingredients in innovative Korean cosmeceuticals. *Journal of Cosmetic Dermatology*, *17*(3), 305–312. https://doi.org/10.1111/jocd.12492

Kambhampati, S., Li, J., Evans, B. S., & Allen, D. K. (2019). Accurate and efficient amino acid analysis for protein quantification using hydrophilic interaction chromatography coupled tandem mass spectrometry. *Plant Methods*, *15*, 46. https://doi.org/10.1186/s13007-019-0430-z

Kaur, H., & Subburayan, B. (2024). *A Comprehensive Evaluation of Assessment Tools for Detecting Corporate Greenwashing Practices in the Beauty and Cosmetics Industry* (SSRN Scholarly Paper 4751446). Social Science Research Network. https://doi.org/10.34293/sijash.v11iS3-Feb.7264

Kelm, G. R., & Wickett, R. R. (2017). The role of fatty acids in cosmetic technology. In *Fatty acids* (pp. 385–404). Elsevier. https://www.sciencedirect.com/science/article/pii/B978012809521800012X

Khan, A. D., & Alam, M. N. (2019). Cosmetics and their associated adverse effects: A review. *Journal of Applied Pharmaceutical Sciences and Research*, 1–6. https://doi.org/10.31069/japsr.v2i1.1

Khunkitti, W., Satthanakul, P., Waranuch, N., Pitaksuteepong, T., & Kitikhun, P. (2014). Method for screening sunscreen cream formulations by determination of in vitro SPF and PA values using UV transmission spectroscopy and texture profile analysis. *Journal of Cosmetic Science*, *65*, 147–159.

Kipgen, T., Sharma, S., Sharma, D. G. K., & Chandrul, D. K. K. (2021). An Overview of Cosmetic Science. *International Journal of Trend in Scientific Research and Development*, *5*(5), 1604–1608.

Kornfeld-Lecanu, S., Zajaczkowski, F., Dubourg, S., Martin, L., Lefort, S., & Siest, S. (2010). Vigilance in industry: Cosmetics and household cleaning products. Balance sheet of case report from 2005 to 2007. *Clinical and Experimental Dermatology*, *35*(8), 874–880. https://doi.org/10.1111/j.1365-2230.2010.03904.x

KP, M. H., K, S. H., Saraswathi, R., Mohanta, G. P., & Nayar, C. (2010). Formulation and evaluation of herbal gel of Pothos scandens Linn. *Asian Pacific Journal of Tropical Medicine*, *3*(12), 988–992. https://doi.org/10.1016/S1995-7645(11)60015-1

Kroke, H. P. (1978). Oily components in cosmetics from a European view. *Journal of the American Oil Chemists' Society*, *55*(4), 444–446. https://doi.org/10.1007/BF02911910

Krongrawa, W., Limmatvapirat, S., Pongnimitprasert, N., Meetam, P., & Limmatvapirat, C. (2018). Formulation and evaluation of gels containing coconut kernel extract for topical application. *Asian Journal of Pharmaceutical Sciences*, *13*(5), 415–424.

Kulawik-Pióro, A., Ptaszek, A., & Kruk, J. (2019). Effective tool for assessment of the quality of barrier creams—Relationships between rheological, textural and sensory properties. *Regulatory Toxicology and Pharmacology*, *103*, 113–123. https://doi.org/10.1016/j.yrtph.2019.01.026

Kusumawati, I., & Indrayanto, G. (2013). Natural antioxidants in cosmetics. *Studies in Natural Products Chemistry*, *40*, 485–505.

Lajtha, K., & Schlesinger, W. H. (1988). The Biogeochemistry of Phosphorus Cycling and Phosphorus Availability Along a Desert Soil Chronosequence. *Ecology*, *69*(1), 24–39. https://doi.org/10.2307/1943157

Latimer, G. W., Jr. (Ed.). (2023). AOAC Official Method 978.04Nitrogen (Total) (Crude Protein) in Plants: Kjeldahl Methods. In *Official Methods of Analysis of AOAC INTERNATIONAL* (p. 0). Oxford University Press. https://doi.org/10.1093/9780197610145.003.1359

Lochhead, R. Y. (2007). The Role of Polymers in Cosmetics: Recent Trends. In *Cosmetic Nanotechnology* (Vol. 961, pp. 3–56). American Chemical Society. https://doi.org/10.1021/bk-2007-0961.ch001

Lodén, M., & Alander, J. (2022). Hydrating Substances. In *Handbook of Cosmetic Science and Technology* (5th ed.). CRC Press.

Lopetinsky, R. J. G., Masliyah, J. H., & Xu, Z. (2006). Solids-Stabilized Emulsions: A Review. In B. P. Binks & T. S. Horozov (Eds.), *Colloidal Particles at Liquid Interfaces* (pp. 186–224). Cambridge University Press. https://doi.org/10.1017/CBO9780511536670.007

Luchs, M. G., Naylor, R. W., Irwin, J. R., & Raghunathan, R. (2010). The Sustainability Liability: Potential Negative Effects of Ethicality on Product Preference. *Journal of Marketing*, *74*(5), 18–31. https://doi.org/10.1509/jmkg.74.5.018

Luco, D. P., Leite-Silva, V. R., Benson, H. A. E., & Lopes, P. S. (2019). In Vitro Methods: Alternatives to Animal Testing. In *Cosmetic Formulation*. CRC Press.

Lukić, M., Pantelić, I., & Savić, S. D. (2021). Towards Optimal pH of the Skin and Topical Formulations: From the Current State of the Art to Tailored Products. *Cosmetics*, *8*(3), Article 3. https://doi.org/10.3390/cosmetics8030069

MacFarlane, B. (2019). Common Cosmetic Ingredients: Chemistry, Actions, Safety and Products. In *Cosmetic Formulation*. CRC Press.

Man, Q., & Rahman, M. (2019). The Impact of Cosmetics Industry Social Media Marketing on Brand Loyalty: Evidence from Chinese College Students. *Academy of Marketing Studies Journal*. https://www.semanticscholar.org/paper/The-Impact-of-Cosmetics-Industry-Social-Media-on-Man-Rahman/c8becc0b5b671eb2cdcd304d3ddd5c026c0cc337

Mann, R. M., & Bidwell, J. R. (2001). The acute toxicity of agricultural surfactants to the tadpoles of four Australian and two exotic frogs. *Environmental Pollution*, *114*(2), 195–205.

Manouze, H., Bouchatta, O., Gadhi, A. C., Bennis, M., Sokar, Z., & Ba-M'hamed, S. (2017). Anti-inflammatory, Antinociceptive, and Antioxidant Activities of Methanol and Aqueous Extracts of Anacyclus pyrethrum Roots. *Frontiers in Pharmacology*, *8*. https://doi.org/10.3389/fphar.2017.00598

Mansur, J. de S., Breder, M. N. R., Mansur, M. C. d'Ascençäo, & Azulay, R. D. (1986). Determinaçäo do fator de proteçäo solar por espectrofotometria. *An. bras. dermatol*, 121–124.

Manzoor, F. (2024). Concept of Cosmetology and its Historical Background in Unani Perspective. *Journal of Drug Delivery and Therapeutics*, *14*(4), Article 4. https://doi.org/10.22270/jddt.v14i4.6498

Marume, A., Khoza, S., Matope, G., Nyakudya, T. T., Mduluza, T., & Ndhlala, A. R. (2017). Antioxidant properties, protein binding capacity and mineral contents of some plants traditionally used in the management of animal wounds. *South African Journal of Botany*, *111*, 23–28.

Matsumoto, I. (1973). On the Instrumental Analysis of Surface-Active Agents in Cosmetics. *Journal of Japan Oil Chemists' Society*, *22*(9), 565–574.

Matwiejczuk, N., Galicka, A., & Brzóska, M. M. (2020). Review of the safety of application of cosmetic products containing parabens. *Journal of Applied Toxicology*, *40*(1), 176–210. https://doi.org/10.1002/jat.3917

Mawazi, S., Ann, J., Othman, N., Khan, J., Alolayan, S., thagfan, S., & Kaleemullah, M. (2022). A Review of Moisturizers; History, Preparation, Characterization and Applications. *Cosmetics*, *9*, 61. https://doi.org/10.3390/cosmetics9030061

Mitsui, T. (1997). *New Cosmetic Science*. Elsevier Science.

Mohiuddin, A. K. (2019). Cosmetics in use: A pharmacological review. *J Dermat Cosmetol*, *3*(2), 50–67.

Moldovan, M., & Ciortea, L. (2010). Efficacy evaluation of different cream formulations on healthy skin properties. *Farmacia*, *58*(6), 787–794.

Munteanu, I. G., & Apetrei, C. (2021). Analytical Methods Used in Determining Antioxidant Activity: A Review. *International Journal of Molecular Sciences*, *22*(7), 3380. https://doi.org/10.3390/ijms22073380

Naidu, J., Ismail, R., & Sasidharan, S. (2014). Acute Oral Toxicity and Brine Shrimp Lethality of Methanol Extract of *Mentha Spicata* L (Lamiaceae). *Tropical Journal of Pharmaceutical Research*, *13*(1), 101. https://doi.org/10.4314/tjpr.v13i1.15

Navarro-Pérez, Y. M., Cedeño-Linares, E., Norman-Montenegro, O., Ruz-Sanjuan, V., Mondeja-Rivera, Y., Hernández-Monzón, A. M., & González-Bedia, M. M. (2021). Prediction

of the physical stability and quality of O/W cosmetic emulsions using full factorial design. *Journal of Pharmacy & Pharmacognosy Research*, *9*(1), 98–112. https://doi.org/10.56499/jppres20.908_9.1.98

Neibecker, H., & Imdahl, I. (2022). Cosmetics as Essential Everyday Companions – the Psychological and Physical Relevance of Cosmetic Products for People. *Snow Journal*, *148*(3), 1–12.

Nicuță, D., Grosu, L., Alexa, I.-C., & Fînaru, A.-L. (2024). Sustainable Characterization of Some Extracts of Origanum vulgare L. and Biosafety Evaluation Using Allium cepa Assay. *Horticulturae*, *10*(5), Article 5. https://doi.org/10.3390/horticulturae10050504

Palefsky, I. (2022). Creams and Ointments. In *Cosmetic Dermatology* (pp. 101–105). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119676881.ch10

Pandey, A., Jatana, G. K., & Sonthalia, S. (2024). Cosmeceuticals. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK544223/

Patil, A., Bhide, S., Bookwala, M., Soneta, B., Shankar, V., Almotairy, A., Almutairi, M., & Narasimha Murthy, S. (2018). Stability of Organoleptic Agents in Pharmaceuticals and Cosmetics. *AAPS PharmSciTech*, *19*(1), 36–47. https://doi.org/10.1208/s12249-017-0866-2

Patil, A., & Ferritto, M. S. (2013). Polymers for Personal Care and Cosmetics: Overview. In *Polymers for Personal Care and Cosmetics* (Vol. 1148, pp. 3–11). American Chemical Society. https://doi.org/10.1021/bk-2013-1148.ch001

Pereira, J., & Pereira, T. (2018). Cosmetics and its Health Risks. *Global Journal of Medical Research*, 63–70. https://doi.org/10.34257/GJMRBVOL18IS2PG63

Pinto, M. B., Pires, P. C., Calhelha, R. C., Silva, A. R., Sousa, M. J., Vilas-Boas, M., Falcão, S. I., Veiga, F., Makvandi, P., & Paiva-Santos, A. C. (2024). Bee Venom-Loaded Niosomes as Innovative Platforms for Cancer Treatment: Development and Therapeutical Efficacy and Safety Evaluation. *Pharmaceuticals*, *17*(5), Article 5. https://doi.org/10.3390/ph17050572

Polati, S., Gosetti, F., & Gennaro, M. C. (2007). Preservatives in cosmetics. Analytical methods. In *Analysis of cosmetic products* (pp. 215–245). Elsevier Amsterdam, The Netherland. https://www.google.com/books?hl=fr&lr=&id=IYf8FDXlD5oC&oi=fnd&pg=PA211&dq=preservatives+in+cosmetics&ots=wmKaenco-R&sig=rsFTo6pT88U8ug75Pr1caoCGIKc

Prasad, K. N., Xie, H., Hao, J., Yang, B., Qiu, S., Wei, X., Chen, F., & Jiang, Y. (2010). Antioxidant and anticancer activities of 8-hydroxypsoralen isolated from wampee [Clausena lansium (Lour.) Skeels] peel. *Food Chemistry*, *118*(1), 62–66. https://doi.org/10.1016/j.foodchem.2009.04.073

Qu, M., Huang, X., García-Caparrós, P., Shabala, L., Fuglsang, A. T., Yu, M., & Shabala, S. (2024). Understanding the role of boron in plant adaptation to soil salinity. *Physiologia Plantarum*, *176*(3). https://doi.org/10.1111/ppl.14358

Rähse, W. (2019). Composition of Creams for Skin Care. In *Cosmetic Creams* (pp. 131–173). John Wiley & Sons, Ltd. https://doi.org/10.1002/9783527812219.ch5

Rasmussen, K., & Mech, A. (2014). Better understanding of the EU regulatory frameworks for cosmetic products. *Science of The Total Environment*, *479–480*, 322–325. https://doi.org/10.1016/j.scitotenv.2014.01.106

Ratz-Lyko, A., Arct, J., & Pytkowska, K. (2012). Methods for evaluation of cosmetic antioxidant capacity. *Skin Research and Technology*, *18*(4), 421–430. https://doi.org/10.1111/j.1600-0846.2011.00588.x

Rawlings, A. V., Harding, C. R., Watkinson, A., Chandar, P., & Scott, I. R. (2002). Humectants. In *Skin Moisturization*. CRC Press.

Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products (Recast) (Text with EEA Relevance), 342 OJ L (2009). http://data.europa.eu/eli/reg/2009/1223/oj/eng

Ribeiro, A. S., Estanqueiro, M., Oliveira, M. B., & Sousa Lobo, J. M. (2015). Main Benefits and Applicability of Plant Extracts in Skin Care Products. *Cosmetics*, *2*(2), Article 2. https://doi.org/10.3390/cosmetics2020048

Ricapito, N. G., Ghobril, C., Zhang, H., Grinstaff, M. W., & Putnam, D. (2016). Synthetic Biomaterials from Metabolically Derived Synthons. *Chemical Reviews*, *116*(4), 2664–2704. https://doi.org/10.1021/acs.chemrev.5b00465

Rico, F., Mazabel, A., Egurrola, G., Pulido, J., Barrios, N., Marquez, R., & García, J. (2024). Meta-Analysis and Analytical Methods in Cosmetics Formulation: A Review. *Cosmetics*, *11*(1), Article 1. https://doi.org/10.3390/cosmetics11010001

Rieger, M. N. (2017). *Surfactants in Cosmetics*. Routledge.

Romanowski, P., & Schueller, R. (2001). Stability Testing of Cosmetic Products. In *Handbook of Cosmetic Science and Technology*. CRC Press.

Rosen, M. J., & Kunjappu, J. T. (2012). Characteristic Features of Surfactants. In *Surfactants and Interfacial Phenomena* (pp. 1–38). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118228920.ch1

Russell, A. D. (2003). Challenge testing: Principles and practice. *International Journal of Cosmetic Science*, *25*(3), 147–153. https://doi.org/10.1046/j.1467-2494.2003.00179.x

Sah, R. N., & Brown, P. H. (1997). Techniques for boron determination and their application to the analysis of plant and soil samples. *Plant and Soil*, *193*(1), 15–33. https://doi.org/10.1023/A:1004251606504

Sakamoto, K., Lochhead, R. Y., Maibach, H. I., & Yamashita, Y. (2017). *Cosmetic Science and Technology: Theoretical Principles and Applications*. Elsevier.

Saraf, S., & Saraf, S. (2015). *Cosmetics: A Practical Manual*. Bsp Books Pvt. Limited.

Sarri, M., Sarri, D., Noui, H., & Hadjer, F. (2018). Note sur une nouvelle station d'Anacyclus monanthos subsp. Cyrtolepidioides (Pomel) Humphries dans la région du Chott el Hodna (M'sila, Algérie). *Acta Botanica Malacitana*, *43*, 1–3. https://doi.org/10.24310/abm.v43i0.4899

Selles, C., Mohammed El Amine Dib, Djabou, N., Beddou, F., Muselli, A., Tabti, B., Costa, J., & Hammouti, B. (2013). Antimicrobial activity and evolution of the composition of essential

oil from Algerian Anacyclus pyrethrum L. through the vegetative cycle. *Natural Product Research*, *27*(23), 2231–2234. https://doi.org/10.1080/14786419.2013.811409

Shim, J., Woo, J., Yeo, H., Kang, S., Kwon, B., Lee, E., Oh, J., Jeong, E., Lim, J., & Park, S. (2024). The Clean Beauty Trend Among Millennial and Generation Z Consumers: Assessing the Safety, Ethicality, and Sustainability Attributes of Cosmetic Products. *SAGE Open*, *14*. https://doi.org/10.1177/21582440241255430

Shingfield, K. J., Reynolds, C. K., Hervás, G., Griinari, J. M., Grandison, A. S., & Beever, D. E. (2006). Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. *Journal of Dairy Science*, *89*(2), 714–732. https://doi.org/10.3168/jds.S0022-0302(06)72134-8

Silva, L., & Silva, F. (2023). *Bioassay with artemia salina l.: A gateway to understanding the toxicity of medicinal plant extracts* (p. 2023). https://doi.org/10.37885/230814206

Singleton, V. L., Rudolf, O., & Rosa M., L.-R. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology* (Vol. 299, pp. 152–178). Academic Press. https://doi.org/10.1016/S0076-6879(99)99017-1

Thiyagarasaiyar, K., Goh, B.-H., Jeon, Y.-J., & Yow, Y.-Y. (2020). Algae Metabolites in Cosmeceutical: An Overview of Current Applications and Challenges. *Marine Drugs*, *18*(6), Article 6. https://doi.org/10.3390/md18060323

Thring, T. S. A., Hili, P., & Naughton, D. P. (2009). Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. *BMC Complementary and Alternative Medicine*, *9*, 27. https://doi.org/10.1186/1472-6882-9-27

Toklu, H. Z., Antigua, A., Lewis, V., Reynolds, M., & Jones, J. (2019). Cosmetovigilance: A review of the current literature. *Journal of Family Medicine and Primary Care*, *8*(5), 1540–1545. https://doi.org/10.4103/jfmpc.jfmpc_447_18

Veeresham, C. (2012). Natural products derived from plants as a source of drugs. *Journal of Advanced Pharmaceutical Technology & Research*, *3*(4), 200. https://doi.org/10.4103/2231-4040.104709

Vera-Maldonado, P., Aquea, F., Reyes-Díaz, M., Cárcamo-Fincheira, P., Soto-Cerda, B., Nunes-Nesi, A., & Inostroza-Blancheteau, C. (2024). Role of boron and its interaction with other elements in plants. *Frontiers in Plant Science*, *15*. https://doi.org/10.3389/fpls.2024.1332459

Wany, A., Kumar, A., Nallapeta, S., Jha, S., Nigam, V. K., & Pandey, D. M. (2014). Extraction and characterization of essential oil components based on geraniol and citronellol from Java citronella (Cymbopogon winterianus Jowitt). *Plant Growth Regulation*, *73*(2), 133–145. https://doi.org/10.1007/s10725-013-9875-7

Woisky, R. G., & Salatino, A. (1998). Analysis of propolis: Some parameters and procedures for chemical quality control. *Journal of Apicultural Research*. https://www.tandfonline.com/doi/abs/10.1080/00218839.1998.11100961

Yang, J., & Hamid, M. B. B. (2024). Sustainable Beauty: A Conceptual Paper of How Sustainable Marketing Impact Consumer Behaviour in the Cosmetic Industry. *Advances in*

Economics, Management and Political Sciences, *93*(1), 54–59. https://doi.org/10.54254/2754-1169/93/20241083

Zhao, H., Zhang, H., & Yang, S. (2014). Phenolic compounds and its antioxidant activities in ethanolic extracts from seven cultivars of Chinese jujube. *Food Science and Human Wellness*, *3*(3–4), 183–190. https://doi.org/10.1016/j.fshw.2014.12.005